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The Effect of Water Deficit on Growth and Some Physiological Responses of Yew (*Taxus baccata* L.), as an Ornamental Tree

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Abstract

Drought is the most important environmental stress, which is usually defined as an external factor that has adverse effects in plants. The endangered yew species is very important for human societies for medicinal and ornamental reasons. The roots and leaves of this plant produce biochemical compounds such as flavonoids, alkaloids and tannins that act as anti-inflammatory, anti-cancer, anti-bacterial and anti-viral. The present research investigated the effect of different levels of drought stress (100, 75, 50 and 25 % of field capacity) on the growth and biochemical characteristics of the yew plant in a greenhouse environment in three replications in the form of a completely randomized design. In this research, which used 3-year-old seedlings, the fresh-to-dry weight ratio of shoots and roots, chlorophyll a and b and the ratio of these two, the relative content of leaf water, the activity of superoxide dismutase and catalase enzymes, and the percentage and yield of essential oil were evaluated. The results showed that the growth traits and photosynthetic pigments and the relative water content of the leaves decreased with the increase in the severity of drought stress. Of course, the activity of catalase and superoxide dismutase enzymes increased with the intensity of drought stress. The highest percentage of essential oil was obtained in semi-severe drought stress conditions (50 % FC). With the increase of drought stress up to 25 % FC, the percentage of essential oil decreased. Most of the growth and physiology parameters of yew plant at 75% FC compared to 100 % FC were not significantly different, so it seems that this plant shows good tolerance in the condition of 75 % FC and has an acceptable yield. Therefore, it is recommended to cultivate this valuable plant in favorable environmental conditions with sufficient water and/or with only in mild stress conditions.

Keywords: Antioxidant enzymes, Common yew, Dehydration stress, Essential oil.

INTRODUCTION

Drought is the most important environmental stress, usually defined as an external factor that leaves adverse effects. In most cases, stress is measured about growth (biomass accumulation) or primary assimilation processes (CO_2 and mineral absorption) related to overall growth (Pamungkas *et al*., 2022). Drought does not immediately create its effect, as plants use protective mechanisms to delay or halt chemical and thermodynamic disturbances within the cell (Shukla *et al*., 2018). Plants use various strategies to shield their tissues from the harmful effects of reactive oxygen species (ROS) and mitigate the potentially risky outcomes of water scarcity. In plants, innate antioxidant systems consist of such as superoxide dismutase and catalase as well as non-enzymatic antioxidants like certain primary metabolites (proline) and secondary metabolites (phenolic compounds, terpenoids, and alkaloids). These antioxidants aid plants retain their proteins and membranes, neutralize ROS, and maintain cellular redox potential (Soares *et al*., 2019).

The yew (*Taxus baccata* L.) is one of the species that is endangered due to extinction. This plant is highly important for human societies due to its medicinal and ornamental reasons. The roots and leaves of this plant produce chemical compounds like flavonoids, alkaloids, and tannins, which serve as anti-inflammatory, anti-cancer, antibacterial, and antiviral agents. The yew plant, with its attractive shape and colorful flowers, along with leaves that change colors in different seasons, can enhance gardens, parks, and green spaces, adding to the beauty and diversity of nature (Alavi *et al*., 2019; Shahmohammadi *et al*., 2023).

Investigating the effect of different soil moisture regimes on morphological, physiological and phytochemical responses in previous researches (Minaei *et al*., 2019; Gholizadeh *et al*., 2020; Arpiwi *et al*., 2023) showed that the fresh and dry weight of the plant decreased and the content of antioxidant enzymes increased. The highest percentage of essential oil and the highest yield of essential oil were obtained in mild treatment. Reduction of leaf water capacity is the primary result of drought in plants, thus reducing photosynthetic activity by stomatal closure, membrane damage, and accumulation of reactive oxygen species (ROS) (Shukla *et al*., 2018). According to previous studies, different levels of drought stress increased the antioxidant and phenolic constituents in olive (Gholami *et al*., 2022) and sweet cherry (Hojjati *et al*., 2023), essential oil content in lemon verbena (Hosseini *et al*., 2021), *Thymus daenensis* and *Thymus vulgaris* (Askary *et al*., 2018).

The main aim of the present study is to investigate the impact of drought stress on yew growth and biochemical characteristics. It also examines the potential for cultivating this ornamental-medicinal tree under different water scarcity stress conditions.

MATERIALS AND METHODS

Experimental design and treatments

The present study was conducted in the spring of 2022 in a greenhouse located in the Vardavard area of Tehran. The greenhouse had a photoperiod of 16 hours of light and 8 h of darkness, with a relative humidity of 65 to 75 percent, maximum temperature of 29 ºC, and minimum temperature of 15 ºC.

The experiment was based on a completely randomized design with three replications under four levels of drought stress treatments (control-100%, 75%, 50%, and 25% of FC). The drought stress was imposed based on the soil field capacity. The relative soil moisture at the field capacity and wilting point was determined using a pressure plate apparatus. Subsequently, the amount of water required based on the moisture treatments at 100%, 75%, 50%, and 25% of the soil FC was applied. Table 1 presents the physical and chemical characteristics of the pot soils.

Table 1. Physical and chemical characteristics of potting soil.

Parameter	The value
Total nitrogen (%)	0.16
Absorbable phosphorus (mg/kg)	14.4
Absorbable potassium (mg/kg)	262
Organic carbon (%)	0.59
Loam (%)	47
Clay (%)	27
Sand (%)	26
Electrical conductivity (m.mh)	1.2
pH	7.1
Class	Clay loam

The ratio of fresh weight to dry weight of aerial parts and roots

Initially, the fresh weight of aerial parts and roots after harvesting was measured using a digital scale with an accuracy of 0.1 g. Then, the samples were dried in an oven at 72 ºC for 24 h, and their dry weight was determined using a digital scale with an accuracy of 0.1 gr. The ratio of fresh weight to dry weight was then calculated (Inbar *et al*., 1994).

Chlorophyll content of leaves

Amount of 0.1 g of fresh needle leaves were completely ground in a mortar with 3 ml of 80% acetone until the final volume of the extract reached 15 ml. The extract was then centrifuged at a speed of 5000 × g for 10 min. A spectrophotometer (Shimadzu UV-160) was used to measure the absorption levels of the samples. The chlorophyll a (Eq. 1) and chlorophyll b (Eq. 2) content were calculated, followed by the calculation of the ratio of chlorophyll a to chlorophyll b (Arnon, 1967).

Equation 1: $[(12.7\times A663) - (2.69\times A645)] \times V / 1000 \times W$ = mg of chlorophyll a per g of fresh leaf
Equation 2: $[(22.9\times A645) - (4.69\times A663)] \times V / 1000 \times W$ = mg of chlorophyll b per g of fresh leaf

In the above equations, A represents the absorption at the specified wavelength, V is the final volume of 80% acetone in ml, and W is the weight of the fresh leaf in g.

Relative water content (RWC)

To measure this parameter, the youngest developed leaf of the plant was separated and quickly weighed with an accurate laboratory scale LiBROR AEL model 40SM with an accuracy of 0.0001. Then, the leaves of each treatment were immersed separately in a closed test tube containing distilled water for 4 to 5 hours, and after this period, the leaves were removed from the test tube and dried using filter paper, and weighed again. Until their weight is obtained in the state of complete turgor. To calculate the dry weight, the leaves were placed in a 70 °C oven for 48 hours and then weighed. Relative leaf water content was calculated from the following equation, where FW, leaf wet weight; DW is the dry weight of the leaf and TW is the saturated weight of the leaf (Ritchie *et al*., 1990).

$$RWC=\frac{FW-DW}{TW-DW}\times 100$$

Superoxide dismutase activity (SOD)

The activity of SOD enzyme was measured on the samples of fresh needle leaves of the plant. The activity of this enzyme was determined by its ability to inhibit the photochemical reduction reaction of nitrobuterazolium (NBT). The reaction mixture contains 50 mM phosphate buffer (pH=7), 13 mM methionine, 0.1 mM Na-EDTA, 75 µmol nitrobuterazolium (NBT), 75 µmol riboflavin, and 100 µL of extract. 3 ml of the above solution without enzyme extract was poured into two test tubes, one was placed in the device away from light and the other was placed in the presence of fluorescent light as a control. Every two min., the absorbance of the solution was read in the photometric mode and the wavelength of 560 nm with a spectrophotometer (Shimadzu UV-160). The activity of this enzyme was calculated in terms of enzyme units per milligram of protein (Giannopolitis and Ries, 1977).

Catalase enzyme activity

In order to extract enzymes, the sample of fresh needle leaves of the plant was crushed in a Chinese mortar with liquid nitrogen and turned into powder. Then, 0.5 ml of sodium phosphate buffer with pH equal to 6 was added to it and centrifuged at 13000 rpm. In order to measure the total protein and enzyme activity of the samples, the supernatant was taken from the solution. A spectrophotometer (Shimadzu UV-160) was used to measure catalase enzyme activity at a wavelength of 240 nm for 30 seconds. 20 mM sodium phosphate buffer with pH equal to 7 and 20 microliters of 30% hydrogen peroxide (H_2O_2) were used as electron acceptors. The amount of catalase activity was expressed in terms of enzyme units per milligram of protein (Luck, 1965).

The percentage and yield of essential oil

In order to measure the dry weight of the aerial parts of the plant (the upper branches which include the needles and part of the sub-stems of the plant) and also to prepare the sample for the preparation of essential oil, the plants after harvesting for two weeks at normal temperature (control), dried in the laboratory environment. In order to calculate the percentage of essential oil, 100 g of the dry matter of the plant was transferred to an essential oil extraction device (Clonger) and the process of extracting the essential oil contained in the plant was carried out for 4 h. Essential oil yield was obtained from the product of essential oil percentage in dry weight (Ghasemi Dehkordi and Taleb, 2018).

Statistical analysis

The obtained data were analyzed in the form of a completely random design, and Duncan's test and SAS 9.4 software were used to compare the mean.

RESULTS AND DISCUSSION

Drought stress was significant on wet-to-dry weight ratio of shoot and chlorophyll b at five percent level and on chlorophyll a at one percent level (Table 2).

Table 2. Results of variance analysis of drought stress treatment on growth characteristics of yew.

S.o.V	df	MS				
		fresh shoot / dry shoot	**fresh root / dry root**	**Chlorophyll a**	**Chlorophyll b**	**Chlorophyll a / b**
Drought stress	3	0.22*	0.13ns	0.13**	0.01*	0.07ns
Error	8	0.07	0.08	0.002	0.002	0.23
CV (%)	-	5.99	7.03	5.42	17.67	12.05

*, ** and ns: significant at $P < 0.05$, $P < 0.01$ and insignificant based on the Duncan's test, respectively.

Based result, with escalating drought stress, the ratio of fresh weight to dry weight of aboveground parts rose. As plant-available water decreased (under severe drought stress (25% FC)), this ratio hit its lowest numerical value (Table 3), suggesting a drop in tissue water content and a rise in the dry weight ratio of aboveground parts.

Table 3. The comparison results of the average effect of drought stress on the fresh-to-dry weight ratio of aerial parts and photosynthetic pigment in yew.

Drought stress	**Fresh shoot / dry shoot**	**Chlorophyll a**	**Chlorophyll b**
		(mg/g F.W.)	
100 F.C	4.38^{ab}	1.05^{a}	0.28^{a}
75 F.C	4.37^{ab}	1.03^{a}	0.25^{ab}
50 F.C	4.57^{a}	0.77^{b}	0.20^{bc}
25 F.C	3.94^{b}	0.62^{c}	0.15^{c}

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the Duncan's test.

Drought stress can significantly impact plant weight through various pathways. Under drought conditions, root, stem, and cell division growth decreases, leading to reduced plant weight due to decreased new tissue production. Drought stress can also alter cell structure, such as cell hardening and shape changes in dry conditions. These structural changes can decrease cell volume and plant weight. (Billah *et al*., 2021).

Photosynthetic pigments

Based on the results of increasing drought stress intensity, the levels of photosynthetic pigments (chlorophyll a and b) decreased. The lowest levels of chlorophyll a and b were linked to the 25% FC drought stress treatment (Table 3). In dry conditions, plants experience water deficiency, leading to reduced water uptake dependent on photosynthesis in the leaves. This reduction in water uptake against evaporation decreases chlorophyll production (Shahmohammadi *et al*., 2023). Under drought stress conditions, free radicals such as hydroxyl radicals (OH-) increase due to improper use of electron transfer in the photosystem and can degrade chlorophyll. This action leads to a reduction in chlorophyll in the plant (Jacomassi *et al*., 2022). Reduced plant growth during drought stress initially occurs with a decrease in the rate of absorption surface growth and subsequent inhibition of photosynthesis. The reduction in the rate of photosynthesis during drought stress can be attributed to stomatal factors (Chowdhury *et al*., 2017; Denaxa *et al*., 2020).

Drought stress resulted in significant differences in relative leaf water content, SOD, and catalase at the 1% level, and in essential oil yield at the 5% level (Table 4).

Table 4. Results of variance analysis of drought stress treatment on biochemical characteristics and essential oil of yew.

S.o.V	**df**	**Relative leaf water content**	**Superoxide dismutase**	**Catalase**	**Essential oil percent**	**Essential oil yield**
Drought stress	3	303.33**	15.05**	0.09**	0005*	4408.43^{ns}
Error	8	7.83	0.43	0.01	0.001	3291.93
CV (%)	-	3.62	14.48	17.19	17.62	28.55

*, ** and ^{ns}: significant at P < 0.05, P < 0.01 and insignificant based on the Duncan's test, respectively.

The relative leaf water content decreased with increasing drought stress intensity in yew. The highest numerical value of relative leaf water content was associated with 100% FC, which statistically grouped with 75% FC (Table 5).

Table 5. Comparison results of the average effect of drought stress on relative leaf water content, enzyme activity and essential oil yield in yew.

Drought stress	Relative leaf water content (%)	Superoxide dismutase	Catalase	Essential oil percent
		(mg/g F.W)		
100 FC	86.67[a]	2.67[c]	0.29[b]	0.15[c]
75 FC	83.67[a]	3.03[c]	0.33[b]	0.19[ab]
50 FC	74.67[b]	4.87[b]	0.54[a]	0.24[a]
25 FC	64.33[c]	7.57[a]	0.65[a]	0.17[b]

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.

Reduction in relative leaf water content is the first effect of drought stress on plants. Decreased leaf water potential due to low relative leaf water content leads to stomatal closure (El Sabagh *et al*., 2019). The reduction in water level causes plant cells to experience decreased turgor pressure, leading to cellular damage, wilting, and reduced plant growth. High relative water content in plants helps neutralize oxygen species and osmotic stress resulting from drought, potentially aiding in higher yields (Gurumurthy *et al*., 2019).

Catalase and superoxide dismutase

In this study, the activity of antioxidant enzymes (superoxide dismutase and catalase) in yew increased under drought conditions (Table 5). Increased enzyme activity, due to their role in removing oxygen from the environment, helps reduce light respiration and the compensation point of carbon dioxide. Additionally, they remove hydrogen peroxide from the environment and compensate for the lack of oxygen resulting from the inhibitory reaction (Kapoor *et al*., 2020). The rate of reactive oxygen species (ROS) production significantly increases during stress. Many biological processes are affected by reactive oxygen molecules, one of which is the function of antioxidant enzymes. In response to this oxidative damage, plants evolve tolerance mechanisms (increasing the activity of antioxidant enzymes such as catalase and superoxide dismutase) and develop resistance to non-biological stress (Mahmud *et al*., 2023).

Essential oil yield

Increasing stress intensity up to 50% FC led to an increase in the percentage of essential oil in yew, while at 25% FC, the percentage of essential oil decreased (Table 5). Glandular trichomes responsible for essential oil production change under stress and fertilization conditions, leading to changes in the amount of essential oil (Oguz *et al*., 2022). Medicinal plants exhibit different responses in essential oil production depending on the type and magnitude of stress. Drought stress at 60% FC led to an increase in the amount of essential oil, while at 40% FC, it led to a decrease in the essential oil of marigold (Bidabadi *et al*., 2020). Consistent with the current research, an increase in the percentage of essential oil under moderate stress and a decrease under severe stress conditions were reported in coriander plants (Amiripour *et al*., 2021).

CONCLUSION

Significant differences were observed in the growth, photosynthetic pigments, and biochemical traits of yew (*Taxus baccata* L.) plants under drought stress. Increased drought stress resulted in decreased growth, chlorophyll a and b levels, and leaf relative water content, while the catalase and superoxide dismutase enzyme activities increased under such stress. In response to drought stress, the yield of essential oil increased up to 50% FC, while under severe drought stress (25% FC), essential oil synthesis decreased, possibly due to the plant's energy being directed towards growth and biochemical activities for adaptation to adverse conditions, thereby reducing the production of compounds such as essential oil. It seems that the yew plant exhibits good tolerance under the condition of 75% FC and has an adequate yield because the majority of its growth and physiological characteristics at 75% FC compared to 100% FC were not significantly different. As a result, it is recommended to cultivate this valuable plant in an ideal habitat with sufficient water and/or under mild stress.

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Effect of 1-Methylcyclopropene and Methyl Jasmonate on Post-harvest Life of Alstroemeria cv. "Calgary" Cut Flowers

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Abstract

Alstroemeria is one of the most important cut flower crops globally, known for its diversity in color, various cultivars, and overall beauty, contributing to its success in the global market. The use of different preservative solutions at various stages of cut flower supply to the market plays a critical role in preventing ethylene production and maintaining the water balance and energy supply required for the plant after being separated from the mother plant. Therefore, this investigation effect of 1-methylcyclopropene (1-MCP) and methyl jasmonate (MeJA) on the post-harvest life of alstroemeria (cv. Calgary) cut flowers was conducted in the Department of Horticultural Science and Landscape Architecture, Faculty of Agriculture, Ferdowsi University of Mashhad. The experiment was done in a factorial design (3 × 3) with five repetitions for each treatment. The results of the main effect of methyl jasmonate (MeJA) application showed that the highest water uptake, relative fresh weight, vase life, chlorophyll a and b, anthocyanin, catalase, peroxidase, and superoxide dismutase were observed at a concentration of 0.2 µL/L of MeJA. The main effect of 1-MCP application showed that the highest water uptake, relative fresh weight, vase life, chlorophyll a and b, anthocyanin, catalase, peroxidase, and superoxide dismutase were observed at a concentration of 1 µL/L of 1-MCP. The results of the interaction effect between MeJA and 1-MCP application showed that the highest water uptake, relative fresh weight, vase life, chlorophyll a and b, anthocyanin, and antioxidant enzymes were observed in the treatment with 4% sucrose + 300 mg l^{-1} 8-HQS + 1 µL/L 1-MCP + 0.2 µL/L MeJA.

Keywords: Anthocyanin, Chlorophyll, Hydroxyquinoline, MeJA, 1-MCP.

INTRODUCTION

Alstroemeria, scientifically known as *Alstroemeria aurantica*, belongs to the Alestromeriaceae family and is native to South America. It is cultivated for the production of cut flowers and potted plants. Most species of alstroemeria have been identified in Chile and Brazil. Today, alstroemeria is primarily grown as a cut flower, with significant cultivation taking place in the Netherlands, Colombia, and the United States. In recent decades, alstroemeria has become one of the most important cut flowers globally due to its beautiful blooms, wide range of patterns and colors, and long-vase life quality. Over the past 20 years, various commercial hybrids of alstroemeria species, often referred to as Alstroemeriaceae or Peruvian lily, have become a significant part of the world cut flower trade (Nikbakht *et al*., 2020).

Alstroemeria flowers are highly sensitive to ethylene, and the ethylene produced in the late stages of their growth can lead to a reduction in their vase life, often causing petal drop (Wagsttaf *et al*., 2005; Chanasut *et al*., 2003). In flowers, which are ethylene-sensitive, ethylene has a major impact on petal senescence. It interacts with other phytohormones to start a signaling pathway and control different developmental stages (Dar *et al*., 2021). Many researchers have demonstrated that the appropriate chemistry of vase solutions can reduce respiration rate and senescence, thus increasing the longevity of cut flowers. These preservative solutions include carbohydrates, ethylene biosynthesis inhibitors such as silver thiosulfate (STS), aminoethoxy vinyl glycine, and antimicrobial agents (Vehniwal and Abbey, 2019).

Multiple stresses and their temporarily nature limit the vase life of cut flowers. The main symptoms of flower senescence are decreased water absorption, depletion of stored carbohydrates, and increases in ethylene production and respiratory activity. There are numerous methods for prolonging the preservation of cut flowers, such as controlling temperature and flower dehydration, using growth regulators, ethylene action inhibitors, and flower preservative treatments. Adding sugar to pulse solution or vase solution prolongs the postharvest life of cut flowers by enhancing water balance and energy or delaying senescence by lowering ethylene production (da Costa *et al*., 2021). To extend the vase life of cut flowers, using sucrose in the holding solution has been shown to increase the level of glucose and fructose in the petals. Sugars as a source of carbon and energy for cut flowers. Sugars also maintain the osmotic balance, promote water uptake, inhibit ethylene production and protein degradation, and ultimately postpone the senescence process (Alam *et al*., 2023).

One of the most crucial preservatives in the floral business is the germicide 8-hydroxyquinoline sulfate (8-HQS), which also serves as an antibacterial and enhances water absorption by decreasing "physiological" stem blockage in sterile tissues (Monya *et al*., 2021). However, adding sugar to 8-HQS improved the efficiency of this treatment. The application of 8-HQS + 3% sucrose at rates of 200 and 300 mg/l caused the longest vase life, lowest bacterial population in the vase solution, and least electrolyte leakages in cut *Alstroemeria* (Mohammadi Kabari and Jadid Soleimandarabi, 2019).

1-Methylcyclopropene (1-MCP) influences various aspects of aging and ripening in plants, including pigments, softening, cell wall metabolism, scent, and aroma. The aim of using 1-MCP for ornamental plants (cut flowers and potted plants) is to increase their vase life.1-MCP may increase JA content by increasing the BrWRKY12-BrLOX4 module-mediated JA biosynthesis in Chinese cabbage. Having potential uses for improving bioactive components, prolonging shelf life, and raising the market value of harvested horticulture products (Yue *et al*., 2023). The application of 1-MCP in lilies inhibits wilting and complexity response, extending the flower's life by half a day (Kim *et al*., 2010). However, the effects of 1-MCP treatment on potted plants like kalanchoe may vary depending on the cultivar or environmental factors.

Limiting effects on the post-harvest life of some , varieties have been observed (Park, 2012).

Jasmonates are derived from jasmonic acid and are a naturally occurring plant volatile compound. They act similarly to jasmonic acid in plants and are active in fruits such as strawberries, plums, lychees, apples, grapes, and peaches, increasing total phenolic content after harvest by activating the enzyme phenylalanine ammonia-lyase responsible for phenolic biosynthesis. Pre-harvest application of methyl jasmonate in grapes leads to the accumulation of anthocyanins in cells by activating proteinase inhibitors and chitinase gene expression (Ruiz - Garcia and Gomez-Plaza, 2013). Methyl jasmonate (MeJA) is favored as a vapor treatment to enhance the post-harvest lifespan of cut roses (Foukaraki *et al*., 2017).

Therefore, we design this research to evaluate effectiveness of 1-methylcyclopropane and methyl jasmonate on morphophysiological and biochemical characters to increase postharvest life of alstroemeria 'Calgary' cut flowers.

MATERIALS AND METHODS

In September 2022, alstroemeria 'Calgary' cut flowers were harvested in the commercial stage from the greenhouse located in Mashhad and transferred to the horticultural lab, Faculty of Agriculture, Ferdowsi University of Mashhad. The flowers were recut to 50 cm in height, and after weighing, they were placed in a 750 ml volume of vase solution containing 4% sucrose and 300 mg l^{-1} 8-hydroxyquinoline sulfate. Then cut flower stems were placed in 200-liter glass aquariums and treated with 1-MCP at concentrations of 0, 0.5, and 1 µL /L for 24 hours (Nergi and Ahmadi, 2014). Ethyl bloc powder prepared from US AgroFresh was used for applying the 1-MCP treatment. Considering the given concentrations, certain amounts of Ethyl Bloc were weighed and placed in Petri dishes, and then warm water (40–50 ˚C) was added to the Petri dishes inside glass aquariums. Immediately, the lids of glass aquariums were hermetically sealed with adhesive tape. For the steam treatment with methyl jasmonate at concentrations of 0, 0.1, and 0.2 µL L^{-1}, the flower stems were placed inside 200 L glass aquariums for 24 hours. Then, depending on the desired treatment concentration, the appropriate amount of liquid methyl jasmonate was mixed with 20 µL L^{-1} of ethanol. The mixture was poured onto filter paper inside the aquarium, and the lids of the aquariums were immediately sealed completely using special adhesive tapes (Darras *et al*., 2005).

During testing, the flowers were kept at 22 ± 2 ˚C with a relative humidity (RH) of 60–70%. At the end of the experiment, various traits such as water uptake, relative fresh weight, vase life, chlorophylls, anthocyanin, catalase, peroxidase, and superoxide dismutase enzyme activity were measured. The experiment was conducted in a factorial (3 × 3) design based on a completely randomized design with 5 replications for each treatment.

Vase life is defined as the leaves yellowing and petals wilting process and is expressed as days.

To measure the chlorophyll content in the leaves, follow the method of Lichtenthaler and Wellburn (1983). Anthocyanin content in petals was carried out according to the method of Liao *et al*. (2013). Catalase enzyme activity was followed by the Cakmak and Horst (1991), method. Measurement of peroxidase enzyme activity was conducted using the method described by Tabatabaei and Ehsanzadeh (2016).

The activity of the superoxide dismutase (SOD) enzyme was determined similarly to the method mentioned for the catalase enzyme (Chance and Maehly, 1995).

The data were analyzed by SPSS software, and the means were compared by the least significant difference (LSD) test ($P < 0.01$ and 0.05). The figures were drawn using Excel software.

RESULTS

The results of the analysis of variance indicate that the main effects of 1-MCP and MeJA, as well as the interaction effect between 1-MCP and MeJA, were significantly different at a 1% probability level for water absorption, relative fresh weight, chlorophyll a, b, and antioxidant enzymes (Table 1). The results of the analysis of variance also show that the main effects of 1-MCP and MeJA were significantly different at a 1% probability level for vase life (Table 1). The results of the analysis of variance also demonstrate that the main effect of 1-MCP was significant at a 1% probability level for anthocyanin (Table 1).

Table 1. Analysis of variance for the effects of 1-MCP and MeJA on measured traits.

S.o.V	df	MS								
		Water uptake	Relative fresh weight	Vase life	Chl. a	Chl. b	Anthocyanin	CAT	POX	SOD
1-MCP (A)	2	1351.57**	806.39**	19.09**	32.02**	30.25**	0.048**	2631.34**	4.01**	3280.19**
MeJA (B)	2	386.48**	485.05**	9.68**	13.89**	10.79**	0.007^{ns}	651.39**	1.83**	828.00**
A×B	4	33.62**	101.38**	0.25^{ns}	4.19**	2.86**	0.0024^{ns}	44.17**	0.61**	74.83**
Error	36	2.91	1.09	0.24	0.11	0.08	0.0016	3.86	0.024	3.73
CV (%)		1.51	1.07	4.69	5.62	5.78	2.81	3.37	8.83	2.96

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

The results of the main effect of MeJA on water uptake, and relative fresh weight, vase life, chlorophyll a, b, and anthocyanin, and antioxidant enzymes showed that with an increase in the concentration of MeJA, there was an increasing trend in the levels of water uptake, relative fresh weight, vase life, pigments, and antioxidant enzymes. The highest and lowest levels of these factors were obtained in treatments with 0.2 and 0 µl/L MeJA, respectively (Table 2).

Table 2. Comparison of the mean main effects of MeJA on measured traits.

MeJA	Water uptake	Relative fresh weight	Vase life	Chl. a	Chl. b	Anthocyanin	CAT	POX	SOD
(µl/L)	(g/cut flower)	(%)	(days)	(µg/mg FW)		(µg/mg FW)	(unit mg^{-1} protein)		
0	99.18c	89.68c	9.533c	4.5991c	3.6339c	0.2402a	43.840c	1.2360c	49.315c
0.1	111.83b	100.18b	11.067b	6.0981b	5.1897b	0.1269c	61.475b	1.7313b	67.895b
0.2	117.76a	103.80a	11.733a	7.5664a	6.4697a	0.1929b	69.775a	2.2693a	78.533a

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

The results of the mean main effect of 1-MCP on water uptake and relative fresh weight showed that with an increase in the concentration of 1-MCP, there was an upward trend in water absorption and relative fresh weight. The highest levels of these factors were observed at a concentration of 1 µl/L 1-MCP (Table 3). There were no significant differences between the treatments with 1 and 0.5 µl/L 1-MCP in terms of water absorption and relative fresh weight (Table 3). The shortest vase life was observed in the control treatment, while the treatment with 1 µl/L 1-MCP had the longest vase life. The results of the mean main effect of 1-MCP on pigments and antioxidant enzyme levels showed that with an increase in the concentration of 1-MCP, the pigments and antioxidant enzyme levels increased. The highest levels of chlorophyll a, b, and antioxidant enzymes were observed at a concentration of 1 µl/L 1-MCP (Table 3).

Table 3. Comparison of the mean main effects of 1-MCP on measured traits.

1-MCP	**Water uptake**	**Relative fresh weight**	**Vase life**	**Chl. a**	**Chl. b**	**Anthocyanin**	**CAT**	**POX**	**SOD**
(µl/L)	**(g/cut flower)**	**(%)**	**(days)**	**(µg/mg FW)**		**(µg/mg FW)**	**(unit mg^{-1}protein)**		
0	103.77b	91.32b	9.933c	4.9878c	4.1532c	0.1747b	50.889c	1.4027c	56.858c
0.5	111.88a	101.18a	10.867b	6.5009b	5.3455b	0.2129a	60.865b	1.7320b	67.892b
1	113.11a	101.16a	11.533a	6.7749a	5.7946a	0.1725b	63.337a	2.1020a	70.994a

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

The results of the interactive effect of 1-MCP and MeJA on water absorption, relative fresh weight, and vase life showed that with an increase in the concentration of 1-MCP in all concentrations of MeJA, water absorption, relative fresh weight, and vase life of the cut flower of alstroemeria increased. The highest level of water absorption, relative fresh weight, and vase life were observed in the treatment with a concentration of 1 µL/L 1-MCP+0.2 µL/L MeJA. While the lowest level of measured traits was observed in the control treatment, there were no significant differences between the treatments (0.1 µL/L MeJA & control) and (1 µL/L 1-MCP+0.1 µL/L MeJA & 0.5 µL/L 1-MCP+0.2 µL/L MeJA) in terms of vase life (Fig. 1).

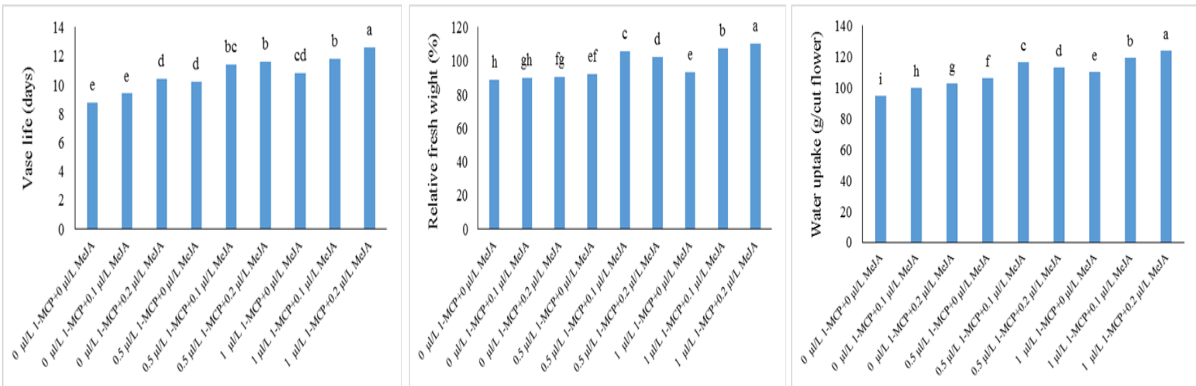

Fig. 1. Interactive effect of 1-MCP and MeJA on uptake of water, relative fresh weight and vase life.

The results of the interactive effect of 1-MCP and MeJA showed that chlorophyll a and b and anthocyanin increased with the increase in the concentration of 1-MCP and MeJA. The highest chlorophyll a and b and anthocyanin content was observed in the treatment with 1 µL/L 1-MCP+ 0.2 µL/L MeJA. The lowest pigment content was observed in the control (Fig. 2).

The results of the interactive effect of 1-MCP and MeJA showed that catalase, peroxidase, and superoxide dismutase increased with the increasing concentration of 1-MCP and at concentrations of 0 µL/L and 0.2 µL/L MeJA. In the presence of 0.1 µL/L of MeJA with concentrations of 0.5 and 1.0 µL/L of 1-MCP, catalase, peroxidase, and superoxide dismutase were higher than at the zero concentration of 1-MCP. However, there were no significant differences between the concentrations of 0.5 and 1.0 µL/L of 1-MCP. The highest levels of catalase, peroxidase, and superoxide dismutase were observed in the treatment with 1.0 µL/L 1-MCP+0.2 µL/L MeJA, while the lowest levels were observed in the control (Fig. 3).

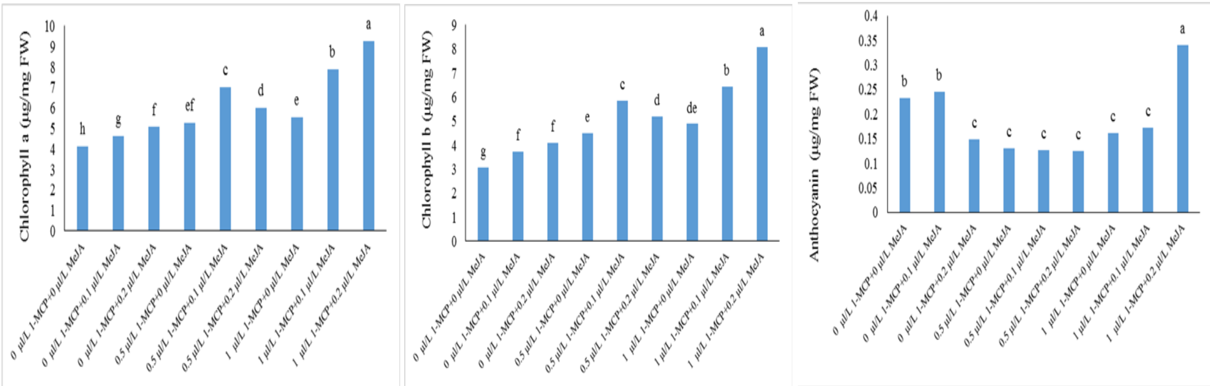

Fig. 2. Interactive effect of 1-MCP and MeJA on chlorophyll a, b and anthocyanin.

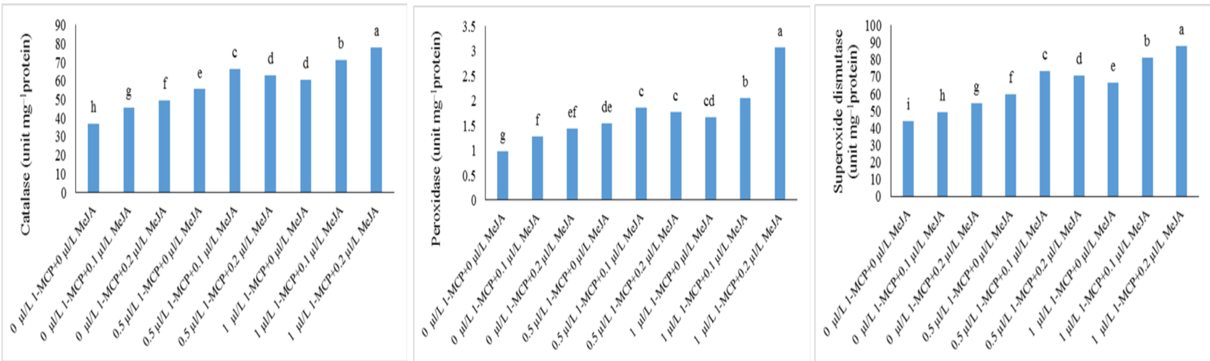

Fig. 3. The interactive effect of 1-MCP and MeJA on antioxidant enzymes.

DISCUSSION

The wilting of cut flowers is related to a hormonal mechanism that involves changes in the physical and biochemical properties of cell membranes. This process is characterized by a rapid decrease in the levels of phospholipids and proteins, an increase in the activity of degrading enzymes, the dispersion of macromolecules, increased respiration activity, and decreased membrane stability. Ultimately, this leads to the uncontrolled loss of soluble substances and water, resulting the wilting and death of the cut flowers (Dhindsa *et al*., 1981). Blockage of the stem by bacteria reduces water uptake, leading to bending and wilting of the stem as well as withering of the petals (Solgi *et al*., 2009). Gray mold fungus is a common pathogen in cut flowers, and it plays a significant role in the onset of aging and determining the vase life of cut flowers. Gray mold fungus can block the cut stems, reduce water uptake, and contribute to wilting (Capdeville *et al*., 2003). The cut flower continues to photosynthesize even after it has been removed from the mother plant; therefore, the cut flower requires a food source to maintain its health and stay fresh for a longer period of time. Treating a cut flower with floral preservation extends its vase's life. These floral preservatives can help keep cut flowers fresher for longer. A flower preservative is a complicated mixture containing sucrose (the primary food material), an acidifier (to lower the pH of the solution), a microorganism inhibitor (to act as a germicide), and a respiratory inhibitor (to decrease the impact of ethylene). A clean vase should be utilized (Monya *et al*., 2021).

Methyl jasmonate application induces the production of secondary metabolites, the expression of defense genes, and the induction of systemic resistance in plants against pathogens (Ahmad *et al*., 2016). The concentration of 0.1 µl MeJA vapor treatment after harvest also had similar results, increasing vase life and quality by reducing the growth of microorganisms in the holding solution (Son *et al*., 2003). Methyl jasmonate had a positive effect on reducing the activity of gray mold and significantly reducing the growth and activity of microorganisms in the holding solution, as well as reducing petal spotting in cut *Freesia* flowers. It induced the expression of defense genes and the synthesis of some defensive compounds, resulting in decreased growth and activity of microorganisms (Darras *et al*., 2005).

Regarding the increase in chlorophyll content as a result of treatment with methyl jasmonate, it can be explained that MeJA leads to an increase in the level of beta-carotene in cells. The primary protective role of beta-carotene and xanthophylls in photosynthetic tissue may involve preventing the production of singlet oxygen, thus protecting chlorophyll from oxidative damage (Farooq *et al*., 2009).

According to research, external application of MeJA can cause an increase in anthocyanin accumulation in plants. Anthocyanins and phenols are plant secondary metabolites that inhibit reactive oxygen species and protect against photodynamic damage. Research has demonstrated that using naturally occurring chemicals, such as methyl jasmonate, might enhance secondary metabolites. For example, preharvest application of 200 μM MeJA on strawberry fruits improved ascorbic acid, total anthocyanin contents, and total antioxidant activity (Asgari *et al*., 2023). Treating blackberry plants with methyl jasmonate significantly increased the flavonoid content in these plants (Wang *et al*., 2008). Gun *et al*. (2023) discovered that nanofiber mats created by utilizing methyl jasmonate and nanosilver active compounds were effective in suppressing microbial growth and prolonging the vase life of cut rose flowers. This study's findings are comparable to those of Hasanzadeh-Naemi *et al*. (2021). They found that MeJA treatments improve post-harvest quality and cut flower vase life. 0.2 mM MeJA enhanced the level of CAT and SOD activity, membrane stability index, anthocyanin, total soluble sugar, and relative water content.

Since post-harvest senescence plays a limiting role in product supply and the marketability of many cut flowers, the application of methods with high reliability has become crucial. Regarding the use of 1-MCP as an ethylene antagonist, it has been established that this compound can effectively compete with ethylene for binding to ethylene receptors, thereby preventing an ethylene response (Seglie *et al*., 2011). 1-MCP has prevented petal abscission in chrysanthemums, although its efficacy depends on transportation conditions, storage temperature, and the number of applications (Djanaguiraman *et al*., 2011). In a study on cut cluster amaryllis flowers, treatment with 1-MCP reduced ethylene production at all concentrations, delaying chlorophyll degradation compared to control plants (Seglie *et al*., 2011). Meng *et al*. (2023) found that fruits treated with 1-MCP or SA alone had increased resistance to pathogen infection, while 1-MCP combined with SA therapy improved self-resistance. Using the preservation approach in combination with 1-MCP and SA could be a viable strategy to extend shelf life. Lilies treated with 1-MCP for 8 hours had maximum anthocyanin content until the twelfth day of storage (Chutichudet *et al*., 2010). The application of 1-MCP to soybean plants reduced hydrogen peroxide levels compared to untreated plants. It also reduced ethylene production and free radicals and increased antioxidant enzyme activity. This treatment extended the post-harvest life of the flowers by inhibiting external ethylene action. Research on cut lilies also suggests that 1-MCP preserves the quality by inhibiting internal ethylene production (Djanaguiraman *et al*., 2011). Therefore, increasing the post-harvest life of cut flowers using 1-MCP is attributed to the inhibition of ethylene action and consequently the suppression of ethylene biosynthesis (Mojdeh, 2020). Application of 0.5 and 1 $\mu L\ L^{-1}$ 1-MCP significantly extended vase life, water uptake, and relative fresh weight of *N. tazetta* cut flowers compared to the control. The effects of 1-MCP in preserving chlorophyll content result from inhibiting ethylene action and, consequently, ethylene biosynthesis, which is the most crucial factor in leaf yellowing in ornamental plants. In a study on cut cluster amaryllis flowers, treatment with 1-MCP reduced ethylene production and delayed chlorophyll degradation compared to control plants (Asil *et al*., 2013). Bayat and Moradinezhad (2020) found that the application of 0.5 and 1 $\mu L\ L^{-1}$ 1-MCP significantly extended vase life, water uptake, and relative fresh weight of *N. tazetta*-cut flowers compared to the control. This result is similar to the current study. Additionally, 1-MCP prevented yellowing in *Dendrobium* and *Amaryllis* varieties (Serek *et al*., 1998), with its effects attributed to blocking ethylene receptors.

Sucrose has been demonstrated to enhance glucose and fructose levels in petals, lending support to the use of sugar-based external holding solutions to extend the vase life of cut flowers. Sugars are a source of energy and carbon for cut flowers and serve a vital role in reducing protein breakdown and ethylene generation, maintaining osmotic equilibrium, enhancing water intake, and finally delaying the senescence process (Sharafshah Rostami and Kaviani, 2023). The addition of sucrose in pulse solution or as a component of vase solution improves flower vase life by enhancing water balance and energy or delaying senescence through reductions in ethylene production (da Costa *et al*., 2021). According to Sun *et al.*'s (2022) results, sugar can improve the quality of cut flowers by affecting the hormone balance of flower tissues and delaying the breakdown of mitochondria in senescent petals. Studies have shown that providing sucrose in the vase solution for cut flowers leads to improved quality and better post-harvest longevity (Gebremedhin, 2020). The use of sugar solutions in the vase water leads to a reduction in ethylene production and enhances the quality and post-harvest longevity of cut flowers, including lilies (Verlinden and Garcia, 2004). The positive effect of sucrose in extending the vase life of other cut flowers such as roses, marigolds, chrysanthemums, waxflowers, snapdragons, lisianthus, statice, alstroemeria, and gladiolus has also been demonstrated (Manzoor *et al*., 2018).

CONCLUSIONS

Finally, it can be concluded that increasing the activity of antioxidant enzymes reduces the aging of flowers. In general, 1-MCP and MeJA vapor treatments had a positive effect on improving physiological and biochemical characteristics, resulting in an extended postharvest longevity of alstroemeria cut flowers. The higher concentrations of 1-MCP and MeJA revealed a better effect in comparison to low concentrations. According to the results, 1-MCP and MeJA vapor treatments, by increasing the protein content and the activities of catalase, peroxidase, and superoxide dismutase, improved the quality and increased the vase life of alstroemeria cut flowers. Based on this study, the postharvest application of 1-MCP acting as an ethylene action inhibitor and MeJA induces the production of secondary metabolites, the expression of defense genes, and the induction of systemic resistance in plants against pathogens, which could be recommended to increase postharvest life and extend the longevity of alstroemeria cut flowers.

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Research Article
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Biological Inhibition of *Thuja* Collar and Root Rot Using Some Antagonistic Bacteria

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Abstract

Root and collar rot of *Thuja* caused by *Fusarium oxysporum* is one of the important diseases in *Thuja* cultivation. In this research, the effect of eight bacterial strains of *Bacillus licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis*, *B. velezensis*, *Pseudomonas flurescens*, *P. koreensi* and *P. putida* in controlling this disease was investigated in the laboratory. Among them, based on the dual culture test and observation of the inhibition zone, *B. velezensis* and *B. subtilis* had the highest inhibition with 9.33% and 5.6%, respectively. In the study of the effectiveness of non-simultaneous antifungal volatile compounds, *P. flurescens* had the highest inhibition with 58.33%. The simultaneous volatile compounds of *P. koreensi*, *P. flurescens*, *B. pumilus*, and *B. megaterium* completely controlled the disease agent. In the study of the effect of filtered extracellular liquid metabolites on the growth of the pathogen colony, it was observed that with the increase in the concentration of the metabolites, the inhibition percentage of the growth of the pathogen colony by all bacterial strains increases. The best strains against *F. oxysporum* were *B. velezensis*, *B. subtilis*, and *B. pumilus* strains, which in 25% concentration were 50, 72.27 and 86.67%, respectively, and in 15% concentration *B. pumilus* strain was 66. 75% and *B. velezensis* strain with 46.66% inhibition, and at 5% concentration, *B. pumilus* strain with 44% inhibited the growth of the fungal colony. In the protease production test, all isolates were able to produce protease. Only *P. fluorescent* strain was able to produce a siderophore. In the microscopic studies, all the investigated strains caused morphological changes, fusion of different parts of the filaments, and destruction.

Keywords: Antagonist, Bacteria, Collar rot, *F. oxysporum*, *Thoja*.

INTRODUCTION

F. oxysporum has a wide host range and causes disease in various crops (Moshayedi and Rahanandeh, 2014). The cause of collar and root rot (*F. oxysporum*) of *Thuja* was first reported in 2022 from the western regions of Gilan province in Iran (Ghadimi and Rahanandeh, 2022). In India in 2007, this fungal agent was reported as the cause of the wilting of the thuja tree (Raghavendra *et al*., 2007). The use of fungicides leads to environmental pollution, endangering the health of humans and other creatures, and they often have low efficiency in controlling these pathogens. Therefore, researchers have been led to develop and use other environmentally friendly and effective methods for an integrated management of *Fusarium*. The use of biological inhibitors (biological control) is one of the most important methods used in the integrated management of fungal diseases (Rahanandeh *et al*., 2012; Grosu *et al*., 2015; Shirmohamadi *et al*., 2022). The effect of biological control agents can be categorized into two types direct and indirect effects; direct mechanisms include the production of siderophore, the secretion of antimicrobial compounds, or the production of toxic and destructive enzymes (Compant *et al*., 2005; Rahanandeh *et al*., 2017). On the other hand, more indirect mechanisms include increasing the resistance of plants, which takes place through two paths acquired resistance (SAR) and induced resistance (ISR) (Doornbos *et al*., 2012). *Bacillus* species such as *B. amyloliquefaciens* and *B. subtilis* are Gram-positive and spore-forming bacteria and have antagonistic activity against plant pathogens. In addition, Bacillus species are suitable for commercialization due to the production of spores that are resistant to adverse environmental conditions such as drought and heat, and they can easily be used in the formulation of sustainable products and as protection against environmental stress (Perez-Garcia *et al*., 2011). Some strains of *P. fluorescens* have prevented the growth of *F. oxysporum* in the laboratory and the greenhouse, they have caused a decrease in the severity of the disease and an increase in the growth of plants (Ebrahimi Kazemabad *et al*., 2012). In one of the researches, a direct relationship between the production of antifungal metabolites of bacterial isolates and the reduction of disease has been observed (Ebrahimi Kazemabad *et al*., 2012). The effect of some isolates of *Pseudomonas* spp., *Bacillus* spp. has been observed in the control of *F. oxysporum* under controlled conditions. The extracellular secretions of *Bacillus* species have inhibitory properties and prevent the germination and mycelial growth of *Fusarium* conidia (Iraqi *et al*., 2009). It has also been determined that *B. subtilius* strains are very effective in protecting crops against *Fusarium* and *Rhizoctonia* and have greatly increased plant growth (Schisler *et al*., 2004). According to Zhang *et al*. (2009), *B. subtilis* has fungicidal properties and can be used as a seed coating against root rot caused by pathogens. The production of antibiotics such as mycotoxin, ethiorin A, bacillomycin, basilin, and subsporin by *B. subtilis* species is the main and determining factor in the biological control of plant diseases by this bacteria (Kim *et al*., 1997). The purpose of this research is to evaluate the identified antagonists against the fungus *F. oxysporum*, which causes collar and root rot of thuja in laboratory conditions.

MATERIALS AND METHODS

Preparation of samples of antagonistic bacteria

To investigate the antagonistic effects, eight bacteria were prepared from Tehran Water and Soil Institute.

Experimental dual-culture

To perform the test, it was done according to Garbeva's method (Garbeva *et al*., 2008).

After seven days of incubation at 27 °C, the average diameter of the inhibitory halo was measured and the rate of pathogen growth inhibition was calculated according to the method of Huang (Huang *et al*., 2017).

$$\text{Growth inhibition} = 100 \times \left(\frac{\text{growth diameter of the control plot} - \text{growth diameter of the treatment plot}}{\text{growth diameter of the control plot}}\right)$$

Investigating the effect of non-synchronous antifungal volatile compounds

The amount of 200 µl of the suspension of bacterial isolates was spread on NA and the Petri dishes were kept at 27 ˚C for 72 h. Then, rings with a diameter of five mm of fungi culture were cultivated in the center of petri dishes containing PDA. Then, the Petri dishes containing the fungus were placed upside down on the Petri dishes containing the antagonist isolates, and the edges of the Petri dishes were placed on top of each other and completely blocked by parafilm tape. Petri dishes were kept at a temperature of 27 ˚C for one week. In the test of volatile compounds, bacteria and fungi were cultured at the same time, and other test steps were performed as above (Kraus and Loper, 1990; Sedaghatfar *et al*., 2002). The amount of inhibition of pathogen growth was calculated according to the method of Huang (Huang *et al*., 2017). This experiment was conducted in the form of a completely randomized design including 8 treatments in 3 repetitions.

Production of extracellular compounds

100 µl of the suspension of bacterial isolates and sterile distilled water were added to the PDA culture medium as a control and spread on the surface of the culture medium with a Pasteur pipette and kept for 72 h at a temperature of 27 ˚C. Then the isolates were washed from the surface of the culture medium and the Petri dishes were exposed to chloroform vapor for 30 min upside down. Then, a five-mm ring of fungi was planted in the center of each petri dish, observing the sterile conditions. Petri dishes were kept at a temperature of 27 ˚C and the diameter of mycelium growth was measured after 10 days (Kraus and Loper, 1990). The amount of inhibition of pathogen growth was calculated according to the method of Huang (Huang *et al*., 2017).

Protease production

Considering the role of protease as one of the biological control mechanisms, the production of this enzyme was evaluated on eight bacterial strains. In this experiment, SMA (Skim milk agar) culture medium containing 5 g of milk powder, 5 g of yeast extract, 4 g of blood agar, and 13.5 g of microbiological agar in one liter. The bacteria were cultured on the surface of the medium in a spot manner and incubated at a temperature of 28 to 30 °C for 48 h. The presence of a colorless halo around the colony is a sign of protease activity. The average radius of this halo in mm was measured in different strains and was used as a criterion to evaluate the ability of protease production in different strains (Maurhofer *et al*., 1992).

Extracellular liquid secretions of antagonist isolates

250 g of potato, 20 g of dextrose were prepared in one liter of distilled water. 50 ml of the above liquid medium were poured into flasks with a capacity of 250 ml and sterilized in autoclave conditions. After cooling the liquid environment, a full loop of 24 h bacterial culture was added to each of the flasks. The flasks containing bacteria were placed on a shaker with 70

revolutions per min at 27 ˚C for one week, and after passing the liquid medium of the isolates through Whatman No. 1 filter paper and centrifuged at 5000 x g for 20 min, each of the isolates were filtered separately by microbiological 0.22 micron and the extract obtained from each of the mentioned isolates was used in the test of inhibiting the growth of mycelium of fungi. To investigate the effect of the obtained extract, first, the PDA culture medium was poured into tube of 19, 17, and 15 ml, and after sterilization in autoclave conditions, 1 ml (concentration of 5% vol: vol), 3 ml (concentration 15%) and 5 ml (concentration 25%) of the extract of each of the antagonists were added to PDA tubes with a temperature of 45 ˚C and mixed well and in sterile conditions to sterile Petri dishes. They were transferred. As a control treatment, the liquid culture medium without bacteria that was passed through a 0.22 micron filter was used. After the coagulation of the culture medium, a ring with a diameter of five ml from the fungi culture was planted in the center of each petri dish. The diameter of the filament was measured every day for 10 days after fungi cultivation. The amount of inhibition of pathogen growth was calculated according to the method of Huang (Huang *et al*., 2017).

Siderophore production

This study was done according to the method of Wheller and Cook (1983). *Pseudomonas fluorescent* isolates were cultured on King B culture medium containing concentrations of 5, 50, and 100 µM of Fe III chloride and were kept for 48 h at a temperature of 25 ˚C. Then the spore suspension of *Geoterichum candidum* from a 48-h culture on PDA medium was sprayed on the surface of the petri dish containing the antagonist isolate. The absence of mushroom growth around the bacteria indicates the production of siderophore.

Investigating the inhibitory effects of antagonistic strains on a microscopic scale

In addition to measuring the macroscopic effect of antagonistic strains based on their inhibitory properties in Petri dishes, their effects on *Fusarium* fungus were also investigated at the microscopic scale. For this purpose, microscopic samples were prepared from the margin of the colon affected by the metabolites of the antagonistic strains and observed under the microscope with 40x magnification. In this study, the condition of mycelium and mushroom growth were compared to the control treatment.

Statistical analysis of data

The statistical analysis of the data was done using SAS and the categorization of treatments was performed by Duncan Multiple Range Test at 0.1 and 0.5 level of significance.

RESULTS

Characteristics of antagonistic bacteria

The bacteria used in this research were obtained from Tehran Water and Soil Research Institute (Table 1).

Dual-culture test and selection of antagonistic strains

Based on this test, two strains were inhibitory to *F. oxysporum* isolate. The B.ve strain exhibited the highest inhibition at 33.9%, while the B. sub isolate showed 5.6% inhibition, placing it in the next statistical group (Fig. 1).

Table 1. Characteristics of the bacteria used.

Abbreviation of bacteria	Name of bacteria and code
B. li	*Bacillus licheniformis* (CCSM-B 00587)
B. me	*Bacillus megaterium* (CCSM-B00702)
B. p	*Bacillus pumilus* (CCSM-B005220)
B. su	*Bacillus subtilis* (CCSM-B01449)
B. ve	*Bacillus velezensis* (CCSM-B484)
P. f	*Pseudomonas flurescens* (CCSM-B00102)
P. k	*Pseudomonas koreensis* (CCSM-B00287)
p. p	*Pseudomonas putida* (CCSM-B00586)

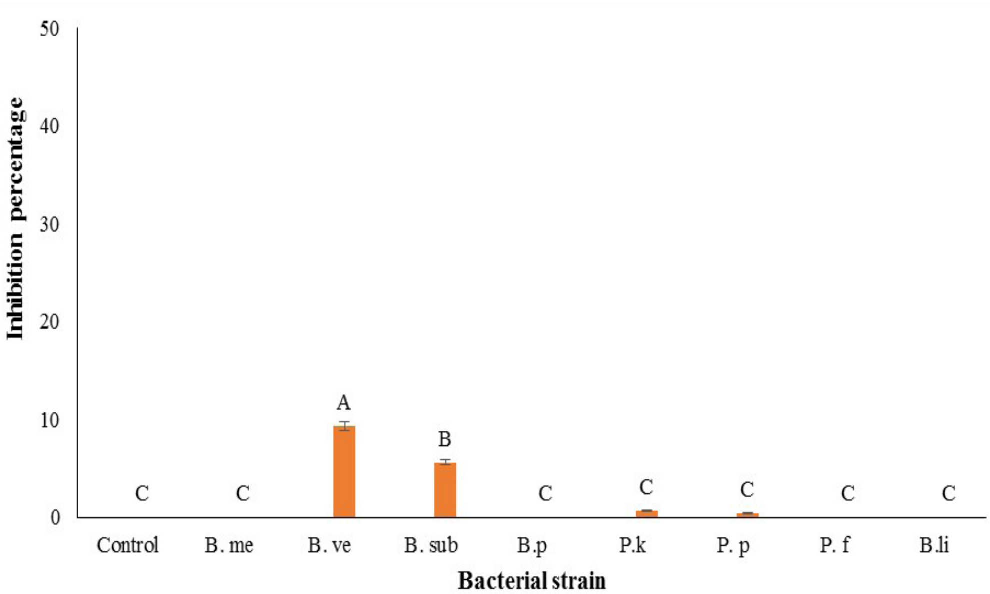

Fig. 1. The effect of antagonistic bacterial strains on the inhibition percentage of the growth of *F. oxysporum*, the causative agent of thuja rot, in the dual-culture test.

The effect of volatile antifungal compounds on bacteria

In the test of the effectiveness of non-synchronous volatile antifungal compounds, strain P.f was the most effective with 58.33 percent inhibition, and other strains were at lower levels based on statistical analysis, and strain B. li was not inhibitory (Fig. 2).

In testing the effect of volatile antifungal metabolites at the same time, B.p., and P.f strains with 50% inhibition of the growth of the disease-causing fungus *F. oxysporum* were the most effective and B. me was the least effective with 8.33% inhibition (Fig. 3).

The effect of the production of diffusible extracellular metabolites of bacterial strains in preventing the mycelium growth of *F. oxysporum*

B. me, B. p, P. f, and P. k strains had a 100 % effect on *F. oxysporum* pathogen compared to the control. B. su isolate with 54.66% inhibition was the least effective isolate in statistical group D (Fig. 4).

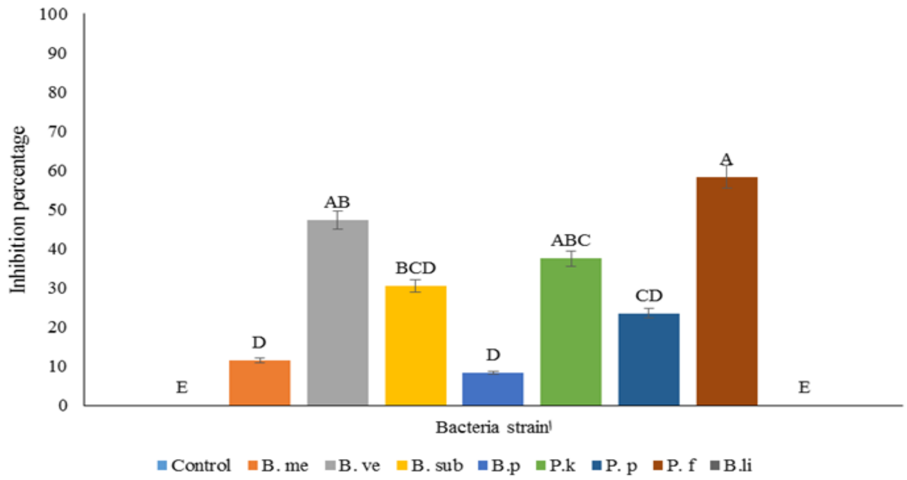

Fig. 2. Inhibition percentage of non-simultaneous volatile compounds of antagonistic bacterial strains.

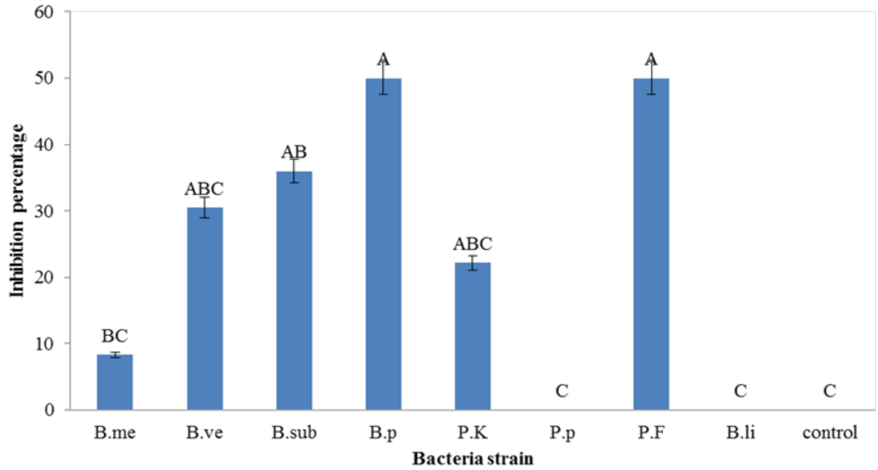

Fig. 3. Inhibition percentage of simultaneous volatile compounds of antagonistic bacterial strains.

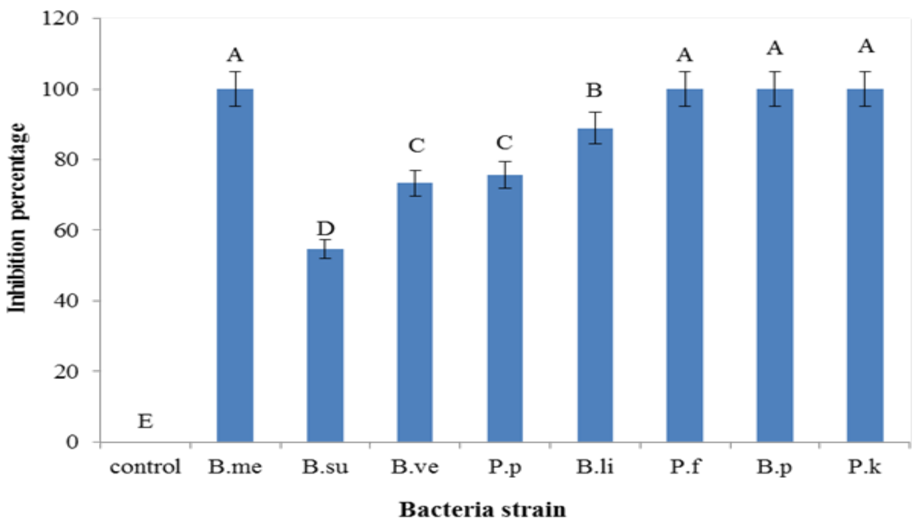

Fig. 4. Inhibition percentage of diffusible extracellular compounds of antagonistic bacterial strains.

Effect of filtered extracellular liquid metabolites of antagonistic bacteria

Increasing the concentration of metabolites, resulted in a higher percentage of inhibition of pathogen growth by all bacterial strains. At a concentration of 25%, the B. ve, B. su, and B. p strains exhibited inhibitions of 50%, 72.27%, and 86.67%, respectively. At a concentration of 15%, the B. p strain showed 75.66% inhibition, while the B. ve strain showed 46% inhibition. Finally, at a concentration of 5%, the B. p strain demonstrated the highest inhibition of fungal growth at 44% (Fig. 5).

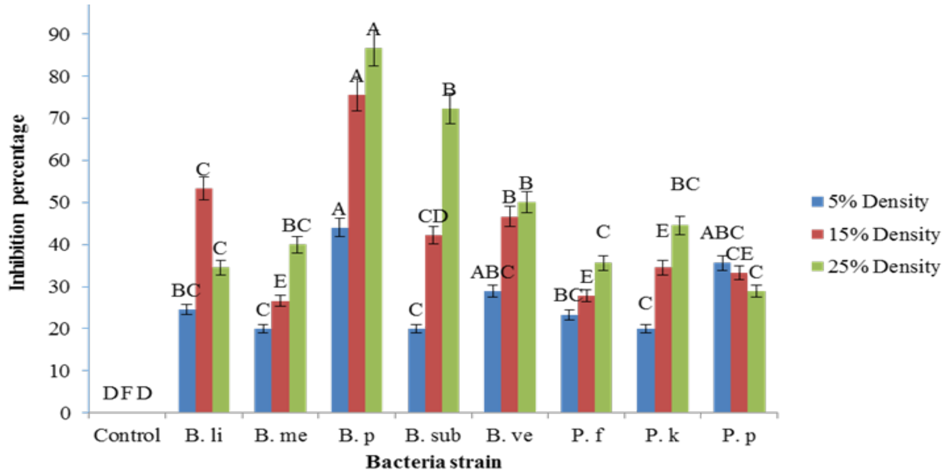

Fig. 5. The effect of filtered extracellular fluid metabolites of bacterial strains on the growth inhibition rate of *F. oxysporum* fungi.

Protease production test

All isolates were able to produce protease. In this test, B. ve bacteria had the largest halo diameter of 8. 167 mm and B. su had the smallest halo diameter of 1. 667 mm (Fig. 6).

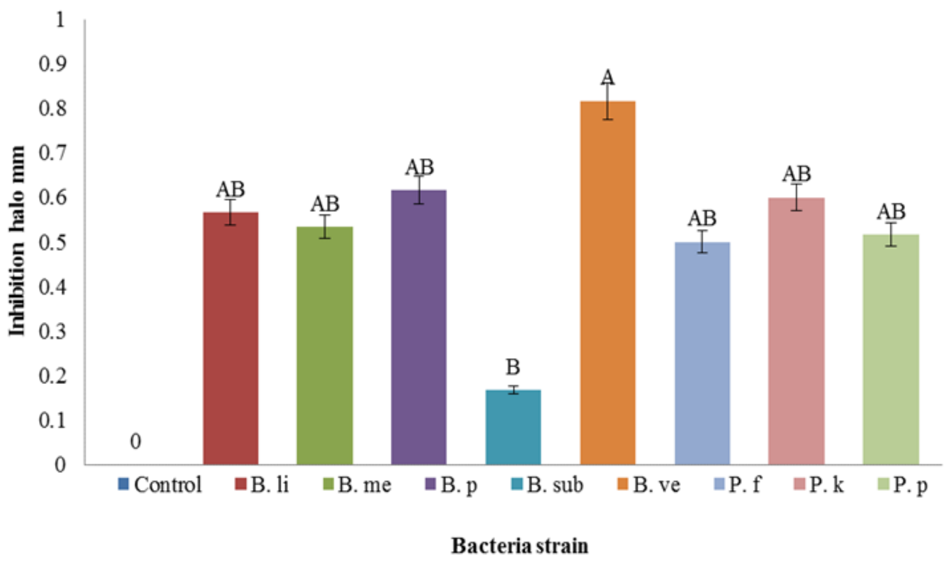

Fig. 6. The amount of halo formed by bacterial isolates in the protease test.

Siderophore production

P. f strain was able to produce siderophore at concentrations of zero, 25, 50, and 100 micromol of iron III chloride on King-B culture medium.

The effect of antagonistic bacterial strains on the microscopic characteristics of pathogenic fungi

In the microscopic examination of all the investigated strains, morphological changes such as the formation of a series of vesicles at the end of the filaments and near the end of the filaments and also along the length of the filaments, lightening of the tip of the hyphae and changes in the amount of pigment production as well as complexity and to co-irradiation of different parts of the root and destruction of the root became the disease (Fig. 7).

Fig. 7. Deformation and vesiculation of fungal hyphae due to antagonistic bacteria.

DISCUSSION

The fungus *F. oxysporum*, which cause of thuja collar and root rot, is one of the important factors that reduce thuja plant production and cultivation per unit area in Iran, since the pathogen is soil-borne and the ineffectiveness of chemical poisons in controlling soil-borne pathogens, there is a need to use methods replacement is required (Ghadimi and Rahanandeh, 2022). In many researches, the high antagonistic effect of *Bacillus* bacteria in controlling pathogenic fungi has been proven. *Bacillus* BAS23 isolate controlled more than 12% of rice pathogenic fungi and caused a decrease of more than 12% in the dry weight of the mycelium of pathogenic fungi (Saechow *et al*., 2018). In this research, B. ve and B. su isolates showed the greatest inhibition of the growth of pathogenic fungi in the dual-culture method. In the test related to the effect of volatile metabolites, most of the isolates were effective. Isolate P. f with 58.33% showed the greatest inhibitory effect on the mycelium growth of the pathogenic fungus. Also, in this study, after opening the Petri lid and placing the fungus at room temperature, the pathogenic fungus was able to start its growth again, and after 9 days, it filled the Petri dish, which indicates the effect of the static fungus on the fugitive metabolites produced by *Pseudomonas* bacteria, and also indicates that the presence and survival of the bacteria is necessary to continue controlling the pathogenic fungus. One of the biocontrol factors in the antagonistic bacteria *Pseudomonas* and *Bacillus* has been the production of volatile compounds in various research (Elshahat *et al*., 2016). Volatile compounds produced by *B. subtilis* caused vacuolization and swelling of *Rhizoctonia solani* mycelium (Fiddaman and Rossall, 1993). Extracellular compounds produced by the *Pseudomonas* strain can cause deformation and destruction of the mycelium of fungi (Gupta and Verhoeven, 2001). In *B. subtilis* bacteria, extracellular compounds are considered to be a more effective mechanism than volatile substances in preventing the activity of pathogenic agents. The production of these compounds in the cultivation environment depends on the presence of some substances. By adding D-glucose or a combination of carbohydrates along with peptone, the production of these substances in the environment increased (Fiddaman and Rossall, 1993). In the current research, it was also shown that all antagonistic bacterial strains can to produce extracellular compounds. In studies related to siderophore production, P. f isolates were able to produce siderophore. Siderophore production can both act as a growth stimulant and control the disease through the competition mechanism (Ahmadzadeh and Sharifi Tehrani, 2021). The biocontrol ability of *Pseudomonas* is related to the production of siderophore (Loper and Schroth, 1986), volatile compounds, and antibiotics (Elshahat *et al*., 2016; Rahanandeh *et al*., 2017). In the test related to the production of protease enzyme, all isolates were able to produce this enzyme, one of the important mechanisms in the biological control of pathogenic fungi is the production of extracellular enzymes, which mainly aim to destroy the cell wall and plasma membrane of the pathogen. They become chitinase enzyme produced by *Bacillus* bacteria is one of the most important chitin degrading enzymes. Therefore, by producing this enzyme, *Bacillus* bacteria destroy the cell wall of the pathogenic fungus and prevent its growth (Shoda, 2000; Rahanandeh *et al*., 2012).

CONCLUSION

Considering the importance of thuja collar and root rot disease in the country and the lack of a suitable management method to control this disease, the present study was conducted to evaluate the effect of *Bacillus* and *Pseudomonas* isolates on the disease-causing fungus. The bacterial isolates used in this research could to inhibit *F. oxysporum* in laboratory tests. Therefore, based on comprehensive investigations and considering prevalence of *Fusarium* disease common in the region, the use of these bacteria can be used as a soil treatment, as recommended an efficient method in sustainable agriculture to manage *Fusarium* diseases and improve thuja growth. However, it is necessary to evaluate the positive and negative characteristics of these bacterial strains as well as their effect on non-target organisms before making recommendations. Additionally, since each of these isolates employs a specific mechanism to control the pathogenic agent, it is advisable to use a combination of them for controlling this fungus. Furthermore, it is important to assess the effect of the bacterial combination on each other and the host plant.

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NanoTiO$_2$, Quantum dot-Graphene Oxide, and CeO$_2$ Foliar Prescription Meliorates Growth and Some Physiological Traits of Gazania (*Gazania splendens* L.) under Salinity

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Abstract

The production and maintenance of ornamental plants are closely related to the high consumption of fresh water. Today, due to the limited water resources, we have to water plants with salt water sources. To evaluate the effects of foliar application of nano TiO_2, CeO_2, and quantum dot-graphene oxide (zero and 1.5 mg/L) and NaCl salinity stress (0, 75, 150 mM) on *Gazania splendens* L.; a factorial experiment was conducted based on completely randomized design. The results revealed that plant dry weight, flower number, proline and flavonoids content, antioxidant enzymes activity, MDA, H_2O_2, Na, N, and P content were influenced by the interaction effects of experimental treatments. The highest leaf dry weight, flower number, and N content were recorded at no-salinity × quantum dot-graphene oxide. The highest data for Na content, ion leakage (56.6%), H_2O_2 (246 nmol/mg FW), malondialdehyde (37 nmol/mg FW), and proline (1.1nmol/mg FW) content were recorded at $NaCl_{150\ mM}$ × no-foliar spray. 150 mM salinity stress × quantum dot-graphene oxide increased catalase activity (8.9 µmol/g FW) in the plant. Superoxide dismutase and ascorbate peroxidase activity were influenced by $NaCl_{150\ mM}$ × quantum dot-graphene oxide and TiO_2 foliar spray. Chlorophyll index, total phenolics, and K/Na ratio were responded to the simple effects of salinity and foliar application. The top ratio of K/Na and chlorophyll index was recorded at quantum dot-graphene oxide foliar spray. 75 and 150 mM salinity improved phenolics content in plants. Foliar spray with all nanoparticles increased phenolics content. The overall results showed that salinity had adverse effects on the growth and physiological characteristics of *Gazania splendens*. Foliar treatments under 150 mM salinity stress; promisingly influenced the antioxidant enzymes activity and root dry weight of plants. All in all, *Gazania splendens* can tolerate up to 75 mM NaCl salinity stress without a remarkable decline in growth and physiological attributes.

Keywords: Elemental content, Enzyme, *Gazania splendens*, Malondialdehyde, Phenolics.

INTRODUCTION

Plants are constantly exposed to biotic and abiotic stresses during their lifetime. Salinity stress is considered one of the most important environmental stresses in arid and semi-arid regions of the world. Salinity as an environmental stress affects various morphological, biochemical, and physiological aspects of the plant (Mahmoud *et al*., 2020; Wang *et al*., 2020). Under salinity stress conditions, sodium and chlorine ions enter the cell due to competition or selective ion permeability by the cell membrane and affect cell metabolism and other ions absorption. The most prominent effects of salinity on plants are osmotic stress, disturbance in photosynthetic potential, decomposition of pigments, imbalance in the absorption of water and nutrients, and increase in biosynthesis of reactive oxygen species (Khare *et al*., 2020; Hasanuzzaman *et al*., 2020).

Nowadays, chemical fertilizers are often used for growing plants, but the long-term use of these fertilizers has harmful effects on human health and the environment (Al-Taey *et al*., 2019). Nanotechnology is one of the novel and creative approaches in the agricultural sector, to moderate the negative effects of stressful conditions and reduce the dangerous effects of chemical fertilizers. Nanoparticles are materials with dimensions of 1 to 100 nanometers that easily penetrate the cell. Nanoparticles have unique electrical, thermal, and physicochemical properties such as high solubility, high specific surface area, and controlled release (Guo *et al*., 2018). Nanoparticles retard soil and water pollution by improving the release of nutrients from the fertilizer source and concomitantly reducing the frequency of fertilizer application (Toksha *et al*., 2021). Titanium oxide (TiO_2) nanoparticles enhance plant growth by increasing nutrient absorption, improving nitrate absorption, and nitrogen fixation, and even enhancing chlorophyll content. Moreover, TiO_2 foliar spray regulates the activity of antioxidant enzymes and partially enhances carbon dioxide acquisition (Yang *et al*., 2008; Mustafa *et al*., 2021).

As a fertilizer, cerium oxide (CeO_2) nanoparticle stimulates root growth, and antioxidant enzyme activity, and prevents peroxidation and membrane ion leakage (Rajeshkumar and Naik, 2018; Cao *et al*., 2018). Under salinity stress, cerium oxide acts as a catalyst in the production of chlorophyll and the removal of oxygen-free radicals and stabilizes the chloroplast structure and cell wall in plants (Jurkow *et al*., 2020).

Graphene nanoparticle, as a carbon-based material, is widely used in various industries due to its exceptional physical and chemical properties, good thermal stability, high electrical conductivity, and mechanical strength (Chen *et al*., 2018). Graphene oxide is one of the most important members of the graphene family and has unique properties, double-layer structure, and high potential for use in industry, medicine, and agriculture. By entering plant tissues and cells, carbon nanomaterials affect the activity of the antioxidant system and cell metabolism in plants (Safikhan and Chaichi, 2018) and increase plant growth and yield (Chakravarty *et al*., 2015).

The interest in living in modern cities and an industrialized lifestyle away from nature has led to an increasing desire to have a small model of green space to meet spiritual needs and relieve daily fatigue. Ornamental plants have a significant impact on human life due to their aesthetic, cultural, health, and economic effects (Benjaw *et al*., 2017). *Gazania splendens* L. belongs to the Asteraceae family and, is an annual or perennial herb flower in spring and summer (Magee *et al*., 2011; Jiashi *et al*., 2016). Due to the long-lasting flowering time, Gazania is one of the most popular plants in urban horticulture. But in recent years, due to the continuation of droughts and increasing salinity stress in most regions (salinization of irrigation water and soil in several areas of Iran), plant producer has faced a serious challenge. Accordingly, this study aims to evaluate the effect of foliar application of nanoparticles on the growth and some physiological traits of *Gazania* under salinity stress. The possible promising results would be advisable for the extension section and urban horticulture to reduce the effects of salinity stress.

MATERIALS AND METHODS

Plant materials

This experiment was conducted at the Research Greenhouse of Azarbaijan Shahid Madani University, Tabriz, Iran, during the spring and summer of 2022. The greenhouse growing conditions were as follows: Lighting period: 16:8, day and night; temperature regime: 27 °C and 23 °C in the day and night, and relative humidity of approximately 65%, respectively. Homogenous *Gazania splendens* plants (in the trifoliate stage) were planted in 5 L pots filled with medium-sized perlite. The plants were nourished with half-strength Hoagland's nutrient solution (pH: 5.5) for 15 days for a reliable establishment until four-leaflet stage. Afterward, the salinity treatments were imposed. The salinity levels were 0 (EC: 2.1 mS/cm), 75 (EC: 9.0 mS/cm), and 150 (EC: 18.0 mS/ cm) mM NaCl for 16 weeks. The addition of salts began at 50 mM and gradually increased to reach the final level within 12 days. The half-strength Hoagland's nutrient solution with NaCl treatments (300 mL pot^{-1}) was applied every four days. Two repeated foliar treatments with zero (distilled water) and 1.5 mg/L of nano CeO$_2$, TiO$_2$ (10–30 nanometer-sized from US-Nano Company, USA), and quantum dot-graphene oxide were applied to the plants. The first foliar treatment was applied with the salinity initiation (four-leaflet stage), and the second one occurred two weeks later. 110 days after the second foliar spray (flowering stage), the leaf samples were taken for morphological and biochemical assays. The dry biomass of the plant was recorded on a digital scale (BB141, Becco, Germany). Separated plants were dried in the oven at 30 °C until they reached a constant weight. Plant height was measured with a ruler and flower diameter was recorded with a digital caliper. A manual chlorophyll meter device (SPAD-502Plus, KONICA) was used to determine the leaf chlorophyll index.

Instrumentation

The Fourier transform infrared (FTIR) spectrum of the Quantum dot-graphene oxide (CQDs) was recorded on a Vector 22 (Bruker, Ettlingen, Germany) Fourier transform infrared spectrometer using KBr as the mulling agent. The dynamic light scattering (DLS) measurements were taken on the Zetasiser instrument ZEN3600 (Malvern, UK MAL 1001767) with a He-Ne laser beam at 511 nm and 25 ºC. A spectrofluorimeter with a xenon arc lamp of 150 watts and a scanning speed of 4000 rpm (Jasco, model FP-6200, Japan) was applied to record the fluorescence spectra of different solutions. An electric muffle furnace (Fan Azma Gostar, model FM8P, Iran) was used for heating purposes. An electronic analytical balance (PFB300-3, Kern, Germany) was applied for weighing the solid materials.

Synthesis of activated carbon nanoparticles

Mulberry leaf was used for green CQDs synthesis through the green route. After harvesting the leaf, the dust on leaves was washed with water and then it was washed again with distilled water for several times. Next, the materials were extracted with ethanol. Later, the extract was heated in an autoclave for 5 h at 150°C. After cooling down (to room temperature), the resultant was centrifuged for 30 min at the speed of 6000 rpm to obtain CQDs suspension.

Elemental composition

A dried leaf sample (85 ºC) of the *Gazania splendens* L. plant was grounded in a Wiley mill to particles less than 0.42 mm. Leaf samples (0.2 g) were acid-digested (2N HCl) and analyzed for nutrient content (Chrysargyris *et al*., 2018). Na and K content was measured by flame photometric method (Corning, 410, England). The Mg and Ca content was recorded by atomic absorption spectroscopy (Shimadzu, AA6300, Tokyo, Japan), phosphorus by vanadate molybdate, and N content by Kjeldahl (Chrysargyris *et al*., 2018).

Electrolyte leakage

One cm^2 of leaf desk was used for measuring cell membrane leakage. First, the samples were incubated in 20 ml of deionized water (at room temperature) for 18h. Then, the leaf sample was boiled for 30 min, and the conductivity of the incubation solutions was measured by using a conductivity meter (EMCEE model 1152, USA) (Lutts *et al*., 1995).

Hydrogen peroxide content

Leaf tissue (0.2 g) was powdered in liquid N_2 and then grounded in ice-cold 0.1% trichloroacetic acid (TCA) and then, centrifuged at 12000 g for 15 min. An aliquot (50 µl) of the supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH: 7.5) and 1 ml of 1M potassium iodide. The H_2O_2 content was evaluated using standards of 5 to 1000 µM of H_2O_2, and the calibration curve was plotted accordingly. The absorbance of samples and standards was measured at 390 nm, and results were expressed as µmol H_2O_2 g^{-1} fresh weight (Alexieva *et al*., 2001).

Malondialdehyde (MDA) content

2 g of leaf tissue was crushed in liquid nitrogen. On the sample, 5 ml of TCA 0.1% (trichloroacetic acid) was added and the resulting mixture was centrifuged at 12000 g for 15 minutes. In the next step, 1 ml of the supernatant solution was mixed with 4 ml of 0.5% thiobarbituric acid (TBA) and 20% TCA and kept at 95 °C for 30 minutes. An ice bath was used to stop enzyme activity. After centrifugation at 10,000 g for 5 min, the absorbance at 532 nm was measured by a spectrophotometer ($T80^+$, China) and corrected for non-specific absorbance at 600 nm. The amount of MDA was determined using an extinction coefficient of 155 mM cm^{-1} (Heath and Packer, 1968).

Superoxide dismutase (SOD) activity

SOD enzyme activity was detected by recording the enzyme inhibition from the nitroblue tetrazolium (NBT) photoreduction. The reaction mixture contained 0.1 mM EDTA, 50 mM sodium phosphate buffer (pH 7.6), 12 mM L^{-1}, methionine, 50 mM sodium carbonate, 50 µM NBT, 10 µM riboflavin, and 100 µM plant sample extract (final volume of 3.0 mL). SOD activity was recorded at a wavelength of 560 nm using a spectrophotometer ($T80^+$, China). One unit (U) of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of NBT (Giannopolitis and Ries, 1997).

Catalase (CAT) activity

For the determination of CAT activity, 0.5 g of leaf samples were homogenized with 0.1 M cold potassium phosphate buffer (pH: 7.5) and 0.5 mM EDTA (Luhova *et al*., 2003). The resulting extract was centrifuged at 15000 g for 15 min at 4 ºC. 0.05 ml of supernatant was added to 1.5 ml of 0.1 mM phosphate buffer (pH: 7) and 1.45 ml of double-distilled H_2O. The reaction was started by adding 0.5 ml of 75 mM H_2O_2, and a decrease in absorption was recorded at 240 nm for 1 min by spectrophotometer (Luhova *et al*., 2003).

Ascorbate peroxidase activity

100 mg of fresh leaf sample, 250 µl of 100 mM phosphate buffer (pH: 7), 250 µl of 1 mM ascorbate, 250 µl of 0.4 m MEDTA, 190 µl of double distilled water, 10 µl of 10 mM H_2O_2 and 50 µl of extracted solution were mixed. Absorption was recorded at 290 nm by spectrophotometer at the start time and one minute later (Murshed *et al*., 2008).

Proline content

0.2 g of gazania leaf sample was crushed in liquid nitrogen. 5 ml of 3% homogenized sulfosalicylic acid was added to the material, and centrifuged at 6000 rpm for 7 min at 20 °C. One ml of supernatant was mixed with the same volume of ninhydrin acid and, 1 ml of glacial acetic acid. Samples were incubated in 100 ºC water bath for one hour, and then in an ice bath for 5 min. Later, on the standard solution and sample, 2 ml of toluene was added and shaken for 30 s. After 30 min, a red phase was formed. Proline content was measured by spectrophotometer ($T80^+$, China) at 520 nm (Fedina *et al*., 2006).

Total phenolics and flavonoids content

50 mg fresh leaf tissues were homogenized in 0.5 ml 80% ethanol and centrifuged. The pellet was washed twice with 0.5 ml ethanol (80%, v/v), then, supernatants were pooled. Total phenolics content was determined using a Folin-Ciocalteu assay. Gallic acid was used as a standard (Zhang *et al.*, 2006). The aluminum chloride calorimetric method was tried for flavonoid content estimation, with quercetin as a standard (Chang *et al*., 2002).

Experimental design and data analysis

The experiment was conducted as a factorial based on a completely randomized design with three replications. Analysis of variance (ANOVA) was performed by SPSS ver. 2023. The significant differences among means were evaluated with the least significance difference test (LSD) at $P< 0.05$. Pearson's correlation and cluster dendrogram heat maps were depicted in R software (R Foundation for Statistical Computing, version 4.1.2).

RESULTS

Characterization of CQDs

The Fourier transform infrared (FTIR) spectroscopy can be used to determine the functional groups on the surface of CQDs. The result of this test for green-synthesized CQDs is presented in Fig. 1. As shown in Fig. 1, the band at 3371 cm^{-1} region includes a relatively wide peak which is related to stretching bands of either -OH or -NH groups. The peaks at 2898-2976 cm^{-1} indicate the presence of methylene or methyl (C-H) functional groups due to the presence of the aliphatic hydrocarbons. The peaks at 2340-2360 cm^{-1} were attributed to C-N bond. The peak at 1400 cm^{-1} could be identified as C-N, N-H, and -COO groups. The bands at 1000-1100 cm^{-1} correspond to C-O-C and C-O stretching, respectively. The narrow band at 883 cm^{-1} and 669 cm^{-1} shows the existence of out-of-plane bending of -CH and -OH bonds, respectively. The availability of C-O-C and C-H functional groups on the surface of the CQDs makes it highly hydrophilic (Velu and Lee, 2022; Akhgari *et al*., 2017).

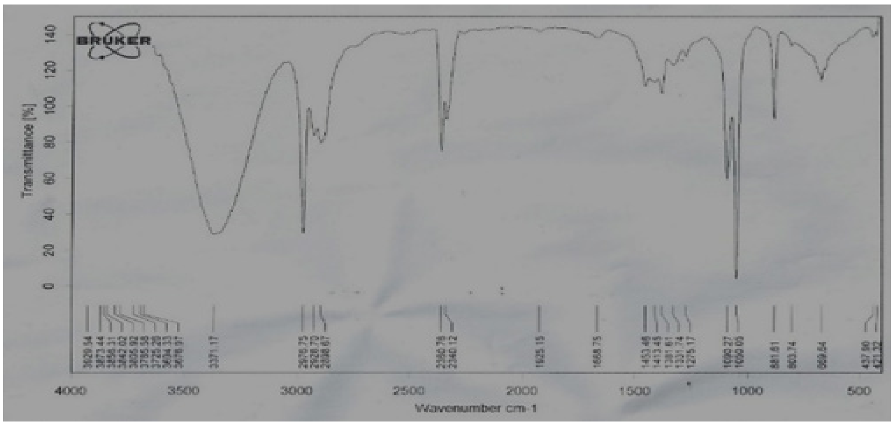

Fig. 1. FT-IR spectrum of CQDs.

DLS technique measured the average hydrodynamic size of green-synthesized CQDs as 8.7 nm (Fig. 2).

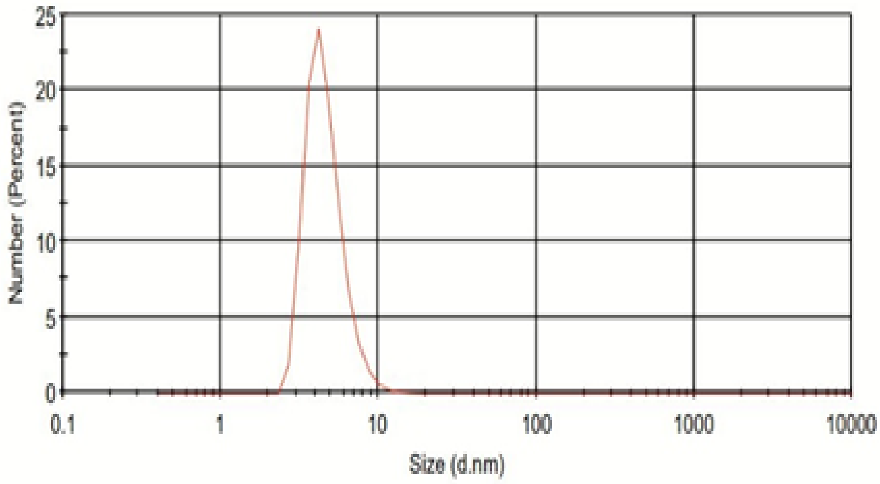

Fig. 2. Size distribution of CQDs.

To investigate the optical properties of green-synthesized CQDs, photoluminescence spectra were obtained at different excitation wavelengths. The results revealed that CQDs display the characteristic "excitation-independent emission" behavior and a relatively narrow emission peak at 685 nm (Fig. 3).

Plant dry weight

The interaction effects of the experimental treatments affected the dry weight of the root and aerial parts of the plant (Table 1). The aerial parts dry weight increased under $NaCl_0$ × quantum dot-graphene oxide spray, which showed a 130% increase compared to the control (Table 2). The root dry weight was raised in $NaCl_{150\ mM}$ × cerium oxide nanoparticles foliar spray. The lowest root dry weight was recorded in $NaCl_{100\ and\ 150\ mM}$ × no foliar spraying. In both salinity stress levels; foliar application reduced the negative effects of stress on root dry weight (Table 2).

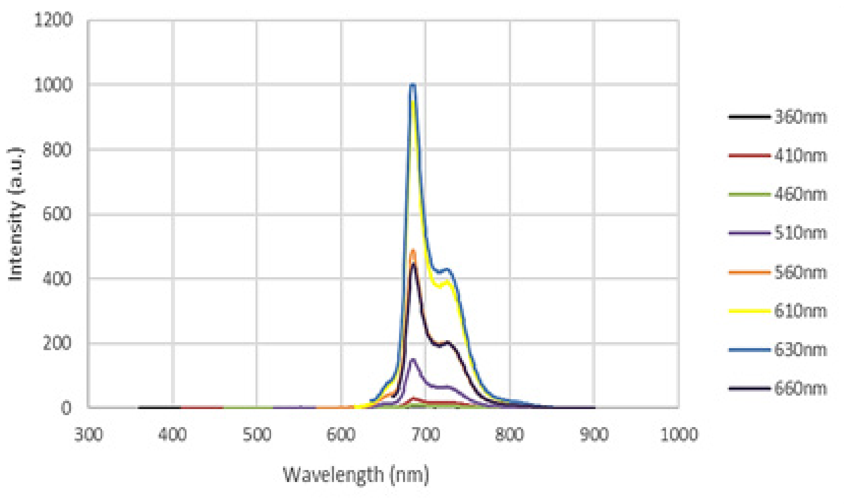

Fig. 3. The emission spectrum of carbon quantum dots at different excitation wavelengths.

Table 1. The analysis of variance for the impact of salinity and foliar sprays on growth and flower parameters of *Gazania splendens* L.

S.o.V	df	Root dry weight	Arial parts dry weight	Flower number	Flowering stem length	Flower fresh weight	Flower diameter	Chlorophyll index
Salinity (A)	2	0.002**	1.3**	4.3**	69**	0.70**	17.2**	426**
Foliar spray (B)	3	0.014**	1.0**	0.32**	20.3**	0.75**	9.7**	726**
A × B	6	0.001**	0.17**	0.29**	2.0*	0.17**	0.5^{ns}	16.6^{ns}
Error	24	0.002**	1.3**	4.3**	69**	0.70**	17.2**	426**
CV (%)		10	12	11	7	12.8	8.5	9

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Flower number and diameter, flowering stem length, and flower fresh weight

Flower number, the length of the flowering stem, and the flower's fresh weight were influenced by the interaction effects of the experimental treatments (Table 1). Flower diameter was affected by the simple effect of salinity and foliar spray (Table 1). Based on the results, no salinity condition, and increased flower diameter to 6.6 cm. With the increase of salinity stress to 150 mm, the flower diameter decreased to 2.4 cm (Table 3). Quantum dot-graphene oxide increased flower diameter in plant (Table 4). The highest flower number, flowering stem length, and flower fresh weight were recorded at $NaCl_0$ × quantum dot-graphene oxide spray. Salinity stress in the condition without foliar spraying caused the decrease of all three traits (Table 2). The flower number was reduced in $NaCl_{150\ mM}$ × no foliar spray. The $NaCl_{75\ and\ 150\ mM}$ × no foliar spray decreased the flower's fresh weight and the length of the flowering stem compared to the control (Table 2).

Table 2. Mean comparison for the interaction effects of salinity and foliar spray on growth and some physiological parameters of *Gazania splendens* L.

NaCl (mM)	Foliar spray	Root dry weight (g/pot)	Arial parts dry weight (g/pot)	Flower number	Flowering stem length	Flower fresh weight (g)	Electrolyte leakage (%)	H_2O_2 (nmol/mg FW)	Malondialdehyde content (nmol/mg FW)
0	0	0.36^{f}	1.0^{ef}	2.0^{b}	10.8^{d}	1.0^{def}	16^{e}	121^{e}	6.1^{h}
0	CeO_2	0.10^{bc}	1.2^{cd}	2.0^{b}	13.0^{b}	1.6^{b}	13^{e}	125de	6.5^{h}
0	CQD	0.08^{cd}	2.3^{a}	3.0^{a}	16.3^{a}	2.0^{a}	12.6^{e}	101^{g}	4.7^{h}
0	TiO_2	0.05^{e}	1.5^{b}	2.0^{b}	13.6^{b}	1.3^{cd}	16^{e}	120^{ef}	5.7^{h}
75	0	0.02^{fg}	0.8^{f}	1.6^{bc}	9.8^{de}	0.8^{fg}	38^{bc}	170^{c}	14^{e}
75	CeO_2	0.10^{b}	1.0^{def}	2.0^{b}	12.3^{bc}	1.1^{de}	28^{d}	124de	11^{f}
75	CQD	0.08^{cd}	1.3^{bc}	2.0^{b}	12.3^{bc}	1.3^{cd}	28^{d}	104^{fg}	7.3^{gh}
75	TiO_2	0.07^{de}	1.1^{cde}	2.0^{b}	11^{cd}	1.6^{b}	32^{cd}	138^{d}	10f^{g}
150	0	0.01^{g}	0.47^{g}	1.0^{d}	7.3^{f}	0.7^{g}	56^{a}	246^{a}	37^{a}
150	CeO_2	0.15^{a}	1.0^{ef}	1.3^{cd}	8.6^{ef}	0.9^{e-g}	38^{bc}	201^{b}	29^{b}
150	CQD	0.11^{b}	1.1^{cde}	1.0^{d}	10^{d}	1.4^{bc}	36^{c}	184^{c}	20^{d}
150	TiO_2	0.07^{de}	0.9^{ef}	1.0^{d}	8.3^{f}	0.9^{e-g}	42^{b}	216^{b}	25^{c}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Flower number and diameter, flowering stem length, and flower fresh weight

Flower number, the length of the flowering stem, and the flower's fresh weight were influenced by the interaction effects of the experimental treatments (Table 1). Flower diameter was affected by the simple effect of salinity and foliar spray (Table 1). Based on the results, no salinity condition, and increased flower diameter to 6.6 cm. With the increase of salinity stress to 150 mm, the flower diameter decreased to 2.4 cm (Table 3). Quantum dot-graphene oxide increased flower diameter in plant (Table 4). The highest flower number, flowering stem length, and flower fresh weight were recorded at $NaCl_0$ × quantum dot-graphene oxide spray. Salinity stress in the condition without foliar spraying caused the decrease of all three traits (Table 2). The flower number was reduced in $NaCl_{150\ mM}$ × no foliar spray. The $NaCl_{75\ and\ 150\ mM}$ × no foliar spray decreased the flower's fresh weight and the length of the flowering stem compared to the control (Table 2).

Table 3. Mean comparison for the effects of salinity on flower diameter, chlorophyll index and total phenolics content of *Gazania splendens* L.

NaCl (mM)	Flower diameter (cm)	Chlorophyll index (SPAD)	Total phenolics content (µmol/g FW)
0	6.6^{a}	35^{a}	4.4^{b}
75	5.0^{b}	32^{a}	8.5^{a}
150	4.2^{c}	26^{b}	8.3^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Table 4. Mean comparison for the effects of foliar spray on flower diameter, chlorophyll index and total phenolics content of *Gazania splendens* L.

Foliar spray (ml)	Flower diameter (cm)	Chlorophyll index (SPAD)	Total phenolics content (µmol/g FW)
0	4.0^{c}	19.3^{d}	5.4^{b}
CeO_2	5.1^{b}	26.1^{c}	7.1^{a}
CQD	6.6^{a}	40.4^{a}	8.6^{a}
TiO_2	5.3^{b}	35^{b}	7.3^{ab}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Chlorophyll index

The independent effect of salinity stress and foliar spraying affected the leaf chlorophyll index (Table 1). The treatment without salinity stress and 75 mM sodium chloride increased the chlorophyll index compared to 150 mM sodium chloride (Table 3). The treatments used in foliar spraying affected the leaf chlorophyll index. The lowest chlorophyll index (19.3 SPAD) was observed under no foliar spraying treatment and the highest in quantum dot-graphene oxide foliar spray (40.4 SPAD). In terms of chlorophyll index, foliar spraying with titanium oxide nanoparticles increased the chlorophyll index by 19% compared to foliar spraying with cerium oxide nanoparticles (Table 4).

Electrolyte leakage, malondialdehyde, and H_2O_2 content

The treatments interactions affected the content of hydrogen peroxide and malondialdehyde (Table 5). $NaCl_{150\ mM}$ × no foliar spray increased the content of hydrogen peroxide (by 103% compared to the control treatment) and malondialdehyde content (Table 2). MDA content was the least under no salinity stress with all levels of foliar applications. In the salinity stress of 150 mM, spraying with any three nanoparticles reduced the negative effects of the stress on the plant (Table 2). The treatment without foliar spraying and foliar spraying with titanium oxide nanoparticles raised ion leakage in the 150 mM salinity stress. Foliar spraying with nanoparticles reduced ion leakage compared to the condition without foliar spraying at the same level of salinity stress (Table 2).

Table 5. The analysis of variance for the impact of salinity and foliar sprays on some physiological characteristics of *Gazania splendens* L.

S.o.V	df	Electrolytes leakage	Malondialdehyde content	H_2O_2 content
Salinity (A)	2	2557**	1637**	3597**
Foliar spray (B)	3	238**	115**	3695**
A × B	6	47**	33**	458**
Error	24	11.2	3.5	95
CV (%)		11	10.3	6.4

*, ** and ns: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test, respectively.

Antioxidant enzymes activity

Catalase, superoxide dismutase, and ascorbate-peroxidase activities were influenced by the interaction effects of experimental treatments (Table 6). $NaCl_{150\ mM}$ × quantum dot-graphene oxide foliar spray increased the catalase activity (8.9 µmol of hydrogen peroxide/mg protein/minute). The lowest activity of catalase was observed in $NaCl_0$ × quantum dot-graphene oxide and titanium oxide nanoparticle foliar application. For CAT activity, treatments of 75 mM NaCl in the condition without foliar spraying, foliar spraying with cerium oxide nanoparticles and titanium oxide were at the same statistical level, but foliar spraying with quantum dot-graphene oxide × 75 mM salt stress increased the activity of catalase (Table 7). Foliar treatment with titanium oxide and quantum dot-graphene oxide nanoparticles × $NaCl_{150\ mM}$ raised the activity of superoxide dismutase and ascorbate peroxidase. The lowest activity of both enzymes was observed in the treatment without salt stress × foliar spraying with all nanoparticles. Increasing the salinity stress to 75 mM with all foliar spraying treatments enhanced the activity of both enzymes (Table 7).

Table 6. The analysis of variance for the impact of salinity and foliar sprays on the antioxidant enzymes activity and proline, phenolics and flavonoids content of *Gazania splendens* L.

S.o.V	df	Catalase activity	Superoxide dismutase activity	Ascorbate peroxidase activity	Proline content	Total phenolic content	Flavonoids content
Salinity (A)	2	122**	21716**	1.3**	0.58**	65**	5.7**
Foliar spray (B)	3	5.16**	1333**	0.16**	0.05**	14**	0.64**
A × B	6	0.78**	654**	0.05**	0.042**	2.2^{ns}	0.19**
Error	24	0.19	158	0.01	0.006	1.08	0.02
CV (%)		11	6.4	12	11	10	12

*, ** and ns: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Table 7. Mean comparisons for the interaction effects of salinity and foliar sprays on enzyme activity and proline content of *Gazania splendens* L.

NaCl (mM)	Foliar application	Ascorbate peroxidase (µmol ASC/ activity mg protein/min)	Superoxide dismutase activity (units mg/protein/ min)	Catalase activity (µmolH_2O_2/mg protein/min)	Proline content (µmol/g FW)
0	0	0.41^{d}	153^{f}	1.1^{g}	0.38^{e}
0	CeO_2	0.50^{d}	159^{f}	1.4^{fg}	0.45^{e}
0	CQD	0.53^{d}	168^{ef}	1.8^{ef}	0.51^{e}
0	TiO_2	0.46^{d}	147^{f}	1.4^{fg}	0.48^{e}
75	0	0.70^{c}	181^{de}	1.9^{ef}	0.92^{b}
75	CeO_2	0.72^{c}	197^{cd}	2.1^{ef}	0.76^{cd}
75	CQD	0.93^{b}	191^{cd}	3.6^{d}	0.66^{d}
75	TiO_2	0.76^{c}	169^{cd}	2.3^{e}	0.76^{cd}
150	0	0.86^{bc}	209^{c}	5.9^{c}	1.1a
150	CeO_2	0.96^{b}	232^{b}	7.2^{b}	0.86^{bc}
150	CQD	1.4^{a}	272^{a}	8.9^{a}	0.66^{d}
150	TiO_2	1.3^{a}	253^{a}	7.7^{b}	0.90^{b}

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

Proline content

The interaction effects of experimental treatments affected the proline content of the plant (Table 6). The highest content of proline was observed in $NaCl_{150\ mM}$ × no foliar spray which showed an increase of 189% compared to the control (Table 7).

Phenolics and flavonoids content

Phenolics content was influenced by the independent effects of experimental treatments, Moreover, flavonoid content was influenced by the interaction effects of experimental treatments (Table 6). Both levels of salinity stress increased the content of total phenolics (Table 3). All three foliar treatments increased the phenolics content compared to the control (Table 4). $NaCl_{150\ mM}$ × quantum dots-graphene oxide foliar spray enhanced the flavonoid content by 350% compared to the control (Fig. 4).

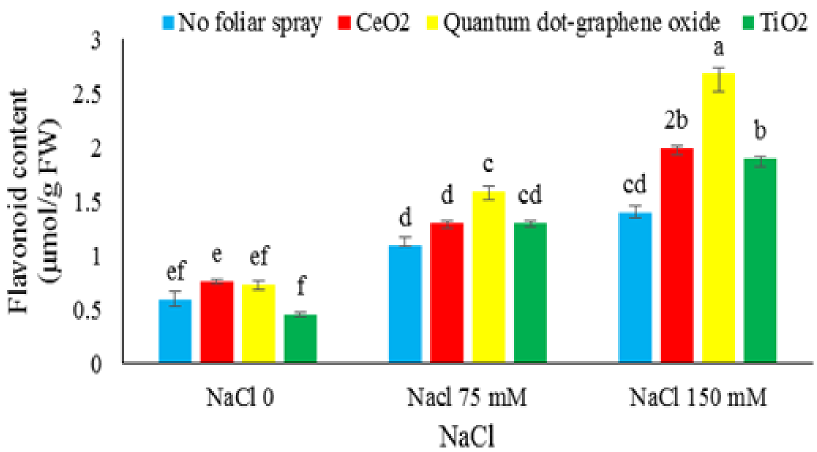

Fig. 4. Mean comparison for the interaction effects of salinity and foliar spray on flavonoids content of *Gazania splendens* L. In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

Elemental content

The nitrogen, phosphorus, magnesium, and sodium content was affected by the interaction effects of treatments. However, potassium and calcium content, and K/Na ratio were influenced by the independent effects of the experimental treatments (Table 8). A 58% increase in nitrogen content was observed in the foliar spray with quantum dot-graphene oxide × $NaCl_0$ compared to the control (Table 9). 75 mM salinity stress × quantum dot-graphene oxide spraying increased the phosphorus and magnesium content of the plant. The lowest phosphorus content (31% reduction compared to the control) and the highest sodium content were observed in $NaCl_{150\ mM}$ without foliar spraying (Table 9). Sodium content was increased in $NaCl_{75\ and\ 150mM}$ × without foliar spray, but foliar application with nanoparticles decreased the sodium content at the same salinity level (Table 9). 75 and 150 mM sodium chloride decreased the potassium and calcium content of the plant. The highest content of potassium (32 mg g $^{-1}$ DW) and calcium (5.5 mg g $^{-1}$ DW) was observed in no salinity condition (Table 10). Foliar spraying with quantum dot-graphene oxide increased the potassium content by 8% compared to the control. Foliar spraying with all three nanoparticles increased calcium content compared to the control (Table 11). Foliar application of quantum dot-graphene oxide enhanced the potassium-to-sodium ratio of the plant (Table 11). 150 mM sodium chloride decreased K/Na ratio by 50% compared to 75 mM salinity stress, and 73% compared to the control (Table 10).

Table 8. The analysis of variance for the impact of salinity and foliar spray on the elemental content of *Gazania splendens* L.

S.o.V	df	N content	P content	K content	Ca content	Mg content	Na content	K/Na
Salinity (A)	2	4.9**	4.4**	80**	6.1**	0.036**	303**	136**
Foliar spray (B)	3	3.5**	2.0**	414**	0.88**	0.024**	49**	36**
A × B	6	0.68**	0.29**	4.7[ns]	0.11[ns]	0.002*	17**	2.00[ns]
Error	24	0.07	0.06	2.3	0.07	0.001	1.8	0.93
CV (%)		6.9	5	5.4	5.6	5.8	9	14

*, ** and ns: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test, respectively.

Table 9. Mean comparison for the interaction effects of salinity and foliar spray on elemental content of *Gazania splendens* L.

NaCl (mM)	Foliar spray (ml)	N content (g/ kg DW)	P content (g/kg DW)	Na content (mg/kg DW)	Mg content (g/kg DW)
0	0	3.8[d-f]	5.4[bc]	4.4[e-g]	0.49[cde]
0	CeO_2	3.9[de]	5.6[b]	3.8[fg]	0.53[bc]
0	CQD	3.9[de]	5.6[b]	3.2[g]	0.56[b]
0	TiO_2	4.8[b]	5.5[bc]	3.4[g]	0.55[b]
75	0	2.9[d-h]	4.6[d]	9.1[c]	0.48[de]
75	CeO_2	3.6[ef]	5.1[c]	6.5[de]	0.54[b]
75	CQD	4.5[bc]	6.2[a]	5.8[d-f]	0.63[a]
75	TiO_2	4.1[cd]	5.2[c]	5.7[d-f]	0.52[bcd]
150	0	2.8[h]	3.7[e]	20[a]	0.38[a]
150	CeO_2	3.6[ef]	4.4[d]	12.2[b]	0.42[fg]
150	CQD	3.4[fg]	5.2[c]	7.9[cd]	0.54[bc]
150	TiO_2	3.7[d-f]	4.4[d]	12.1[b]	0.44[ef]

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Table 10. Mean comparison for the effects of salinity on K, Ca content, and K/Na ratio of *Gazania splendens* L.

NaCl (mM)	K content (g/kg DW)	K/Na ratio	Ca content (g/kg DW)
0	32[a]	9.0[a]	5.5[a]
75	28[b]	4.5[b]	4.8[b]
150	26[b]	2.4[c]	4.7[c]

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Table 11. Mean comparison for the effects of foliar spray on K and Ca content, and K/Na ratio of *Gazania splendens* L.

Foliar spray (ml)	K content (g/kg DW)	K/Na ratio	Ca content (g/kg DW)
0	20.8[c]	2.9[c]	4.4[b]
CeO_2	30.6[b]	5.0[b]	4.7[ab]
CQD	37.3[a]	7.8[a]	5.1[a]
TiO_2	28.2[b]	5.4[b]	4.9[a]

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

DISCUSSION

Considering the high economic value of ornamental plants; producers often use high-quality water to manage ornamental plants installations. But today, due to the population burst and the need to provide food and the limitation of water resources, the access to fresh water for the production of ornamental plants has decreased, and producers are forced to use salty water in this sector (Garcia-Caparros and Teresa Lao, 2018). Salinity is one of the most important environmental limitations directly related to climate changes, which harms plant growth and yield. The morphological, physiological, nutritional, and biochemical alterations, limitation in water absorption from the soil, ionic toxicity, and osmotic stress are the effects of salinity stress on plants, which reduce plant growth in main part by creating oxidative stress on the whole plant and cells (Shabala *et al*., 2010; Joshi *et al*., 2015). Salinity stress reduced the growth and flower quality of *Tagetes erecta* (Vojodi Mehrabani *et al*., 2017). The results confirm that the growth characteristics of the plant decreased with any salinity stress, but foliar spraying with nanoparticles improved the growth-related traits compared to the treatment of no foliar spraying at the same salinity level. The response to salinity stress is not only different among diverse varieties of plants but also among different plant organs. Depending on the intensity of stress, a variety of physiological responses are observed in stomatal conductance, transpiration, respiration, photosynthesis, chlorophyll content, and root and leaf growth characteristics. In the present study, 150 mM salinity caused a sharp decrease in the number of flowers, flower diameter, and weight of flowers, which confirms the previous reports. Salt stress harmed the number and length of roots, root-to-branch ratio, and chlorophyll content of wheat. Treatment of plants with graphene-quantum dots attached to iron and manganese increased the aforementioned traits and reduced the effects of salt stress on the plant (Haydar *et al*., 2023).

The use of appropriate concentration of graphene oxide increases cell division and growth and thus helps to improve plant growth (Ruiz *et al*., 2011). Salinity stress hurt the growth and yield of *Dracocephalum moldavica* L., (Hasan Zadeh Mohammadi *et al*., 2021) and *Calendula officinalis* (Jahani *et al*., 2022). Foliar spray with cerium oxide nanoparticles under stress conditions improved the growth characteristics of the plant, which may be done via reducing the effects of oxygen free radicals on the plant and maintaining the integrity of the cell membrane. Application of carbon nanoparticles in *Sophora alopecuroides* plant (Wan *et al*., 2020) through leaves under salt stress increased root biomass, total soluble solids content, disease resistance, and plant protein content. Seemingly, improvement in the root biomass by the application of both cerium oxide nanoparticles and quantum dot-graphene oxide may be related to a reliable decline in the negative effects of free radicals on the plant. It seems that under stress conditions, root development played an important role in the subsequent absorption of nutrients by the plant.

The reduction in chlorophyll biosynthesis potential occurs due to the accumulation of toxic ions in the chloroplast and the incidence of oxidative stress in the plant (Hatami, 2017). Inhibition of photochemical reactions and downstream regulation of chloroplast coding genes under the salt stress decreased the chlorophyll content (Saleethong *et al*., 2011). The use of graphene-quantum dots in tomatoes and beans (Feng *et al*., 2019) and titanium oxide in *P. hybrida* (Kamali *et al*., 2018) increased the chlorophyll content of the plant via acting as a photocatalyst and inducing oxidation-reduction reactions in plants. The continuation of this action causes the formation of chlorophyll, stimulates the activity of the Rubisco enzyme and enforce the photosynthesis capacity and enhances plant growth. By increasing light absorption, titanium oxide accelerates the transfer and conversion of light energy, protects chloroplasts against light damage, and helps to enhance the photosynthesis potential (Yang *et al*., 2008; Higashimoto, 2019). In the present study, an increase in the chlorophyll index was observed as a result of foliar spraying with titanium oxide nanoparticles, cerium, and graphene oxide quantum dots, which indicates the positive effect of all three nanoparticles in increasing the chlorophyll content of plants. By eliminating oxygen free radicals and increasing the performance of light-harvesting complexes, cerium oxide nanoparticles improve the content of chlorophyll, accelerate electron transfer, and add up photosynthesis capacity (Kataria *et al*., 2019). The results of the present study confirmed the above studies regarding the increase in chlorophyll content due to the use of nanoparticles and indicated the positive effect of the mentioned compounds on chlorophyll biosynthesis.

Stress causes a drop in the plant's energy level (spending the plant's energy to fight the effects of stress) and, a decrease in protein and carbohydrate production, due to a sharp decline in leaf area and photosynthesis (Bandehagh and Taylor, 2020). The decomposition of the Rubisco enzyme due to stress reduces the protein content (Ishida *et al*., 1997). Titanium oxide affects the molecular mechanism of carbon reactions, activates genes involved in Rubisco enzyme activity, and by increasing protein levels, helps to improve photosynthesis potential (Sheikhalipour *et al*., 2021; Mustafa *et al*., 2021). Under salinity stress, foliar spraying with carbon nanoparticles in the *Sophora alopecuroides* plant amplified the protein content of the plant (Wan *et al*., 2020). The appropriate concentration of graphene oxide is possibly necessary for cell growth due to reducing the negative effects of hydrogen peroxide and protecting cell wall proteins (Anjum *et al*., 2014). The use of cerium oxide nanoparticles raised the protein content of *Calendula officinalis* (Jahani *et al*., 2022). The results of the above study are also in line with the findings of this study, although, in the present study, the highest protein content was obtained in the foliar treatment with carbon nanoparticles.

Under salinity stress conditions, the accumulation of free radicals in plants causes lipid peroxidation and damage to macromolecules. Hydrogen peroxide causes cell membrane damage and programmed cell death by multiplying the production of hydroxyl radicals (Molassiotis and Fotopoulus*,* 2011). Increased production of oxygen free radicals due to stress causes lipids peroxidation and triggers the production of malondialdehyde (Sobhan *et al*., 2016). Salinity stress increased ion leakage in *Tagetes erecta* (Vojodi Mehrabani *et al*., 2017). In bean plants, foliar spraying with quantum dots reduced the content of malondialdehyde (Feng *et al*., 2019). Under salinity stress, titanium nanoparticles foliar use improved the stability of cell membrane, the relative water content and the activity of antioxidant enzymes and, reduced ion leakage and hence enhanced the tolerance against salinity stress in *Stevia* plants (Sheikhalipour *et al*., 2021). Similar results regarding the reduction of ion leakage under salinity along with foliar application of cerium oxide nanoparticles have been reported in *Dracocephalum moldavica* L. (Hasan Zadeh Mohammadi *et al*., 2021). Although, all three nanoparticles used in the present study reduced the effects of stress on the plant, the best result in the present study was obtained from the foliar treatment with carbon nanoparticles at 75 and 150 mM salinity levels.

Salt stress causes oxidative damage through the production of free radicals and their accumulation in the cell. The antioxidant enzymes under these conditions act as a defense mechanism against free radicals (Saleethong *et al*., 2011) In the study conducted on *Tagetes erecta* plant, it was found that zinc nanoparticle foliar application (Vojodi Mehrabani *et al*., 2017), and in bean plant (Feng *et al*., 2019) foliar application with quantum dot under salt stress increased catalase and SOD enzymes activity. Superoxide dismutase enzyme removes superoxide radicals by reducing the production of hydrogen peroxide. The activity of other antioxidant enzymes is very important in the continuation of this process (Hasanuzzaman *et al*., 2020). Catalase plays an important role in converting hydrogen peroxide to water and molecular oxygen. Ascorbate peroxidase scavenges hydrogen peroxide and its role is similar to that of catalase, except that ascorbate peroxidase performs this action through the glutathione-ascorbate cycle (Gharsallah *et al*., 2016). Reducing the effects of salinity stress in plants was reported by spraying both titanium oxide and cerium oxide nanoparticles under salinity stress in cabbage (Rossi *et al*., 2016). An increase in the activity of antioxidant enzymes (catalase, peroxidase, and NADPH oxidase) was reported as a result of treatment with graphene quantum dot bound to iron and manganese under salinity stress in wheat (Haydar *et al*., 2023).

Non-enzymatic antioxidant compounds play an important role in controlling the negative effects of salinity stress on plants. The detachment of hydrogen attached to the active methyl group in the unsaturated fatty acid chain by free radicals causes the oxidation of membrane fats and the outside leakage of cell contents (Shabala, 2010). Protection of cellular systems against the toxicity of active oxygen species is the responsibility of sugars, carotenoids, proline, and phenolic compounds (Morteza *et al*., 2013; Mustafa *et al*., 2021). Accumulation of sucrose in the cell, under stress conditions, causes the preservation of cell membrane phospholipids through the formation of hydrogen bonds between the carboxyl groups of sugars and the polar chains of proteins and prevents structural changes in proteins (Parvaiz and Satyawati, 2008). Proline acts as an osmotic regulator, metal chelator, antioxidant, and signaling molecule in plants. Accumulation of proline under stress conditions is important due to its role in osmotic regulation, maintenance of cell turgescence, detoxification of oxygen free radicals, maintenance of cell membrane integrity, activity of antioxidant enzymes, and protection of protein structure (Mozafari and Ghaderi, 2018; Mbarki *et al*., 2018). Foliar spraying of titanium oxide nanoparticles in wheat (Badshah *et al*., 2023) and graphene oxide in *Vicia faba* L. (Anjum *et al*., 2014) and *Silybum marianum* (Safikhan *et al*., 2018) under salt stress increased the proline, soluble sugars and amino acids content, and superoxide dismutase enzyme activity, and also enhanced the plant tolerance to stress. Polyphenolic compounds play a vital role in reducing oxygen free radicals produced in mitochondria and chloroplasts (Rico *et al*., 2015). The soil-based application of graphene oxide and foliar spraying with iron nanoparticles in grapes (Aazami *et al*., 2022) under stress and, the foliar application of titanium nanoparticles in corn (Mustafa *et al*., 2021) increased the flavonoids and phenolics content of the plant. The use of cerium oxide nanoparticles in marigolds (Jahani *et al*., 2022) and lavender (Vojodi Mehrabani, 2023) enhanced the content of phenolic compounds. Accordingly, it can be concluded that nanoparticles play an important role in plant survival by reducing the effects of environmental stress on plants and by increasing the production of enzymatic and non-enzymatic antioxidant compounds. The results of the present study also showed the positive effect of nanoparticles in enhancing the antioxidant levels in the plant.

The entry of high amounts of sodium ions into the plant under salt stress occurs due to the non-selective nature of the passage of sodium ions through ion channels and the Casparian strip. An increase in sodium ion concentration in the plant causes damage by changing the osmotic balance of the cell, the integrity of the cell membrane, and the hydraulic conductivity of the membranes (Joshi *et al*., 2015). Disturbance of the ionic balance due to salt stress and increasing the ratio of sodium and chlorine ions in the plant creates a competition between sodium, potassium, and calcium for the binding sites to the plasma membrane transporters, and by increasing sodium absorption, it causes plasma membrane instability and cell death (Guo *et al*., 2015; Abrar *et al*., 2020). The use of nanoparticles under stress conditions plays crucial action in the absorption of nutrients by the roots. The use of titanium nanoparticles under salinity stress improves the absorption of high-use and low-use nutrients by plants and helps to improve photosynthesis capacity, growth potential and yield components (Rahneshan *et al*., 2018). Titanium oxide nanoparticles regulate the activity of enzymes involved in nitrogen metabolism in plants. Titanium oxide nanoparticles help the plant absorb nitrate and convert inorganic nitrogen into organic, which can be used for protein and chlorophyll biosynthesis, and hence enhance the biomass and productivity (Yang *et al*., 2008; Mishra *et al*., 2014). An increase in phosphorus absorption was observed in the plant due to salinity stress, which may be one of the reasons for the plant's loss of control over the absorption and transfer of phosphorus to the aerial part of the plant. Phosphorus plays an important role in the photosynthesis, and under salt stress, photosynthesis potential greatly declines due to the lack of phosphorus (Tuna *et al*., 2008). It seems that nanoparticles by changing the expression of genes such as OSNHX1 help to increase potassium absorption under stress conditions (Subramanyam *et al*., 2019). The increase in the K/Na ratio is one of the indicators of plant tolerance to salinity stress (Joshi *et al*., 2015). The use of nanoparticles under salinity stress by enhancing the K/Na ratio assists to enhance the osmotic potential of the plant and helps the survival of plant by reducing the negative effects of stress (Farhangi-Abriz and Torabian, 2018). The application of graphene oxide under salinity stress reduced sodium absorption by multiplying the activity of aquaporins, which help to improve the water relations of the plant (Pandy *et al*., 2018). In the present study, increasing the salinity stress to 150 mM decreased the content of elements except sodium, which indicated the negative effect of stress on the absorption of nutrients from the root medium. Although, the nanoparticles used increased the absorption of nutrients compared to the conditions without foliar application at 75 and 150 mM salinity levels; the highest absorption rate was observed in conditions without salt stress with quantum dot-graphene oxide foliar application.

CONCLUSIONS

The results of the present study showed that the 150 mM salinity stress harmed the growth and reproductive traits of gazania by increasing the content of sodium, malondialdehyde, hydrogen peroxide, and ion leakage. The foliar treatment with quantum dot-graphene oxide under 150 mM NaCl, raised the flavonoids content and catalase activity of the plant. 150 mM sodium chloride along with foliar spraying with quantum dot-graphene oxide and titanium oxide nanoparticles enhanced the activity of superoxide dismutase and ascorbate peroxidase enzymes in leaves. Foliar spraying with quantum dot-graphene oxide nanoparticles improved the number of leaves, flower diameter, chlorophyll index, potassium content, and K/Na ratio in conditions without salinity stress. In general, the results of this study showed that gazania is relatively tolerant to salinity (up to 75 mM sodium chloride) and long-term exposure of the plant to salty soil or water will reduce the number and quality of flowers. More detailed studies with a broad range of salinity levels and diverse foliar treatments are needed to decide on the suitability of this plant for the saline harsh environments and to advise the results to the extension section.

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The Effect of Ornamental Plant Species in Reducing Pollution and Purifying the Air of Sports Facilities

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Abstract

Ornamental plants are one of the cheapest and most natural elements for air purification and pollution reduction. The ability of this type of plant species to absorb pollutants and purify the air is undeniable. Today, due to industrialization and technological progress, air and environmental pollution has become an important concern in developing countries. The purpose of this research is the effect of ornamental plant species in reducing pollution and purifying the air of sports venues. Pollutants that affect the cleanliness of sports places include: Benzene, formaldehyde, carbon monoxide, trichloroethylene, ammonia, fume and xylene. Eight plant species include: English ivy (*Hedera helix*), *Aloe vera*, devil's ivy (*Epipremnum aureum*), dwarf date palm (*Phoenix roebelenii*), spider plant (*Chlorphytum comosum*), variegated snake plant (*Sansevieria trifasciata* 'Laurentii'), boston fern (*Nephrolepis exaltata* 'Bostoniensis') and bamboo palm (*Chamaedorea seifrizii*). In order to purify the air and reduce pollution, pollutants were investigated for air purification and reduction of pollutants in this research. According to the results of this research ($P < 0.05$), the necessary information to predict the correlation of air purification with different plant species statistically showed that, based on the model, eight studied plant species have a natural correlation with air purification and *Sansevieria trifasciata* has the greatest effect in reducing pollution and purifying the air of sports venues (Beta = 0.348, Value = 0.966). The results showed that in any part of the world, the studied plant species are among the most effective species in air purification, so they are suitable for streets, parks and sports places. It is suggested that by using the results of this research, the relevant officials and stakeholders should pay more attention and use the mentioned plants to improve the air quality of the city and sports facilities.

Keywords: Ornamental plants, Sports, Sports complex, Weather.

INTRODUCTION

Creating an urban green space and using plants in the home and work environment is one of the simplest and most important measures to reduce air pollution, because today the role of plants and trees in cleaning the air is not hidden from anyone (Dabiri and Bashiribod, 2014). Urban forests are an essential part of urban ecosystems and provide a myriad of ecological services that contribute to enhancing human welfare, but at the same time, they are profoundly influenced by urbanization (Barona, 2015). Trees and shrubs are a valuable addition to most properties. Adequately planted, well-maintained trees add beauty, wind protection, shade, wildlife habitat, visual screening, and other benefits to the landscape (Kuhns and Rupp, 2000). Plants form the basis of all ecosystems and can partially alleviate the risk of pollution in green spaces (Lohe *et al*., 2015). Biomonitor plants are active collectors that reflect the accumulated effect of environmental pollution and the accumulation of toxicants from atmospheric pollution deposition, binding, and solubility of metals on the leaf surface and soil pollution concentration and bioavailability of elements in soil (Petrova, 2011). Plants have the ability to absorb elements of heavy metals and can reduce pollution (Mohammad Khabbaz, 2014). An ornamental plant is a plant that is grown in a flower garden or as a house plant, in addition, some of them are also air purifiers and are effective in cleaning the air. They are usually planted for their flower display. In recent years flowers and ornamental plants industry in Iran has developed extensively (Mahmoodisafa *et al*., 2016).

The rapid growth of industry and urbanization has produced dust, particulates, and million tons of pollutants that threaten the environment and human health (Hakimzadeh Ardekani *et al*., 2014). Contrary to the common ideas of most people, the dangers caused by pollutants in closed places are more than in open environments. Based on the research and studies of the American Environmental Protection Agency and its scientific advisory board in recent years on the impact of air pollution on humans, it indicates the fact that the amount of air pollution in indoor places can be 2 to 5 times higher. This has made the risk of indoor pollution one of the five main threats to human health (Pluschke, 2012). The Situation is significantly worsening in densely populated cities, which are highly influenced by anthropogenic activities. Artificially built environments are replacing natural vegetation cover leading to poorer self-monitoring and regulation of urban ecosystems, thereby contributing to poor air quality in cities (Hakimzadeh, 2014). These closed places also include spaces such as sports facilities. In the definition of sports facilities, they say: Spaces that are created by fences and in order to create a place for sports and sports activities. Green spaces change the environment by controlling climate, controlling pollution and increasing air quality, protecting against ultraviolet radiation, wind, reducing pollution, and protecting water and soil quality, and this can also indicate the environmental values of green spaces for sports facilities (Sajjadi, 2017). It is recommended to grow plants that have the capacity to absorb air pollution inside sports facilities (Immig and Rish, 1997).

Trees and green spaces in sports facilities should be designed and built in such a way that the sports center officials can monitor and control the users and athletes and coordinate with the relevant institutions regarding the provision of green space maintenance costs. The studied plant species are introduced in this section:

Hedera helix: This ornamental plant is a flowering, twisting and climbing plant that has an effect on air purification. If it is close to a tree, wall or rock it will climb it, otherwise it will crawl on the ground. This plant has green and heart-shaped leaves and has many medicinal uses, including the treatment of acute respiratory tract inflammation caused by cough, and more than 3 medicinal products are made from this plant in Iran (Hamedani, 2022).

Aloe vera: This plant has long been cultivated as an ornamental plant and a medicinal plant, and it can be kept as a plant in an apartment or in a pot. This ornamental plant is a plant without a stem or with a very short stem and grows 60 to 100 cm. Its leaf is thick and fleshy spear with white jagged edges and the color of the leaf is green to gray. Its flowers grow in summer and are sometimes up to 90 cm long, and it absorbs air pollutants. Different species of this plant are resistant to heat, drought and even salinity, and according to the climatic conditions of Iran, our country has a high capacity to produce this valuable plant (Najafi *et al*., 2016).

Epipremnum aureum: This ornamental plant is one of the most suitable and popular indoor plants and it is kept and propagated in warm greenhouses. Its decorative value is due to its bright yellow and creamy white mixed colors and it is considered a climbing plant, because after the stems rise and the leaves grow, a path can be determined for it, and it has the ability to follow the desired path. You can grow without soil and only by staying in water and turn the desired place into a beautiful and pleasant place. In addition to beautifying the surrounding environment, this plant also helps purify the air and plays a significant role in oxygen production and air purification, and is very efficient in removing indoor pollutants such as formaldehyde (Amirimehr, 2020).

Phoenix roebelenii: This ornamental plant is an air purifying plant. It is simple and easy maintenance has made it a very suitable choice for the interior of homes and organizations. These ferns are native to tropical regions and some others are native to temperate regions. This ornamental plant is an evergreen plant and is used both in pots and hanging. This plant needs 1000 lux of light, temperature of 25 degrees Celsius, medium to high irrigation, air humidity of 70%-90% and alkaline soil, and it continues to grow in less light conditions (Tajik *et al*., 2022).

Chlorphytum comosum: This plant is mainly cultivated as an ornamental plant and is considered one of the most popular houseplants that grows in the warm regions of America, Africa and Europe. The ability of this plant to reduce air pollution makes it one of the best air purifying plants. In addition, the plant takes root, leaves and flowers quickly, and the use of nitrate increases the yield and quality of these plants (Osku *et al*., 2022).

Sansevieria trifasciata: This plant is one of the native plants of Africa, Madagascar and South Asia and is actually a very diverse group of plants. This group has more than 70 species, different colors and sizes. Unlike many other ornamental plants, this hardy plant is long-lived. These plants have short-legged and long-legged cultivars and tolerate low water and low light conditions well, and excessive watering should be avoided. One of the characteristics of this plant is the absorption of air formaldehyde, hence it helps to purify the air (Tajik *et al*., 2022).

Nephrolepis: This plant is one of the valuable plants that has been known for centuries as an ornamental plant that purifies the air. These plants do not like the cold, so to keep these plants in the climate of temperate regions, they are placed outside in the shade of the sun in the summer and brought indoors in the winter. In northern Iran, this plant and some other palms are planted in outdoor gardens (Tajik *et al.*, 2022).

Chamaedorea seifrizii: This plant is native to parts of Africa and grows in dense rows with reed stalks and can reach a height of 40 meters. This plant is one of the shade-loving and ornamental plants and can continue to live in low light conditions (Eslamian *et al*., 2009).

A review of research literature shows: Akhavan Markazi *et al*. (2021) showed in a research entitled "Comparison of plant remediation power of two ornamental plants pothos and snake plant when faced with indoor air pollution" that indoor air pollution is one of the most important environmental issues in the world and every The two species used in the research are capable of plant remediation of closed environments, and this ability was observed in pothos more than snake plant due to its higher adaptability to the conditions. Also, when exposed to benzene, the most stress and tissue damage were caused to the plants and the least when treated with acetone. Cai and Li (2019) showed that in a detailed survey to determine the levels and sources of heavy metal pollution in street dust in Shijiazhuang, China, the mixed group of traffic and industry accounted for the largest amount of heavy metals in dust in the city and natural solutions to remove pollution should be identified. Salimi and Sahraei (2016) showed that efforts should be made to purify the air inside the building, because the quality of the air inside the building in conditions of dust and lack of proper ventilation can increase the pollution inside the building, and in this case there will be no way to purify the air and remove pollutants, inside of the building. Mohammad Khabbaz (2014) showed in a research entitled "The role of plants in reducing air pollution" that many plants have the ability to absorb elements of heavy metals and can clean the air and reduce pollution. Fatehifar *et al*. (2012) showed in a research investigated indoor air pollution and ways to reduce it and identified the type of activity in closed environments, especially the type of fuel consumed in heating systems, ventilation of places and their design. He declared the type of activity effective on the amount of pollutants and the reduction of air quality in closed environments. Wolverton *et al*. (1995) showed that leaves, roots, soil and microorganisms associated with plants are a means of reducing air pollution and stated that plant roots and microorganisms associated with them destroy a variety of viruses, bacteria and disease chemicals and they clean the air.

It is obvious that with the large increase of pollutants in urban life and the need to use safe and secure sports places in Rasht city, the need for the presence of plant species in these places is essential for air purification. Therefore, the current study was conducted with the aim of the effect of ornamental plant species in reducing pollution and purifying the air of sports facilities in Rasht city in 2023.

The city of Rasht, capital of Guilan Province in northern part of Iran, is located in a temperate humid region in the foothills of Northern Alborz range in seaward plains (Lat. 37◦ 16′ N., Long. 49◦ 36′ E., Alt. 7 m.) to the South of the Caspian Sea. Its precipitation rate is over 1300 mm, the number of rainy days is 135, and the average relative humidity is 82.6 %. The maximum temperature occurring in July-August is 31.6 ºC and the minimum occurring in December-January is 3.5 ºC (Meteorological statistics of Rasht based on the data of Meteorological Organization of Guilan Province for 2010–2015). The city of Rasht has a population of 640,000 located in the center of Guilan Province. It has 58 parks and its per capita green space is about 2.6 m2 (Annuals Statistics, 2017). This research was conducted from June 2023 to September 2023 in the 6000 person Shohada sports complex in Rasht and Sampling was done purposefully. The location of the study area is shown in Fig. 1.

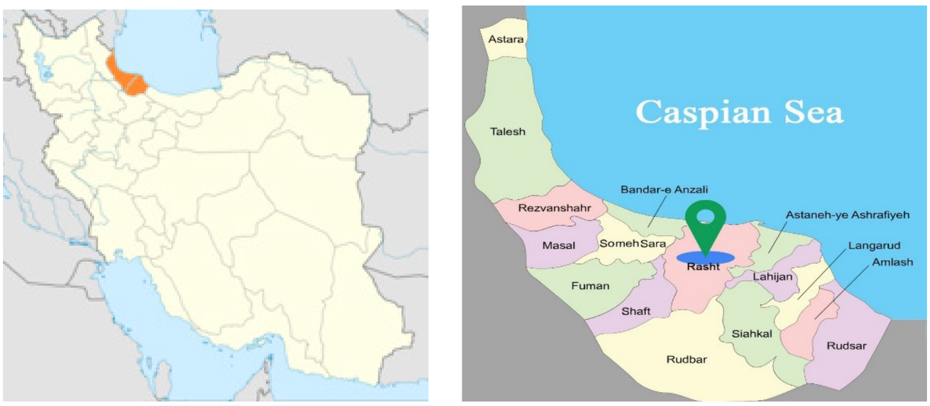

Fig. 1. The location of the study area (Annuals Statistics, 2017).

Sampling and analysis

The samples were taken from the sports facilities of Rasht city. The number of Eight plant species in sports facilities was investigated to reduce pollution and purify the air. In this research, library resources and mass communication tools have been used to collect data. The internal reliability of the data was determined using Cronbach's alpha test. The value of Cronbach's alpha coefficient was 78%, so we can rely on the reliability of the data. For the statistical tests of the research, first, the Kolmogorov-Smirnov test was used for the normality of the data, and the distribution of the data was reported to be normal. Linear regression has been used to examine the contribution of the power of predicting the changes of the dependent variable through the independent variable. Linear regression has been used to determine the effect of the independent variables of plant species on the dependent variable of air purification. The data normality analysis showed that the data distribution was normal. The studied plant species are shown in Fig. 2.

Fig. 2. Plant species studied (Research findings, 2023).

RESULTS AND DISCUSSION

Green spaces and ornamental plant species have a leading role in human life. In this regard, it is necessary to use ornamental plant species to purify the air and reduce pollution. This research was done with the aim of The Effect of Ornamental Plant Species in Reducing Pollution and Purifying the Air of Sports Facilities.

Table 1, shows the studied plant species that are effective in air purification and pollution reduction. These pollutants are: Benzene, formaldehyde, CO, TCE, NH_3, fume and xylene.

The preliminary analysis of the research shows that the assumptions of normality, homogeneity and correlation have not been rejected in it. Then the relationship between plant species variable and air purification variable was investigated. Table 2, shows the positive and normal correlation between research variables.

Table 1. Effective plant species in reducing pollution and purifying the air of sports facilities.

Plant species	Benzene	Formaldehyde	CO	TCE	NH3	Fume	Xylene
Hedera helix	*	*		*		*	
Aloe vera	*	*					
Epipremnum aureum		*	*			*	
Phoenix roebelenii					*	*	
Chlorphytum comosum	*	*	*				*
Sansevieria trifasciata	*	*	*	*		*	
Nephrolepisa		*	*				*
Chamaedorea seifrizii	*	*		*	*		

It shows the effectiveness of plant species in reducing pollutants and significant at $P < 0.05$.

Table 2. Correlation coefficient of research variables.

Pearson,s R	Value	Approx. Sig.
	0.425	0.05
N = 8		

Table 3, the summary of the regression model of the independent variable of plant species and the dependent variable of air purification is presented as shown below (R2 = 0.0093, P < 0.05) explains the total amount of changes in the dependent variable of air purification by the independent variable of plant species.

Table 3. Summary of the regression model of the variables.

Model	R	R Square	MSE
	0.425	0.0180	0.56766

Table 4, ANOVA, which is used to compare the mean of two or more groups, the linear regression model significantly predicted the research variables. The value of 0.966 shows the statistical normality of the model and the regression value, which has value. The significance level ($P < 0.05$) shows that, in general, the regression model used in the research has predicted the significant value of the main research variable.

Table 4. ANOVA.

ANOVA	SS	df	F	Value
	0.622	7	1.032	0.966

Significant at $P < 0.05$.

The necessary information to predict the correlation of air purification with different plant species is provided in table 5. Statistically, based on the model, the eight studied plant species have a natural correlation with air purification and reducing the pollution of sports spaces and have a value (Beta = 0.348, Value = 0.966).

Table 5. Predict the correlation of air purification with various plant species.

Model	Unstandardized coefficients		Standardized coefficients	t	Value
	B	Std. Error	Beta		
	0.285	0.188	0.348	0.882	0.966

Significant at $P < 0.05$.

In addition to directly purifying the air from pollutants, plants also have an indirect effect in purifying the air from harmful factors for living organisms. There are various plant species that can clean and purify the air inside sports facilities by absorbing pollution and producing oxygen, and remove a significant amount of environmental pollution. The studied plant species *Hedera helix*, *Aloe vera*, *Epipremnum aureum*, *Phoenix roebelenii*, *Chlorphytum comosum*, *Sansevieria trifasciata*, *Nephrolepisa* and *Chamaedorea seifrizii* showed that they play an important role in reducing pollution and purifying the air of sports venues. For example, the "*Sansevieria trifasciata*" has a high ability to eliminate most toxins and produces oxygen even at night.

The results showed, *Hedera helix* is an ornamental and evergreen plant that is highly adaptable to environmental conditions and can grow and clean the air in high and low temperatures and low and high light. This plant has the ability to absorb pollutants such as: Benzene, formaldehyde, TCE and fume. The results of these researches are in line with Hamedani (2022), Sajjadi (2017), Lohe *et al.* (2015) and Mahmoodisafa *et al.* (2016). *Aloe vera* is an ornamental plant with many properties, one of its most famous benefits is absorbing pollutants such as formaldehyde and benzene and purifying the air. The research results about this plant are in line with these researches of Najafi *et al.* (2016), Lohe *et al.* (2015), Mohammad Khabbaz (2014), Immig and Rish (1997) and Wolverton *et al.* (1995). *Epipremnum aureum* is one of the popular ornamental and apartment plants that is very easy to maintain at home. This plant is a resistant plant that has a great ability to absorb environmental pollution. This plant can be easily fume, formaldehyde and CO to absorb and purify the air and has many uses in sports facilities. The results of the research are in line with researches Akhavan Markazi (2021), Amirimehr (2020), Sajjadi (2017) and Dabiri and Bashiribod (2014). *Phoenix roebelenii* is a valuable ornamental plant that has been used as an agricultural plant for centuries, and the ability of this plant to purify the air and reduce pollution is undeniable. This plant can absorb NH3 and fume and clean the ambient air from pollutants. The results of these researches are in line with researches Tajik *et al.* (2022), Lohe *et al.* (2015), Dabiri and Bashiribod (2014), and Mohammad Khabbaz (2014).

Chlorphytum comosum is one of the most resistant and cheapest ornamental plants. This air purifying plant absorbs and removes many abnormal elements of the environment such as xylene, formaldehyde, benzene and CO. In addition to being considered a greenhouse plant, this plant is also compatible with open and closed environments such as sports facilities. The research results about this plant are in line with these researches of Osku *et al.* (2022), Sajjadi (2017), Mohammad Khabbaz (2014) and Wolverton *et al.* (1995). *Sansevieria trifasciata* is a beautiful and luxurious plant, with high resistance and evergreen, which is very easy to maintain and grow this ornamental plant. This plant has many fans due to its ability to absorb toxins such as CO, benzene, TCE, fume and formaldehyde and can be used in all open and closed spaces such as apartments, offices and sports facilities. The results of these researches are in line with researches Tajik *et al.* (2022), Akhavan Markazi (2021), Dabiri and Bashiribod (2014), Mohammad Khabbaz (2014) and Immig and Rish (1997). Nephrolepisa is an air purifying plant and can absorb pollutants such as formaldehyde, xylene and CO, the simple and easy maintenance of this plant has made it a very suitable option for reducing indoor pollution and can be used in it is used in outdoor and other indoor places. The results of the research are in line with researches Tajik *et al.* (2022), Amirimehr (2020), Sajjadi (2017) and Lohe *et al.* (2015). *Chamaedorea seifrizii* is one of the other ornamental plants that has the ability to absorb toxins such as formaldehyde, benzene, TCE and NH_3 and has an effect on air purification and reducing pollution in buildings and sports facilities. The research results about this plant are in line with these researches of Sajjadi (2017), Mahmoodisafa *et al*. (2016), Eslamian *et al.* (2009) and Wolverton *et al.* (1995).

The results obtained from that research provided useful information about the air purification of sports facilities through the studied ornamental plant species. The research results indicate a significant and important effect of ornamental plant species on reducing air pollution. These plants can be used as the most natural elements in air purification and reducing pollution in sports facilities. But currently, due to the small number and many limitations in related studies, it is recommended to conduct more research in this field. These findings are consistent with the results obtained from research on the relationship between plant components and pollution reduction.

CONCLUSION

Today, air pollution is one of the important challenges we are facing and its negative effects affect the quality of life and human health. In particular, we are always looking for ways to reduce air pollution. Plants are one of the best options for air purification and pollution reduction. Eight ornamental plant species: English ivy (*Hedera helix*), *Aloe vera*, devil's ivy (*Epipremnum aureum*), dwarf date palm (*Phoenix roebelenii*), spider plant (*Chlorphytum comosum*), variegated snake plant (*Sansevieria trifasciata* 'Laurentii'), boston fern (*Nephrolepis exaltata* 'Bostoniensis') and bamboo palm (*Chamaedorea seifrizii*) are given which are effective in purifying the air and reducing pollution. These pollutants are: Benzene, formaldehyde, CO, TCE, NH_3, fume and xylene. According to the results of this research, statistically, the correlation of air purification with the use of ornamental plant species showed that, based on the model, the eight studied ornamental plant species have created a natural correlation in air purification and reducing the pollution of sports facilities (Beta = 0.348, value = 0.966) and the results of this research confirm the significance of using ornamental plant species in air purification and reducing pollution in sports facilities.

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Studying the Effect of Seed Priming on the Growth and Yield of Marigold (*Calendula officinalis* L.) Under Different Levels of Vermicompost

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Abstract

The present study aims to investigate the effect of different levels of vermicompost on the growth and yield of the essential oil of marigold plant under seed priming with *Azospirillum* and humic acid under different levels of vermicompost factorial experiment carried out based on a randomized complete block design (RCBD) in the research farm of Islamic Azad University, Astara Branch. The test treatments included seed priming including *Azospirillum brazilense* bacteria, humic acid, *Azospirillum* + humic acid and control and different levels of vermicompost (0, 5 and 10 t ha^{-1}). The results showed that some traits such as chlorophyll a, anthocyanin, essential oil yield and biological yield were affected by vermicompost interaction and seed priming. But plant height, number of seeds and flowers, flower weight, seed weight, essential oil amount and total chlorophyll were affected by the main effect of vermicompost, but seed priming was not significant on some of these traits. The highest amounts of chlorophyll a and total, biological yield, essential oil yield and dry weight of flowers were obtained from humic acid + *Azospirillum* treatment under the application of 10 t vermicompost ha^{-1}.

Keywords: Anthocyanin, *Azospirillum*, Essential oil, Marigold, Vermicompost.

INTRODUCTION

The marigold belongs to the Asteraceae family, which usually flowers in spring. Today, marigold cultivation has been developed all over the world as an ornamental-medicinal plant. Marigolds are edible and are widely used for coloring food and salad, making tea and extract of marigolds, in addition to cosmetic and health industries, in the production of ointments, creams, eye drops, and oils. One of the most famous marigold products is calendula ointment, which is used to heal insect bites or superficial wounds (Catalano *et al*., 2022).

Optimum soil quality plays an important role not only in the production and growth of plants, but also in the quality and quantity of effective substances of medicinal plants (Ghadimi *et al*., 2021). Organic farming has gained a special position in most countries. In Europe, from 2015 to 2020, it has increased from 100,000 ha to 2.8 million ha. Its basis is the increase of organic matter in the soil and as a result maintaining soil fertility in the long term by applying organic management (Mandal *et al*., 2023).

The application of compost in the soil is generally in order to maintain and increase the soil stability, the fertility of agricultural and garden soils, which has been of special importance in the past decades (Liu *et al*., 2017). On the other hand, the use of urban waste compost in the soil has caused some concerns due to the aspects of phytotoxicity, the uncertainty of the nutritional value of the available nutrients for the plant and the environmental consequences (Tejada and González, 2009). Vermicompost contains nutrients such as phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in a form that can be easily absorbed and accessed by plants (Amanullah, 2016). Some researchers also reported that organic matter increases the P available to plants and indirectly prevents the precipitation of P in alkaline pHs, which is unabsorbable for plants (Mosavi-Azandehi *et al*., 2023). In addition, significant amounts of micronutrient elements (Amanullah, 2016), humic acid (Maji *et al*., 2017) and plant growth promoting substances such as auxins, gibberellins and cytokinins Mosavi-Azandehi *et al*., 2023) and dissolving bacteria P, enzymes and vitamins (Bakhshi *et al*., 2023), are present in vermicompost. The existence of these compounds leads to the implementation of vegetative growth and economic yield of the plant (Joshi *et al*., 2015). The results of various studies on the application of vermicompost showed that the highest plant weight, essential oil content and yield of Moldavian dragonhead (*Dracocephalum moldavica* L.) oil were obtained in the treatment of using 10 t ha^{-1} of vermicompost (Darzi *et al*., 2016). Dry matter yield in chamomile (*Matricaria recutita* L.) (Fallahi *et al*., 2013), basil (*Ocimum basilicum* L.) (Makkizadeh *et al*., 2011) and thyme (*Thymus daenensis* Celak L.) (Safaei *et al*., 2014) using vermicompost in increased compared to the control.

Humic acid as an organic growth stimulant has been commercially available to farmers for several decades in order to increase crop growth and economic efficiency (Olk *et al*., 2018). Humic acid is produced by the decomposition of organic materials, especially those of plant origin, and is found in soil, coal, and peat. Humic acid forms a stable and insoluble complex with microelements. Humic acid, a mixture of very large molecules with the ability to chelate nutrients, increase their absorption and soil fertility, along with folic acid, are the most important components of soil humus (Mackowiak *et al*., 2001). The results of the application of humic acid organic fertilizer on marigold showed that the application of humic acid increased plant height, fresh flower yield, and number of flowers, seed yield, petal yield and thousand seed weight compared to the control (Abedini *et al*., 2015).

Biological fertilizers also include sufficient amounts of one or more types of beneficial soil microorganisms, which are supplied with suitable preservatives and play a positive role in meeting the nutritional needs of plants and improve their growth conditions. The most important nitrogen-fixing bacteria are symbiotic Rhizobium and non-symbiotic *Azotobacter* and *Azospirillum*, which are present in the soil rhizosphere environment (Ahmadian *et al*., 2011). These microorganisms, when used on the seed, root surface or in the soil, stimulate root growth and increase the plant's growth by increasing the plant's accessibility to minerals (Vessey, 2003). It has been reported that *Azospirillum* increased stem weight and capitol diameter (Shokrani *et al*., 2012) and essential oil yield (Hosseini Mazinani and Hadipour, 2014) in marigold. The positive effect of biological fertilizers on the growth of garden thyme (*Thymus vulgaris* L.), savory (*Satureja hortensis* L.) and rosemary (*Salvia rosmarinus* L.) has also been reported by some researchers (Leithy *et al*., 2006; Nasiri-Dehsorkhi *et al.*, 2018).

Considering the importance of producing medicinal plants in a sustainable production system, protecting natural resources and producing healthy medicinal products, the purpose of this research is to investigate the effect of using vermicompost, *Azospirillum* and humic acid and their interaction on some of the ecophysiological properties and essential oil content of marigold.

MATERIALS AND METHODS

The field experiments was conducted at an experimental farm in Astara, Guilan (Long. 48°52' E., Lat. 38°22' N., the elevation of 10 meters from sea level) in 2022. The study site has been reported to have a temperate and humid climate based on the climatic classification. Its annual precipitation and mean annual temperature have been reported at about 1326 mm and 19.8 °C, respectively. Table 1 depicts the climatic conditions of Astara including temperature and precipitation. Before the experiments, the farm soil was sampled to determine its properties. The results are presented in table 2.

Table 1. Average, maximum and minimum temperature and monthly rainfall in the Astara region during the plant growth period.

Months	Monthly sunny hours	Precipitation (mm)	Temperature (°C)		Mean
			Max	Min	
May	179.3	45.4	24.9	19.4	22.1
June	237.7	76.5	27.1	20.3	23.7
July	264.2	69.1	28.6	21.8	25.2
August	292.2	72.2	27.4	20.2	23.85
September	218.4	89.3	26.6	18.1	22.35

The study carried out as factorial experiment based on a randomized complete block design with three replications. The first factor was the vermicompost of cow manrure (VM) at a rate of 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}; seed priming treatments at four levels including *Azospirillum* brasilense (PA), humic acid 15 g l^{-1} (Ph), A. brasilense + humic acid (PA+Ph) and control (P0).

Table 2. Physical and chemical characteristics of the experimental field soil.

pH	Electrical conductivity	Organic carbon (OC)	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Sand	Silt	Clay
	(dS m-1)	(%)		(mg kg^{-1})			(%)	
7.18	0.67	1.04	0.218	19.9	234	17.3	37.5	45.2

Agronomic practices

The seeds of the marigold of the Gitanana F1 cultivar with yellow petals, which were obtained from Pakan Seed Co., Isfahan. The *Azospirillum* brasilense was provided by the Soil Biology Research Department of the Soil and Water Research Institute. Each experimental plot was composed of six 5–m–long sowing rows with an inter–row spacing of 40 cm and on–row spacing of 10 cm. After the plots were prepared, the vermicompost was distributed according to the planting plan and was completely mixed with the soil. To inoculate the seeds, they were first impregnated with gum arabic, and the bacteria and humic acid were added to them. After the seeds were inoculated and dried, they were sown manually at a depth of 2 cm on May 10 and were immediately irrigated by the sprinkler method.

Plant sampling

In order to evaluate the traits related to a single plant (plant height, number of sub-stems per plant, diameter of the main stem, number of flowers per plant, leaf greenness index), seven plants from each experimental plot, taking into account the margin effect, were randomly selected and traits were measured. A caliper was used to measure the diameter of the main stem.

To estimate chlorophyll, the leaves were cut before the flowering stage, their chlorophyll was measured by Mazumdar and Majumdar›s (2003) method. Finally, leaf chlorophyll a, b and total was calculated by the following equation:

$$\text{Chlorophyll a (mg g}^{-1}\text{ FW)} = 9.93\ (A660) - 0.777\ (A642) \qquad (1)$$
$$\text{Chlorophyll b (mg g}^{-1}\text{ FW)} = 17.6\ (A642) - 2.81\ (A660) \qquad (2)$$
$$\text{Total chlorophyll (mg g}^{-1}\text{ FW)} = 7.12\ (A660) - 16.8\ (A642) \qquad (3)$$

Petal anthocyanin was estimated by spectrophotometry and the following formula:

$$\text{Petal anthocyanin (mg 100 g}^{-1}\text{ DW)} = (e\times b\times c)/(d\times a)\times 100$$

Where, a, b, c, d, and e were the reading, sample size, whole solution, taken sample size, and sample weight, respectively.

After the flowers opened, harvesting was done manually from the plants of the middle two rows of each plot and once every seven days. The harvesting operation started on July 20 and continued until the end of September. After each harvest, in order to maintain the quality, the harvested flowers were immediately dried in a suitable place away from direct sunlight, and after drying, they were kept in suitable paper envelopes until essential oil extraction. The total dry weight of harvested flowers in all harvests was considered as flower yield per unit area. After the end of the flower harvesting period, the bushes whose flowers were already harvested were cut from the top of the crown and weighed after drying. The total weight of harvested plants and flowers was considered as biological yield.

To determine the seed yield in each plot, one row was selected and the seeds were harvested and weighed at the ripening stage and recorded as seed yield, and then the weight of 1,000 seeds harvested was also measured. To determine the percentage of essential oil, a mixture of harvested dry flowers (sum of harvests) was used. In order to extract the essential oil, the water distillation method was used based on the method suggested by the European Pharmacopoeia and the Clevenger device, so that from each sample, 100 g of crushed flowers were extracted for four hours using the Clevenger device (Farjami and Nabavi, 2014). The following relationships were also used to determine the percentage and yield of the essential oil:

Equ.1: Concentration of essential oil = (Weight of extracted essential oil (g))/(Dry weight of flowers (100 g))×100

Equ. 2: Weight of flowers (g m^2) × Percentage of essential oil (%) = Yield of essential oil (g/m^2)

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) for statistical analysis. The significance of the treatment effect was determined by the magnitude of F–value ($P < 0.05$). When the F–test revealed the significance of the treatments, the means were separately compared using the LSMEANS method with LSD adjustment at $P = 0.05$. The statistical analysis of the results was performed by the general linear model (GLM) in the SAS (Ver. 9.2) software suite.

RESULTS

Leaf chlorophyll

The results of analysis of variance showed that the content of chlorophyll a, b and total chlorophyll were affected by the interaction of vermicompost × seed priming (Table 3). The chlorophyll b increased with the increase of vermicompost application, so that the chlorophyll b at 5 and 10 t/ha levels increased by 24.6 and 39.3%, respectively, compared to the without vermicompost application treatment (VM0) (Table 6). The mean comparison showed that the highest chlorophyll a and total were obtained from the combined treatment of *Azospirillum* + humic acid (PA + Ph) under the application of 10 t/ha of vermicompost (VM2) (Table 7).

Petal anthocyanin

The results of analysis of variance showed that the anthocyanin was affected by the interaction of vermicompost × seed priming (Table 3). The mean comparison showed that treated plants increased the amount of anthocyanin by 13.3 to 42.8% compared to control plants. In all levels of vermicompost, the highest content of anthocyanin was obtained from PA (*Azospirillum*) and PA + Ph (*Azospirillum* + humic acid) (Table 7).

Plant height and number of branches

The results of analysis of variance showed that the main effect of seed priming and vermicompost on the plant height was significant (Table 4). The mean comparison showed that there was no significant difference between 5 (VM1) and 10 (VM2) t ha^{-1}, but compared to the VM0, they increased the plant height by 27.5 and 25.3% (Table 5). Seed priming treatments also increased plant height by 24.3 to 27.4% compared to control (Table 6). The number of branches was only affected by vermicompost and the application levels of vermicompost (MV_1 and MV2) increased the number of branches by 35.7 and 64.4% compared to the MV0 (Table 5).

Table 3. Analysis of variance in the effect of different levels of vermicompost and seed priming on physiological traits.

S.o.V	df	MS					
		Chlorophyll a	Chlorophyll b	Total chlorophyll	Anthocyanin	Concentration of essential oil	Yield of essential oil
Block	2	0.553^{ns}	0.671**	5.02**	481^{ns}	0.0025^{ns}	0.036^{ns}
Vermicompost (V)	2	11.93**	0.8109**	17.41**	9274**	0.0074^{ns}	0.513**
Priming (P)	3	12.63**	2.374**	31.42**	26004**	0.023**	0.773**
V × P	6	22.18**	0.336*	23.69**	49619**	0.019**	0.276*
Error	22	0.378	0.089	0.412	562.1	0.0042	0.051
CV (%)		14.64	25.12	11.28	7.94	8.03	16.83

*, ** and ns: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Leaf greenness index

The results of analysis of variance showed that the leaf greenness index was only affected by vermicompost (Table 4). The mean comparison showed that with the increase in vermicompost application, the leaf greenness index also increased (Table 5).

Number of flowers

Based on the results of analysis of variance, the main effect of vermicompost and seed priming on the number of flowers was significant at the 5 and 1% probability level, respectively (Table 4). The results of mean comparison of vermicompost levels showed that with the increase of vermicompost application, the number of flowers plant^{-1} increased so that the most flowers were obtained from VM2 level (Table 5). The results of the comparison of seed priming also showed that seed priming had a significant advantage over the non-prime treatment (P0) and the highest number of flowers was obtained from the *Azospirillum* + humic treatment (34.3 flowers plant^{-1}) (Table 6).

Table 4. Analysis of variance in the effect of different levels of vermicompost and seed priming on morphological traits.

S.o.V	df	MS							
		Plant height	Number of stems	Number of flower plant^{-1}	Leaf greenness index	Dry weight of flower	Seed number capitol^{-1}	Seed weight	Biological yield
Block	2	112.0*	29.4^{ns}	25.03^{ns}	5.12**	1437^{ns}	0.487^{ns}	97.4^{ns}	835^{ns}
Vermicompost (V)	2	268.7**	146**	118.9*	18.84**	9610**	51.7**	1987**	7848**
Priming (P)	3	837**	32.7^{ns}	315**	1.17^{ns}	4425*	95.63**	2210**	16848**
V × P	6	39.8^{ns}	58.2^{ns}	18.9^{ns}	0.39^{ns}	2147^{ns}	2.87^{ns}	879*	14823**
Error	22	26.07	33.16	49.7	0.863	1870	10.56	263.4	1725
CV (%)		3.92	13.44	9.35	22.10	39.55	29.40	33.25	9.23

*, ** and ns: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test, respectively.

Table 5. Means comparison for the main effect of different levels of vermicompost on the measured traits.

VM	Plant height (cm)	Number of stems	Number of flower plant^{-1}	SPAD	Weight of dry flower (g m^{-2})	Seed number capitol^{-1}	Biological yield (g m^{-2})	Chlorophyll b (mg ml^{-1})
VM0	37.21d	9.44c	16.44c	36.73c	163b	26.4	641.0c	0.846c
VM1	40.78b	12.72b	22.3b	38.05b	269ab	33.2	707.5b	1.124b
VM2	41.97a	14.96a	29.96a	49.83a	282a	35.6	744.9a	1.406a

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}.

Dry weight of flowers

Based on the results of analysis of variance, the main effect of vermicompost and seed priming on the dry weight of flowers was significant at the 5 and 1% probability level, respectively (Table 4). The highest dry weight of flowers (282.3 g m^{-2}) was obtained from the application of 10 t ha^{-1} vermicompost (VM2), which showed an increase of 57.8% compared to the VM0, and the application of 5 t ha^{-1} of vermicompost (VM2) was ranked next with the production of 269.2 g dry flowers m^{-2} (Table 5). Also, the results of the mean comparison of seed priming showed that the Azospirillum + humic acid (PA + Ph) treatment had the highest dry weight of flowers (247.6 g m^{-2}), which caused a 55.4% increase in the dry weight of flowers compared to the control (Table 6).

Table 6. Means comparison for the main effect of seed priming treatments on the measured traits.

Priming treatments	Plant height (cm)	Number of flower plant^{-1}	Yield of dry flower (g m^{-2})	Seed number capitol^{-1}	Biological yield (g m^{-2})	Chlorophyll b (mg mL^{-1})
P0	37.82d	15.24c	137.6b	23.98d	57.28a	0.7083d
Ph	39.99c	18.38b	191.9ab	28.86c	54.279b	1.0058c
PA	41.08b	23.92b	217.8ab	34.44b	51.470c	1.2817b
PA+Ph	43.15a	34.9a	247.2a	39.86a	48.664d	1.7550a

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming.

Number of seeds per capitol

The results of the analysis of variance showed that the main effect of vermicompost and seed priming on the number of seeds per capitol was significant at the 1% probability level (Table 4). Based on the results of mean comparison, the application of 10 t ha^{-1} of vermicompost (VM2), with 35.6 seeds per capitol, increased the value of this trait by 24.5% compared to the VM0 (Table 5). *Azospirillum* + humic acid (39.86 seeds per capitol) also increased its amount by 74.1% compared to the control (Table 6).

Seed weight

The results of analysis of variance showed that the interaction effect of vermicompost × seed priming on seed weight was significant at the 5% probability level (Table 4). Based on the mean comparison results, the highest seed weight was obtained in *Azospirillum* (271 g m^{-2}) and PA + Ph (260 g m^{-2}) treatments under the application of 10 t/ha of vermicompost and these two treatments compared to the control increased the seed weight by 68.6 and 67.1%, respectively (Table 7).

Biological yield

Based on ANOVA results in table 4, it was observed that the interaction of vermicompost × seed priming was significant on biological yield (Table 4). The mean comparison of the treatments showed that at all levels of vermicompost, the priming treatments had higher biological yield than the control. All priming treatments showed the highest biological yield at the MV2 level, and PA + Ph (576 g m^{-2}) and Azospirillum (551 g m^{-2}) treatments were significantly superior to other treatments (Fig. 1).

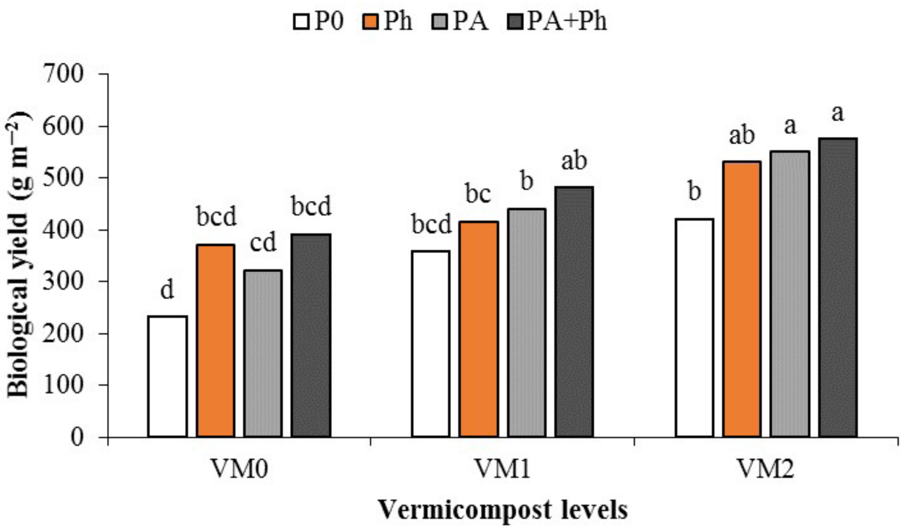

Fig. 1. Mean comparison of interaction effect of vermicompost × seed priming on biological yield. Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Concentration of essential oil

The results of analysis of variance showed that the interaction effect of vermicompost × seed priming was significant on the concentration of essential oil (Table 4). The PA+Ph treatment under VM0 level (0.132%) and Ph treatment under VM1 (0.168%) and VM2 (0.185%) levels showed the highest concentration of essential oil. Among all treatments, the control plants (P0 + MV0) showed the lowest concentration of essential oil (Table 7).

Yield of essential oil

The results of analysis of variance showed that the interaction effect of vermicompost

× seed priming was significant on the yield of essential oil (Table 4). Based on the means comparison results, the highest yield of essential oil was obtained in of PA + Ph (0.943 g m^{-2}) and *Azospirillum* (0.775 g m^{-2}) treatments, under the application of 10 t ha^{-1} of vermicompost, and these two treatments were 79.6 and 68.6%, respectively, compared to the control (Fig. 2).

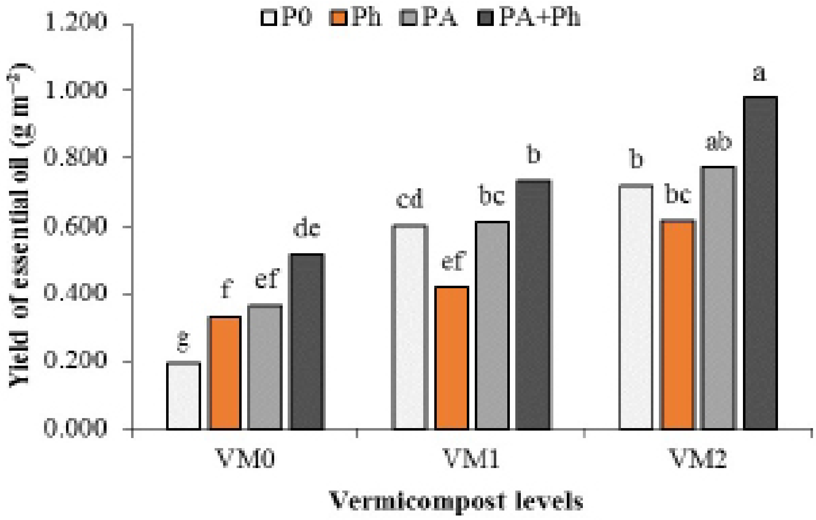

Fig. 2. Mean comparison of interaction effect of vermicompost × seed priming on yield of essential oil. Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Table 7. Means comparison for the interaction effects vermicompost × seed priming on the measured traits.

VM	PT	Chlorophyll a (mg ml^{-1})	Chlorophyll b (mg ml^{-1})	Chlorophyll total (mg ml^{-1})	Anthocyanin (mg 100 g^{-1})	Concentration of essential oil (%)	Seeds weight (g m^{-2})
VM0	P0	1.910h	0.446f	2.826h	210.1h	0.062i	85.00i
	Ph	2.820fgh	0.970de	3.830fgh	283.2ef	0.095h	129.3defg
	PA	3.670defg	0.706ef	4.770def	314.0cde	0.113fg	124.0efg
	PA+Ph	2.656gh	0.993de	5.206cde	241.6gh	0.132e	96.67hi
VM1	P0	3.556e-g	1.153c-e	3.443gh	300.8de	0.101gh	106.0ghi
	Ph	4.080c-e	1.643bc	5.816cd	333.1bcd	0.168b	119.6fgh
	PA	4.926bc	1.123de	4.623ef	248.0f-h	0.139cde	174.0c
	PA+Ph	4.486bcde	1.640bc	5.886c	323.0cd	0.152c	147.6de
VM2	P0	5.480ab	2.016ab	7.523b	372.9a	0.116f	154.3cd
	Ph	4.600bcd	1.056de	8.913a	250.8fg	0.185a	214.3b
	PA	5.340b	1.363cd	4.493efg	343.0abc	0.137de	271.3a
	PA+Ph	6.466a	2.240a	8.703a	369.1ab	0.170b	260.3a

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test. Priming treatment levels: P0: Control, Ph: Humic acid seed priming, PA: Bio priming by *Azospirillum*, PA+Ph: Bio priming by *Azospirillum* + Humic acid seed priming. Vermicompost (VM) levels: 0 (VM0), 5(VM1), 10 (VM2) t ha^{-1}.

DISCUSSION

Soil organic matter is a key factor in soil fertility and has been included in the UN Environmental Program as a fundamental environmental challenge at a global scale (Jhariya *et al*., 2022). In addition to soil health and quality, the presence of organic matter in soil is a proper indicator of soil fertility, which is the result of the interaction of physical, chemical, and biological processes (Ghadimi *et al*., 2021). Organic fertilizers, especially composted animal manure, have a large amount of organic substances, which are a rich source of N, P and K and over time, these elements are provided to the plant and increase the vegetative growth. These increases may be due to the fact that the application of animal manure facilitates the plant's access to water and sufficient nutrients, especially nitrogen, and as a result, it is effective in increasing the height of the plant and the number of branches through the effect on the division and enlargement of cells (Adamipour *et al*., 2019). Mahboub Khomami *et al.* (2021) reported in their research that the application of organic fertilizers caused a significant increase in the height of the ornamental foliage plants. In relation to the increase in plant height due to the use of humic acid and its combination with *Azotobacter chroococcum,* it seems that humic acid, through hormonal effects, influences the metabolism of plant cells, and by having the power of chelating and increasing the absorption of nutrients, causes it increases the growth and height of the plant (Sharafabad *et al*., 2022). The increase in the height of the vitex (*Vitex trifolia* L.) with the use of humic acid has been reported in another study (Ashour *et al*., 2020). In a research on the peppermint (*Mentha piperita* L.), the results indicated that the use of humic acid by creating better nutritional conditions for the vegetative growth of the plant caused a significant increase in traits such as plant height, shoot dry weight and dry matter yield (Askari *et al*., 2012).

The number of flowers is considered as one of the most important components of yield in the marigold, and with its increase, the weight of flowers also increases. The increase in the number of flowers and the weight of flowers with the use of vermicompost can be related to the effect of this fertilizer in increasing soil nutrients and providing the ability to absorb them in the plant (Mashayekhi *et al*., 2019), as a result of which the absorption efficiency of nutrients also increases. The reason for the increase in the number of flowers and flowers weight with the use of humic acid and *Azospirillum* can be because humic acid has increased fertility and production in the plant through chelating essential elements and increasing the absorption of nutrients (Nasiri Dehsorkhi *et al*., 2018). In addition to fixing N, *Azospirillum* causes the release of plant hormones such as gibberellic acid and auxin, and under these conditions, root growth and the availability and absorption of nutrients such as N and P increase, which ultimately increases the height of the plant, the number of secondary stems, and the number of flowers. An increase in the number of flowers and the dry yield of flowers has been reported in coneflower (*Echinaceae purpurea* L.) with the use of *Azotobacter chroococcum* biofertilizer (Agha Alikhani *et al.*, 2013) and in chamomile (*Matricaria chamomilla* L.) with the use of humic acid (Mashayekhi *et al*., 2019).

The use of larger amounts of vermicompost through increasing the water absorption power and the optimal provision of high-use and low-use nutrients has a positive effect on the amount of chlorophyll in the leaves and causes an increase in chlorophyll, and in addition, by providing the nutritional needs of soil microorganisms, it increases the number of and their activity increases, and as a result, the amount of absorption of micro elements such as iron, manganese, and magnesium, which play an important role in the production of chlorophyll, is increased by the plant, and finally they increase the synthesis of chlorophyll (Khosravi Shakib *et al*., 2019). It seems that the application of 10 t ha^{-1} of vermicompost improves and increases the content of organic matter in the soil, maintenance and proper provision of moisture and nutrients such as N, P and K, on the increase in the biological yield of marigold. It has an effect on the shape and weight of the seeds and has improved the yield of the seeds. In relation to the use of *Azospirillum*, it seems that the inoculation treatment increased the biological yield through the obvious effect that increased the number of branches plant^{-1}. On the other hand, probably, humic acid also increases the absorption of nitrate (NO^{-}_{3}) and the activity of the ATPase enzyme in the plasma membrane of root cells, leading to an increase in the absorption of nutrients from the soil, and as a result, by increasing the photo-center in the plant, it has led to an increase in yield (Manivannan and Nanthakumar, 2021).

It seems that adding vermicompost to the soil improves the activity of soil microorganisms and provides the necessary conditions for P solubility and more access to soil N, and as a result, P and N needed for the biosynthesis of ATP and NADPH to make terpenoid compounds (essential oils) in placed near the plant and essential oil production increases (Nasiri Dehsorkhi et al., 2018; Upadhya *et al*., 2022).

Since the use of vermicompost, *Azospirillum* and humic acid has led to an increase in the plant weight and percentage of essential oil of marigold, therefore, the yield of essential oil, which is a result of the last two parameters, also increases. In accordance with the results of this research, Massey *et al.* (2021) observed that the use of appropriate amounts of vermicompost in the cultivation of lemongrass (*Cymbopogon flexuosus* L.) significantly increased the yield of essential oil. They stated that the addition of organic matter to the soil has increased the growth of aerial organs and the production of dry matter and finally improved the yield of essential oil. Kumar *et al.* (2019) also reported that the use of organic fertilizers in marigold increases the yield of essential oil. Regarding the positive effect of the use of humic acid on the percentage and yield of essential oil, it can be argued that humic acid, by increasing the activity of the Rubisco enzyme, increases the photosynthetic activity of the plant and, as a result, the production of photosynthetic products, and because essential oils belong to the chemical group of terpenes, and because glucose is considered as a suitable precursor in the synthesis of essential oil and especially monoterpenes, photosynthesis and the production of photosynthetic products are directly related to the production of essential oil (Chaupoo and Kumar, 2022). Sajjadi *et al.* (2020) reported that the percentage of essential oil increased as a result of seed inoculation with *Azotobacter chroococcum* and *A. lipoferum* bacteria due to the increase of N absorption by the plant.

CONCLUSION

In general, the use of vermicompost can help improve plant growth conditions by increasing organic matter and storing soil nutrients. The use of *Azospirillum* also increases the plant's access to nitrogen and other nutrients and stimulates plant growth, and the use of humic acid increases the concentration of chlorophyll, anthocyanin, growth characteristics, flowers weight, concentration and the yield of marigold essential oil.

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مطالعه اثر پرایمینگ بذر بر رشد و عملکرد اسانس همیشه‌بهار (*Calendula officinalis* L) تحت سطوح مختلف ورمی‌کمپوست

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چکیده

مطالعه حاضر با هدف بررسی اثر سطوح مختلف ورمی‌کمپوست بر رشد و عملکرد اسانس گیاه همیشه‌بهار تحت پرایمینگ بذر با آزوسپریلوم و هیومیک اسید تحت سطوح مختلف ورمی‌کمپوست بر رشد و عملکرد اسانس همیشه‌بهار، به‌صورت فاکتوریل بر پایه طرح بلوک‌های کامل تصادفی در مزرعه تحقیقاتی دانشگاه آزاد اسلامی واحد آستارا، انجام شد. تیمارهای آزمایش شامل پرایمینگ بذر شامل باکتری آزوسپریلوم (*Azospirillum brazilense*)، هیومیک اسید، آزوسپریلوم + هیومیک اسید و شاهد و سطوح مختلف ورمی‌کمپوست (صفر، ۵ و ۱۰ تن در هکتار)، بود. نتایج نشان داد که برخی از صفات مانند کلروفیل a، آنتوسیانین، عملکرد اسانس و عملکرد بیولوژیک تحت تاثیر برهمکنش ورمی‌کمپوست و پرایمینگ بذر، قرار گرفتند. اما ارتفاع بوته، تعداد دانه و گل، وزن گل، وزن دانه، مقدار اسانس و کلروفیل کل تحت تاثیر اثر اصلی ورمی‌کمپوست قرار گرفت اما پرایمینگ بذر روی برخی از این صفات معنی‌دار نبود. بیشترین مقدار کلروفیل a و کل، عملکرد بیولوژیک، عملکرد اسانس و وزن خشک گل‌ها از تیمار اسید هیومیک + آزوسپریلوم تحت کاربرد ۱۰ تن در هکتار ورمی‌کمپوست، به‌دست آمد.

کلید واژه‌ها: آنتوسانین، آزوسپریلوم، اسانس، همیشه بهار، ورمی‌کمپوست.

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تأثیر گونه‌های گیاهی زینتی در کاهش آلودگی و تصفیه هوای اماکن ورزشی

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تاریخ دریافت: ۰۹ دی ۱۴۰۲ تاریخ پذیرش: ۰۶ اسفند ۱۴۰۲

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چکیده

گیاهان زینتی یکی از ارزان‌ترین و طبیعی‌ترین عناصر برای تصفیه هوا و کاهش آلودگی هستند. توانایی این نوع از گونه‌های گیاهی در جذب آلاینده‌ها و تصفیه هوا غیرقابل انکار است. امروزه به‌دلیل صنعتی‌شدن و پیشرفت فن‌آوری، آلودگی هوا و محیط‌زیست به یک دغدغه مهم در کشورهای درحال توسعه تبدیل شده است. هدف از این تحقیق تأثیر گونه‌های گیاهی زینتی در کاهش آلودگی و تصفیه هوای اماکن ورزشی می‌باشد. آلاینده‌هایی که پاکیزگی هوای اماکن ورزشی را تحت تأثیر قرار می‌دهند، شامل: بنزن، فرمالدئید، منوکسیدکربن، تری‌کلرو اتیلن، آمونیاک، دود و زایلن می‌باشند. هشت گونه گیاهی شامل: پاپیتال، آلوئه‌ورا، پوتوس، نخل خرما، گیاه عنکبوتی، سانسوریا، سرخس بوستون و نخل خیزران برای تصفیه هوا و کاهش آلودگی آلاینده‌ها مورد بررسی قرار گرفتند. با توجه به نتایج تحقیق($P < 0.05$) ، اطلاعات لازم برای پیش‌بینی همبستگی تصفیه هوا با گونه‌های مختلف گیاهی از نظر آماری نشان داد که براساس مدل، هشت گونه گیاهی موردمطالعه با تصفیه هوا و کاهش آلودگی فضاهای ورزشی همبستگی طبیعی دارند و سانسوریا بیشترین تأثیر را در کاهش آلودگی و تصفیه هوای اماکن ورزشی دارد (بتا = ۰/۳۴۸، مقدار ارزش = ۰/۹۶۶). نتایج نشان داد که در هر نقطه از جهان، گونه‌های گیاهی موردمطالعه از مؤثرترین گونه‌ها در تصفیه هوا بوده و بنابراین برای خیابان‌ها، پارک‌ها و مکان‌های ورزشی مناسب هستند. پیشنهاد می‌شود که با استفاده از نتایج این تحقیق، مسئولان و دست اندرکاران مربوطه، توجه و بهره‌گیری بیشتری را در استفاده از گیاهان مذکور برای بهبود کیفیت هوای شهر و اماکن ورزشی داشته باشند.

کلید واژه‌ها: گیاهان زینتی، ورزش، مجموعه ورزشی، آب و هوا.

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بهبود رشد و برخی صفات فیزیولوژیک گازانیا تحت تنش شوری با محلول‌پاشی نانوذره اکسید تیتانیوم، کوانتوم دات -گرافن اکسید و اکسید سریم تحت تنش شوری

لمیا وجودی مهربانی[1]، اصغر ابراهیم زاده*[2]، مینا تونتاب قدیمی[2]، محمدباقر حسن پوراقدم[2]، فرزاد رسولی[2]
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تاریخ دریافت: ۰۸ دی ۱۴۰۲ تاریخ پذیرش: ۱۹ فروردین ۱۴۰۳

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چکیده

تولید و نگه‌داری گیاهان زینتی ارتباط تنگاتنگ با مصرف بالای آب تازه و شیرین را دارد. امروزه بدلیل محدودیت منابع آبی ناچار به آبیاری گیاهان با منابع آب شور هستیم. منظور بررسی تاثیر محلول پاشی نانوذره‌های اکسیدسریم، اکسید تیتانیوم و کوانتوم دات -گرافن اکسید (صفر و ۱/۵ میلی‌گرم در لیتر) تحت تنش شوری کلریدسدیم (صفر، ۷۵ و ۱۵۰ میلی‌مولار) در گیاه گازانیا، آزمایشی بصورت فاکتوریل بر مبنای طرح کاملا تصادفی اجرا شد. بیشترین وزن خشک برگ، تعداد گل و محتوای نیتروژن در تیمار بدون تنش شوری با محلول پاشی نانوذره کوانتوم دات- گرافن اکسید مشاهده شد. بیشترین محتوای سدیم، نشت یونی، پراکسید هیدروژن، پرولین و مالون دی آلدئید در تیمار تنش شوری ۱۵۰ میلی مولار کلریدسدیم در شرایط بدون محلول پاشی مشاهده شد. بیشترین محتوای کاتالاز در تیمار تنش شوری ۱۵۰ میلی مولار کلریدسدیم با محلول‌پاشی کوانتوم دات- گرافن اکسید مشاهده شد. فعالیت آنزیم های سوپراکسید دیسموتاز و اسکوربات پراکسیداز تحت تنش شوری ۱۵۰ میلی مولار کلریدسدیم با محلول پاشی کوانتوم دات-گرافن اکسید و نانوذره اکسید تیتانیوم افزایش یافت. شاخص کلروفیل، محتوای فنل کل و نسبت پتاسیم به سدیم تحت تاثیر اثرات مستقل تنش شوری و محلول‌پاشی قرار گرفت. تیمار بدون تنش شوری موجب افزایش نسبت پتاسیم به سدیم شد. بالاترین نسبت پتاسیم به سدیم، و شاخص کلروفیل در تیمار محلول‌پاشی با کوانتوم دات-گرافن اکسید مشاهده شد. افزایش در محتوای فنل کل در تنش شوری ۷۵ و ۱۵۰ میلی‌مولار کلریدسدیم مشاهده شد. محلول‌پاشی با هرسه نانوذره محتوای فنل کل گیاه را افزایش داد. نتایج حاصل از بررسی انجام شده نشان داد که تنش شوری تاثیر منفی بر برخی صفات رشدی و فیزیولوژیک گازانیا داشت. تیمار محلول پاشی مورد استفاده در بررسی حاضر در تنش شوری ۱۵۰ میلی مولار کلریدسدیم تاثیر مثبت بر فعالیت آنزیم های آنتی اکسیدانی و وزن خشک ریشه گیاه را داشت و مشخص شد که گیاه گازانیا قادر به تحمل تنش شوری ۷۵ میلی‌مولار کلریدسدیم می‌باشد.

کلید واژه‌ها: محتوای عناصر، آنزیم، گازانیا، مالون‌دی‌آلدئید، فنل.

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مهار زیستی پوسیدگی طوقه و ریشه توبا با استفاده از برخی باکتری‌های آنتاگونیست

شعیب قدیمی[1] و هادی رهاننده[2]*
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تاریخ دریافت: ۱۵ مرداد ۱۴۰۲ تاریخ پذیرش: ۰۲ اسفند ۱۴۰۲

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چکیده

بیماری پژمردگی و پوسیدگی ریشه و طوقه توبا با عامل *Fusarium oxysporum* یکی از بیماری‌های مهم در کشت توبا می‌باشد. در این پژوهش اثر هشت سویه باکتریایی *Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus subtilis, Bacillus velezensis, Pseudomonas flurescens, Pseudomonas koreensi* و *Pseudomonas putida* در مهار این بیماری در آزمایشگاه بررسی شد. در میان آن‌ها براساس آزمون کشت متقابل و مشاهده هاله بازدارندگی، دو استرین *B. velezensis* و *B. subtilis* به‌ترتیب با۹/۳۳ و ۵/۶ درصد بیشترین بازدارندگی را داشتند. در بررسی میزان تاثیر ترکیبات فرار ضد قارچی غیر همزمان *P. flurescens* با ۵۸/۳۳ درصد بیشترین بازدارندگی را داشت. ترکیبات فرار همزمان باکتری‌های *B. pumilus*، *P. flurescens*، *P. koreensi* و *B. megaterium* بطور کامل باعث کنترل عامل بیماری شدند. در بررسی تأثیر متابولیت‌های مایع خارج سلولی فیلتر شده روی رشد کلنی بیمارگر، مشاهده گردید که با افزایش غلظت متابولیت‌ها، درصد بازداری از رشد پرگنه بیمارگر توسط تمامی استرین‌های باکتریایی افزایش می‌یابد. بهترین استرین‌ها در مقابل *F. oxysporum* استرین‌های *B. subtilis*، *B. velezensis* و *B. pumilus* بودند که در غلظت ۲۵ درصد به‌ترتیب با ۵۰، ۷۲/۲۷ و ۸۶/۶۷ درصد، در غلظت ۱۵ درصد استرین *B. pumilus* با ۷۵/۶۶ درصد و استرین *B. velezensis* با ۴۶/۶۶ درصد بازدارندگی و در غلظت ۵ درصد استرین *B. pumilus* با ۴۴ درصد بیشترین بازداری از رشد پرگنه قارچی را به خود اختصاص دادند. در تست تولید پروتئاز در بررسی های به عمل آمده، تمامی جدایه‌ها قادر به تولید پروتئاز بودند. تنها استرین *P. fluorescent* قادر به تولید سیدروفور بود. در بررسی‌های میکروسکوپی تمامی استرین‌های مورد بررسی باعث تغییرات مورفولوژیکی و به هم تابیدگی قسمت‌های مختلف ریسه و تخریب شدند.

کلید واژه‌ها: آنتاگونیست، باکتری، پوسیدگی طوقه، فیوزاریم آکسیسپورم، توبا.

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تاثیر ۱- متیل سیکلوپروپن و متیل جاسمونات بر عمر پس از برداشت گل‌های شاخه بریده آلسترومریا رقم Calgary

عتیق الله صدیقی[1]، علی تهرانی فر[1*]، محمود شور[1]، حسین نعمتی[1]
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تاریخ دریافت: ۱۶ آذر ۱۴۰۲ تاریخ پذیرش: ۰۱ اردیبهشت ۱۴۰۳

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چکیده

آلسترومریا یکی از مهمترین محصولات گلکاری دنیا است که صفاتی مانند تنوع بسیار زیاد در رنگ، ارقام مختلف و زیبایی باعث موفقیت گل آلسترومریا در تجارت جهانی می‌باشد. استفاده از محلول‌های مختلف نگهدارنده گل در مراحل مختلف عرضه گل‌های بریدنی به بازار موجب جلوگیری از تولید و فعالیت اتیلن، فراهم نمودن آب و تأمین انرژی مورد نیاز گیاه پس از جدا شدن از پایه مادری شده که باعث افزایش عمر گل و حفظ کیفیت گل‌های بریدنی می‌گردد. بنابراین آزمایش بصورت فاکتوریل (۳×۳) بر مبنای طرح کاملا تصادفی با ۵ تکرار در هر تکرار یک شاخه گل در گروه علوم باغبانی دانشکده کشاورزی دانشگاه فردوسی مشهد انجام شد. در این پژوهش اثر ۱-متیل سیکلوپروپن و متیل جاسمونات بر عمر پس از برداشت گل‌های شاخه بریده آلسترومریا (کلگری) مطالعه شد. نتایج اثر اصلی کاربرد متیل جاسمونات نشان داد که بیشترین میزان جذب آب، وزن تر نسبی، عمر گلجای، کلروفیل a، b، آنتوسیانین، کاتالاز، پراکسیداز و سوپراکسیددیسموتاز در غلظت ۰/۲ میکرولیتر در لیتر متیل‌جاسمونات مشاهده شد. نتایج اثر اصلی کاربرد ۱-متیل سیکلوپروپن نشان داد که بیشترین میزان جذب آب، وزن تر نسبی، عمر گلجای، کلروفیل a، b، آنتوسیانین، کاتالاز، پراکسیداز و سوپراکسیددیسموتاز در غلظت ۱ میکرولیتر در لیتر ۱-متیل سیکلوپروپن مشاهده شد. نتایج اثر متقابل کاربرد متیل جاسمونات و ۱- متیل سیکلوپروپن نشان داد که جذب آب، وزن تر نسبی، عمر گلجایی، کلروفیل a وb، آنتوسیانین و آنزیم‌های آنتی‌اکسیدانی در تیمار ۴ درصد ساکارز + ۳۰۰ میلی گرم در لیتر ۸-هیدروکسی کوئینولین سولفات + ۱میکرولیتر در لیتر ۱- متیل‌سیکلوپروپن + ۰/۲ میکرولیتر در لیتر متیل جاسمونات بیشترین میزان بود.

کلید واژه‌ها: آنتوسیانین، کلروفیل، هیدروکسی کوئینولین، متیل‌جاسمونات،۱- متیل‌سیکلوپروپن.

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بررسی اثر تنش کم‌آبی بر رشد و برخی ویژگی‌های فیزیولوژی درخت زینتی سرخدار (*Taxus baccata* L.)

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چکیده

به‌منظور بررسی اثر تنش خشکی بر رشد و ویژگی‌های بیوشیمیایی سرخدار، تحقیقی در محیط گلخانه با ۴ سطح تنش خشکی (۱۰۰، ۷۵، ۵۰ و ۲۵ درصد ظرفیت زراعی) در سه تکرار در قالب طرح کاملا تصادفی انجام شد. در این تحقیق که از نهال ۳ ساله استفاده شد، نسبت وزن تر به خشک اندام هوایی و ریشه، کلروفیل a و b و نسبت این دو، محتوای نسبی آب برگ، فعالیت آنزیم سوپراکسید دیسموتاز و کاتالاز و درصد و عملکرد اسانس ارزیابی شد. نتایج نشان داد صفات رشدی و رنگیزه‌های فتوسنتزی و محتوای نسبی آب برگ با افزایش شدت تنش خشکی، کاهش پیدا کردند. البته فعالیت آنزیم‌های کاتالاز و سوپراکسید دیسموتاز با شدت تنش خشکی افزایش یافتند. بیشترین درصد اسانس در شرایط تنش خشکی نیمه شدید (۵۰ درصد ظرفیت زراعی) حاصل شد. با افزایش میزان تنش خشکی تا ۲۵ درصد ظرفیت زراعی درصد اسانس کاهش یافت. بیشتر شاخص‌های رشد و فیزیولوژی گیاه سرخدار در سطح ۷۵ درصد ظرفیت زراعی در مقایسه با ۱۰۰ درصد ظرفیت زراعی تفاوت معنی‌دار نداشت بنابراین به نظر می‌رسد این گیاه در شرایط ۷۵ درصد زراعی تحمل خوبی نشان دارد و از عملکرد قابل قبولی برخوردار است. از این رو توصیه می‌شود کشت و پرورش این گیاه ارزشمند در شرایط مساعد محیطی و آب کافی و نهایتا در شرایط تنش ملایم صورت گیرد.

کلید واژه‌ها: آنزیم‌های آنتی‌اکسیدانی، سرخدار، تنش اسمزی، اسانس.

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