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Research Article
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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

Lamia Vojodi Mehrabani[1], Asghar Ebrahimzadeh[*2], Mina Tontab Ghadimi[2], Mohammad Bagher Hassanpouraghdam[2], Farzad Rasoli[2]

[1]Department of Agronomy and Plant Breading, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran
[2]Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

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***Corresponding author's email:** acebrahimzadeh@gmail.com

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$_2$O$_2$, 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$_2$O$_2$ (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 ^{o}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).

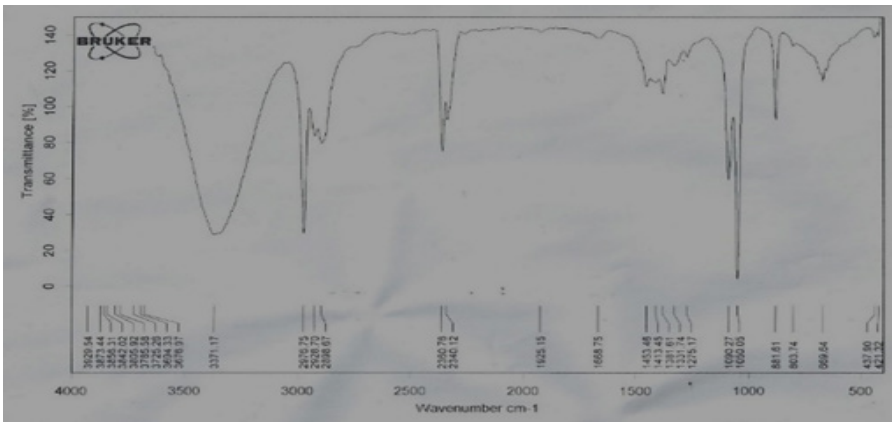

Fig. 1. FT-IR spectrum of CQDs.

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

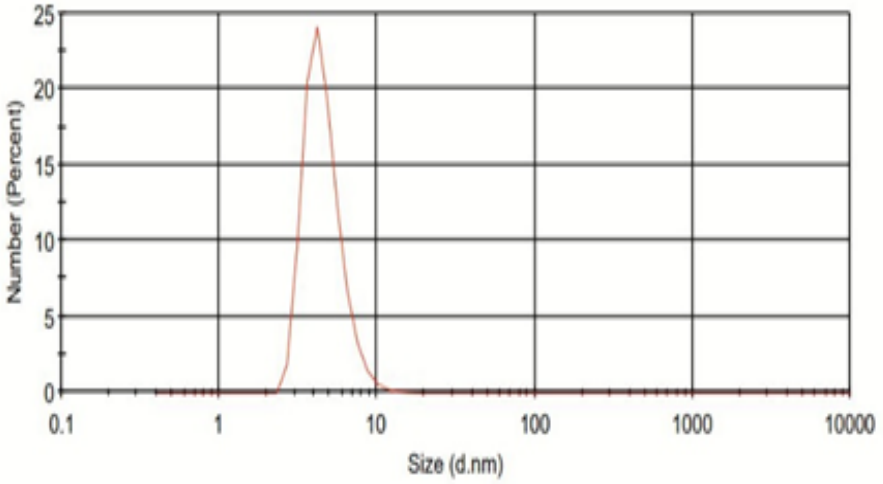

Fig. 2. Size distribution of CQDs.

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).

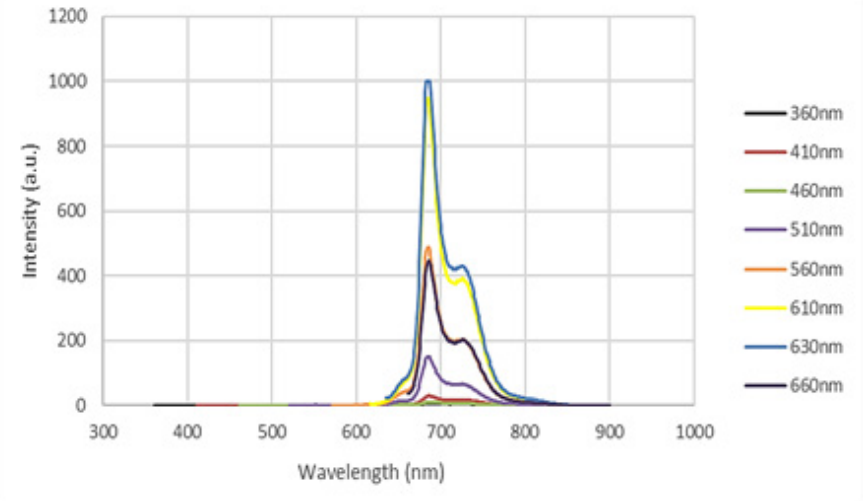

Fig. 3. The emission spectrum of carbon quantum dots at different excitation wavelengths.

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}	181de	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).

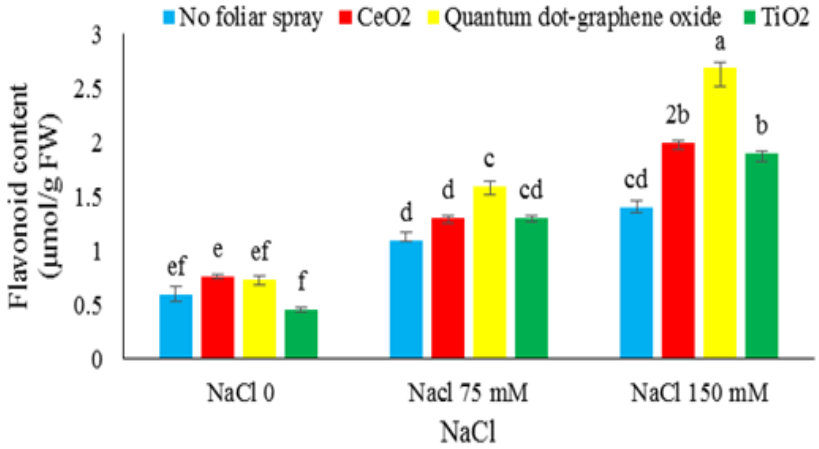

Fig. 4. Mean comparison for the interaction effects of salinity and foliar spray on flavonoids content of *Gazania splendens* L. In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

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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