

**Turkish Journal of Agriculture and Forestry** 

http://journals.tubitak.gov.tr/agriculture/

**Research Article** 

Turk J Agric For (2023) 47: 510-528 © TÜBİTAK doi:10.55730/1300-011X.3105

# Foliar application of graphene oxide, nano-Fe, and selenium mitigates salinity depression on Ocimum basilicum

Lamia VOJODİ MEHRABANİ<sup>1,\*</sup><sup>(0)</sup>, Mohammad Bagher HASSANPOURAGHDAM<sup>2</sup><sup>(0)</sup>, Farzad RASOULI<sup>2</sup><sup>(0)</sup>, Zühal OKCU<sup>3</sup>, Romina ALINA MARC<sup>4</sup>

<sup>1</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran <sup>2</sup>Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, Maragheh, Iran <sup>3</sup>Department of Gastronomy, Faculty of Tourism, Atatürk University, Erzurum, Turkey

<sup>4</sup>Food Engineering Department, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Received: 03.08.2022	•	Accepted/Published Online: 17.02.2023	٠	Final Version: 28.08.2023
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Abstract: An experiment was conducted to investigate the effects of salinity stress and foliar applications of graphene oxide, nano-Fe, and selenium on basil's growth and physiological responses. The results revealed that the treatments affected plant dry weight, essential oil, and malondialdehyde content, relative water content as well as hydrogen peroxide, proline, sodium, iron, and phosphorus content, and sodium to potassium ratio. The highest plant dry weight and essential oil content were recorded under no-salinity and 50 mM salinity × iron nanoparticle foliar use, while 100 mM salinity stress with no foliar spraying increased the levels of malondialdehyde, sodium content, and sodium-to-potassium ratio. The experimental treatments independently affected catalase activity and chlorophyll, calcium, nitrogen, potassium, manganese, zinc, and magnesium content. Foliar treatment of nanoparticles improved chlorophyll b, potassium, manganese and nitrogen content, and catalase activity. Under the control conditions and 50 mM salinity, the magnesium, calcium, and chlorophyll contents were enhanced. Graphene oxide and selenium foliar applications enhanced the calcium and magnesium content of plants. The foliar treatments were partially effective in mitigating the salinity defects under mild-stress conditions. Otherwise, with exposure to the salinity of 100 mM, the foliar treatments could not control the adverse effects of salt stress on the plant.

Key words: Graphene oxide, selenium, salinity, hydrogen peroxide

## 1. Introduction

Salinity is one of the most important abiotic stressors that retard the production of agricultural products in arid and semiarid regions of the world (Van Zelm et al., 2020). The excess accumulation of salts in the soil triggers disturbances in the morphophysiological traits, and ionic balance in plants (Munns and Tester, 2008; Zhao et al., 2019). The strength of stress damage on plants depends on the duration of exposure to salinity and salt concentration (Munns and Tester, 2008). The reactive oxygen species (ROS) are produced to play a role as a cellular signal and as secondary messengers in the signaling pathway of stress responses. Salt stress drastically reduced the growth and physiological characteristics of grapes. In contrast, the foliar spraying with iron and selenium nanoparticles and the soil-based application of graphene oxide improved grapes' growth and physiological attributes under salinity (Aazami et al., 2022).

Plants employ enzymatic and nonenzymatic mechanisms to face ROS overproduction. Under saline environments, the hastened entrance of Na<sup>+</sup> inside the cells and the raised Na<sup>+</sup>/K<sup>+</sup> ratio widely damage the cells' structure and function (Marschner, 1995).

The ever-expanding advances in nanotechnology have provided novel compounds to combat biotic and abiotic stress factors in plants (Latef et al., 2017). The size of nanoparticles varies between 1 and 100 nm. The high surface-to-volume ratio and unique physicochemical characteristics of nanoparticles have caused these particles to interact specifically with the plant and attained a wide application in the agricultural sector. Nanoparticles are able to adjust plant morphophysiological parameters under stress conditions (Das and Das, 2019; Hassanpouraghdam et al., 2019). The external application of nanoparticles increases plant growth, improves biochemical characteristics, and enhances the absorption of mineral



<sup>\*</sup> Correspondence: vojodilamia@gmail.com

elements in plants (Singh et al., 2021, Aazami et al., 2022; Hassanpouraghdam et al., 2019). The performance of nanoparticles in plants depends on their application method and dosage. The appropriate doses of those particles reduce the damage caused by oxygen free radicals and induce the proper signaling cascades in the plant to combat stress effects (Hossain et al., 2016).

Iron and graphene oxide nanoparticles are often used to improve plant growth under stress conditions. Iron plays a vital role in chloroplast structure, photosynthesis function, enzyme activity, hormonal regulation, and oxidation and reduction processes in plants (Marschner, 1995). Studies have shown that using iron under stress conditions reduced the production of oxygen free radicals by activating several mechanisms related to abiotic stress signaling in plants (Shah et al., 2021). In Moringa, salt stress caused a disturbance in the balance of nutrients in the plant. Still, the foliar iron nanoparticle application improved the absorption of nitrogen, phosphorus, potassium, magnesium, and iron (Soliman et al., 2015). Selenium is a beneficial element required by plants, which takes roles in the activity of antioxidant enzymes, photosynthesis, and potassium absorption (Marschner, 1995). The study on lettuce showed that selenium treatment under stress conditions improved the absorption of elements, balanced the ratio of several nutrients, and improved plant growth (Do Nascimento da Silva et al., 2017). Graphene oxide (GO) is a water-soluble compound that is widely used in the agricultural sector under stress conditions (Safikhan et al., 2018). In Silybum marianum (L.) Gaertn., graphene oxide foliar application under stress conditions improved the yield, chlorophyll content, and cell membrane stability (Safikhan et al., 2018).

Basil is an herbaceous plant from the family Lamiaceae that originated from the Asian continent. Basil is considered an aromatic and medicinal plant with high antioxidant and antimicrobial properties widely used in the food, pharmaceutical, and perfumery industries (Barbieri et al., 2012). Chemical constituents such as anthocyanin, linalool, methyl eugenol, eugenol, and 1,8-cineole are responsible for the above-mentioned activities (Purushothaman et al., 2018). The essential oil contains mono- and sesquiterpenes, which are used in treating diseases such as diarrhea, cough, headache, warts, cardiovascular disorder, diabetes, and kidney failure (Ghasemi Dehkordi, 2002; Purushothaman et al., 2018).

This study, for the first time, aims to evaluate the effects of the concurrent foliar application of selenium, iron nanoparticles, and graphene oxide on the growth and physiological responses of basil plants to smoothen the salinity depression.

## 2. Materials and methods

This experiment was conducted during the spring and summer of 2021 at the Research Greenhouse of Azarbaijan

Shahid Madani University, Tabriz, Iran. The greenhouse environmental conditions were; the lightning period: 16:8 day and night, temperature regime, 30:25 °C day and night, and the relative humidity of about 70%.

# 2.1. Reagents and materials for the synthesis of graphene oxide (GO)

All chemicals used were of analytical grade.  $NaNO_3$ ,  $KMnO_4$ , and graphite powder (50 meshes) were acquired from Merck (Germany). Ethanol was purchased from Hamon Teb Markazi (Zarandieh, Iran).

### 2.1.1. Instrumentation

Fourier transform infrared (FTIR) spectrum of the graphene oxide (GO) was recorded on a Vector 22 (Bruker, Ettlingen, Germany) FTIR using KBr as the mulling agent and X-ray diffraction analysis (XRD) of powders was carried out on Bruker D8 Advance (Bruker AXS, Karlsruhe, Germany) instrument with Cu-K<sub>a</sub> radiation source (1.54 Å) between 8° and 80° generated at 40 kV and 35 mA at room temperature (25 °C). In addition, the morphology of the GO was observed under a scanning electron microscope (SEM, model MIRA3, Tescan, Czech Republic).

## 2.1.2. Synthesis of graphene oxide

Graphene oxide was prepared from graphite powders using the modified Hummers' method. This modified method is commonly utilized in the preparation of graphene oxide (Shahriary and Athawale, 2014). In brief, 10 g of graphite powder and 5 g of NaNO<sub>3</sub> were put in 230 mL of cont.  $H_2SO_4$  and  $KMnO_4$  (30 g) were then gradually added to the solution with stirring and cooling to prevent overheating and explosion. After adding KMnO<sub>4</sub>, the solution was allowed to stand overnight at room temperature (25 °C). The next day, the solution was poured into 500 mL of H<sub>2</sub>O and heated at about 100 °C, and the reaction was allowed to progress for a certain time. Finally, the formed graphite oxide was washed with ethanol and dried at 65 °C for 24 h in an oven (Nakajima et al., 1988). Later, the graphite oxide is subjected to ultrasound irradiation for 2 h to obtain the GO.

# 2.2. Reagents and materials for the synthesis of $\text{Fe}_3\text{O}_4$ magnetic nano-particles (Fe $_3\text{O}_4$ MNPs)

All chemicals (iron (III) chloride hexahydrate and iron (II) sulfate heptahydrate) were obtained from Merck (Darmstadt, Germany).

#### 2.2.1. Instrumentation

FT-IR spectrum of Fe<sub>3</sub>O<sub>4</sub> nanoparticles was recorded on a Vector 22 (Bruker, Ettlingen, Germany) instrument using KBr as the mulling agent. X-ray diffraction analysis (XRD) of Fe<sub>3</sub>O<sub>4</sub> nanoparticles was done on Bruker D8 Advance (Bruker AXS, Karlsruhe, Germany) instrument with Cu-K $\alpha$  radiation source (1.54 Å) between 8° and 80° generated at 35 mA and 40 kV at (25 °C) room temperature.

## 2.2.2. Synthesis of Fe<sub>3</sub>O<sub>4</sub> MNPs

For the preparation of  $Fe_3O_4$  MNPs; 50 mL of deionized water was degassed into an ultrasonic bath for 10 min, and 4.86 g FeCl<sub>3</sub> 6H<sub>2</sub>O, and 3.34 g FeSO<sub>4</sub> 7H<sub>2</sub>O were added. The solution was heated at 100 °C and vigorously stirred to dissolve the iron salts, and 12 mL of concentrated ammonia solution was rapidly added under vigorous stirring and allowed to complete the reaction (2 h) and to form a black iron oxide MNPS. Fe<sub>3</sub>O<sub>4</sub> MNPs were cooled at room temperature and collected using a magnet. Later, Fe<sub>3</sub>O<sub>4</sub> MNPs were first washed with a mixture of ethanol: water (50:50 v/v) and then with pure ethanol. Finally, Fe<sub>3</sub>O<sub>4</sub> MNPs were dried at 80 °C for 5 h in an oven.

## 2.3. Plant material and experimental setup

Ocimum basilicum seeds were surface-sterilized with sodium hypochlorite (10%) for 5 min, followed by three times washing with distilled H<sub>2</sub>O. The seeds (local clone supplied by Pakan Bazr Seed Company, Esfahan, Iran) were planted in 5-L pots containing medium-sized perlite. The plants were nourished with half-strength Hoagland's nutrient solution (Malhotra et al., 2014) during the early growth stages and acclimation. When plants had three real leaves, salinity treatments were imposed. The salinity levels were 0, 50, and 100 mM of NaCl. Salinity levels began at 25 mM and gradually increased to reach the final level within 10 days (Chrysargyris et al., 2018). The optimal pH of nutrient solution (NS) was 5.8, recorded daily, and adjusted accordingly using  $H_2SO_4$  (5% v/v). Following the salinity application, the EC of the nutrient solution was 2.1 mS cm<sup>-1</sup> (0 mM NaCl), 6.0 mS cm<sup>-1</sup> (50 mM NaCl), and 12.0 mS cm<sup>-1</sup> (100 mM NaCl). The pots were regularly washed with tap water once every week to avoid the salinity stand-up on the pot surfaces. Foliar treatments included: dH<sub>2</sub>O, nanographene (0.05 g L<sup>-1</sup>) (Hassanpouraghdam et al., 2022), Si, and nano-Fe (3 mg L<sup>-1</sup>) (Hassanpouraghdam et al., 2023; Aazami et al., 2022). Foliar applications were applied twice. The first application was just after salinity exposure (3 leaves stage), and the second foliar spray was 2 weeks later. The plant samples were further analyzed one month after the second foliar treatment. The experiment consisted of 12 treatment combinations and three replications. Each experimental unit had three pots. In total, the experiment was composed of 108 pots. The pots were spaced 30 cm between rows and 20 cm within rows. The pots were manually irrigated with 250 mL of nutrient solution every 4 days.

## 2.3.1. The dry weight of plants

The dry biomass of plant organs was recorded on a digital scale (BB141, Boeco, Germany). After harvesting, the plants above and below ground parts were separated and air-dried at the ambient temperature of about 25 °C until reaching a constant weight. The final moisture content of plant material was about 10%–12% which was determined by oven drying.

## 2.3.2. Essential oil (EO) extraction

To extract the *Ocimum basilicum* L. essential oils, 50 g of the air-dried (at room temperature of 25 °C) aerial parts were partially grounded and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus (British Pharmacopoeia model). To remove the possible water drops in the essential oils, the samples were dehydrated over anhydrous sodium sulfate (0.5 g) and then kept at 4 °C. The essential oil content (EO%) was calculated considering dry mass (Hassanpouraghdam et al., 2022).

## 2.3.3. Total soluble solids content (°Brix)

Total soluble solids (TSS) content of leaves (1 g for each replication) was recorded by a hand refractometer (Erma, Tokyo, Japon), and the data are presented as °Brix.

## 2.3.4. Relative water content (RWC)

The relative water content was calculated from the equation: RWC (%) =  $100\% \times (FW - DW) / (TW - DW)$ ,

where FW: DW, and TW refer to leaf fresh, dry, and turgid weight, respectively (Smart and Bingham 1974).

## 2.3.5. Chlorophyll content

Leaf samples (0.2 g for each replication) were extracted by dimethyl sulfoxide (DMSO, Sigma Aldrich, Germany) with 80% acetone in the dark for 4 h at 65 °C until the tissue became colorless. chlorophylls a (Chl a) and b (Chl b) contents were quantified with the method described by Shinano et al. (1996) at 665 and 648 nm.

**2.3.6.** Hydrogen peroxide content and lipid peroxidation The content of  $H_2O_2$  was assessed according to Alexieva et al. (2001). Next, 0.2 g of leaf tissue was powdered in liquid  $N_2$  and then grounded in ice-cold 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min. An aliquot (0.5 mL) 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<sup>-1</sup> fresh weight.

Lipid peroxidation was determined as described by Heath and Packer (1968) regarding malondialdehyde (MDA) content. Leaf tissue (0.2 g) was homogenized in 10 mL of 0.1% TCA (trichloroacetic acid,) and the extract was centrifuged at 15000 g for 5 min. 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added to 1 mL of the supernatant and incubated at 95 °C for 30 min and then cooled on an ice bath. After centrifugation at 10,000 × g for 10 min, the absorbance was determined at 532 nm and corrected for nonspecific absorbance at 600 nm. The MDA amount was determined using the extinction coefficient of 155 mM cm<sup>-1</sup>. Results were expressed as nmol of MDA g<sup>-1</sup> fresh weight.

## 2.3.7. Catalase (CAT) activity

First, 0.5 g of basil leaf sample was homogenized with 0.1 M cold potassium phosphate buffer (pH: 7.5) and 0.5 mM EDTA based on the method of Luhova et al. (2003). From the resulting supernatant, 0.05 mL 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 hydrogen peroxide, and a decrease in absorption was recorded at 240 nm for 1 min (Luhova et al., 2003).

## 2.3.8. Proline content

Five milliliters of 3% homogenized sulfosalicylic acid was added to 0.2 g of leaf sample. The extract was centrifuged at 6000 rpm for 7 min. Next, 1 mL of supernatant was mixed with the same volume of ninhydrin acid and 1 mL of glacial acetic acid. Later, the samples were incubated in a 100-°C water bath and then in an ice bath for 30 s. After 30 min, the red phase formed above the sample was used for the proline content measurements based on Fedina et al. (2006) at 520 nm. The proline content was computed using a standard curve of proline, and the results were expressed as micromole of proline per gram of plant fresh weight.

## 2.3.9. Elemental composition

Leaf (3 replications/treatment) of basil plant was dried at 75 °C for a day, weighted, and grounded in a Wiley mill to particles less than 0.42 mm. Subsamples (0.2–0.3 g) were acid digested (2 N HCl) and analyzed for nutrient content as described by Chrysargyris et al. (2018). The flame photometric method quantified Na and K content (Corning, 410, England). The content of Mn, Mg, Zn, Ca, and Fe was measured by atomic absorption spectroscopy (Shimadzu, AA6300, Tokyo, Japan) as previously described by Honarjoo et al. (2013), phosphorus by Vanadate Molybdate, and N content by the Kjeldahl method (Honarjoo et al., 2013).

## 2.3.10. 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 MSTAT-C v. 2.1. Also, the significant differences among means were evaluated with the least significance difference test (LSD) at p < 0.05 and p < 0.01. Pearson's correlation and cluster dendrogram heat maps were depicted in R software (R foundation for statistical computing, version 4.1.2).

## 3. Results

## 3.1.1. Characterization of GO

The FT-IR spectrum of GO (Figure 1) shows a broad peak around 3400 cm<sup>-1</sup> in the high-frequency area corresponding to the stretching vibration of OH groups of water molecules adsorbed on graphene oxide. The peaks at 2923 cm<sup>-1</sup> and 2853 cm<sup>-1</sup> represent the symmetric and antisymmetric stretching vibrations of CH<sub>2</sub>, while the presence of the peak observed in the medium frequency area at 1628 cm<sup>-1</sup> can be attributed to the stretching vibration of C=C and C=O of carboxylic acid groups present at the edges of graphene oxide (Shahriary and Athawale, 2014). Finally, the peak at 1384 cm<sup>-1</sup> corresponds to the stretching vibration of C-O of carboxylic acid. The presence of these oxygen-containing groups reveals that the graphene has been oxidized (Figure 2).

The SEM image of synthesized GO is given in Figure 3. From the figure, it can be observed that graphene oxide has a layered structure, which affords ultrathin

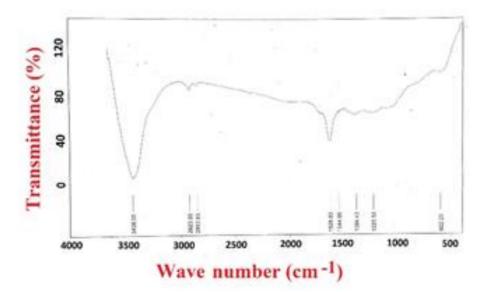


Figure 1. FT-IR spectrum of graphene oxide.

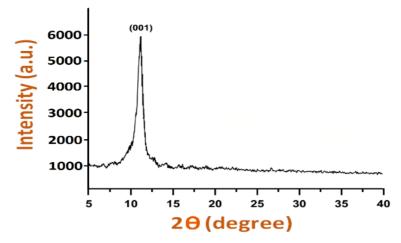


Figure 2. XRD pattern of graphene oxide.

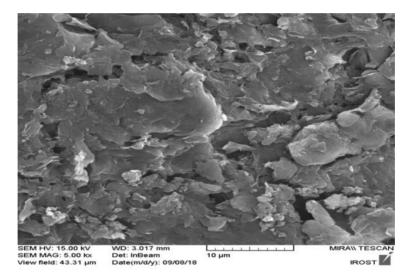


Figure 3. SEM image of graphene oxide.

and homogeneous graphene oxide films. SEM image of synthesized GO shows that the size of GO flakes was about 50 nm.

### 3.1.2. Characterization of Fe<sub>3</sub>O<sub>4</sub> MNPs

The FT-IR spectrum of Fe<sub>3</sub>O<sub>4</sub> MNPs (Figure 4a) shows a strong peak at around 582 cm<sup>-1</sup>, possibly related to the Fe–O bond in Fe<sub>3</sub>O<sub>4</sub>. This peak was shifted to a high wavenumber compared to the Fe–O bond peak of bulk magnetite at 570 cm<sup>-1</sup> due to NPs size. Therefore, this verifies that Fe<sub>3</sub>O<sub>4</sub> MNPs have been successfully synthesized. Moreover, Figure 4b shows the X-ray diffraction pattern (XRD) of Fe<sub>3</sub>O<sub>4</sub> MNPs. The diffraction peaks in 2 $\theta$  region of 5°–80° (30.007°, 35.601°, 43.239°, 53.782°, 57.372°, and 63.058°), which are marked by their indices (220, 311, 400, 422, 511, and 440), confirmed the formation of Fe<sub>3</sub>O<sub>4</sub> MNPs (Camel, 2003).

## 3.2. Dry weight of the plant

The dry weight of root and aerial parts was influenced by the interaction effects of salinity stress and foliar treatments (Table 1). Based on the results, the treatment without salt stress  $\times$  iron nanoparticle foliar application increased the root dry weight. The foliar treatment with iron nanoparticles under no salinity stress and 50 mM salinity enhanced the dry weight of the aerial parts (Table 2). Under 100 mM salinity stress  $\times$  without foliar spraying, the dry weight of the root and aerial parts decreased. There was also a difference between the foliar treatments regarding salinity stress levels. Besides iron nanoparticles, selenium played an influential role in controlling salinity stress compared to graphene oxide. Moreover, selenium, compared to graphene nanoparticles, increased the dry weight of the plant by up to 16% under no salinity.

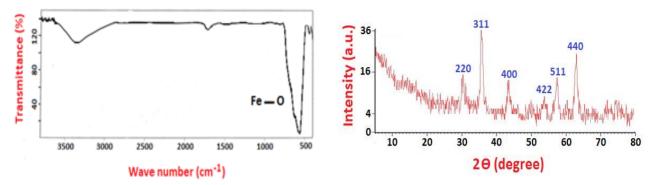


Figure 4. FT-IR spectrum (left) and XRD pattern (right) of Fe<sub>3</sub>O<sub>4</sub> MNPs, respectively.

**Table 1.** ANOVA for the effects of salinity (0–50 and 100 mM NaCl) and foliar applications (no-foliar, graphene oxide, selenium, and nano-Fe) on *Ocimum basilicum* growth characteristics.

Significant	Plant dry weight	Root dry weight	Plant height		Total soluble solids content	Relative water content	Chlorophyll a content	Chlorophyll b content
Salinity (S)	**	**	**	**	**	**	**	**
Foliar (F)	**	**	**	**	**	**	**	**
S×F	**	**	ns	**	ns	**	ns	ns

Table 2. Mean comparisons for the interaction effects of salinity and foliar applications on Ocimum basilicum growth characteristics.

Salinity	Foliar spray	Plant dry weight (gm <sup>-2</sup> )	Root dry weight (gm <sup>-2</sup> )	Essential oil content (%)	Relative water content
0	No-spray	286 ± 0.07e	$4.4 \pm 0.00$ d-f	$0.10 \pm 1.02d$	86 ± 0.81ab
0	Graphene oxide	311 ± 0.09d	5.0 ± 0.01cd	0.13 ± 1.41bc	91 ± 0.47a
0	Selenium	$372 \pm 0.09b$	$6.5 \pm 0.00b$	0.11 ± 1.24cd	89 ± 0.47a
0	Nano-Fe	409 ± 0.13a	8 ± 0.009a	0.18 ± 1.69a	91 ± 0.47a
50	No-spray	241 ± 0.24g	$3.4 \pm 0.00 h$	0.13 ± 1.17bc	78 ± 0.81e
50	Graphene oxide	300 ± 0.12e	$4.3 \pm 0.00 \text{ef}$	0.13 ± 1.00c	80 ± 0.47cd
50	Selenium	345 ± 0.12c	$4.9 \pm 0.00 \text{ef}$	$0.15 \pm 0.04b$	87 ± 0.47b
50	Nano-Fe	394 ± 0.04a	5.2 ± 0.0c-e	0.18± 0.08a	83 ± 0.81bc
100	No-spray	$207 \pm 0.04 h$	$2.4 \pm 0.00i$	0.07± 0.94e	60 ± 0.81g
100	Graphene oxide	229 ± 0.08g	3.5 ± 0.00gh	0.12 ± 0.93cd	$69 \pm 0.47 f$
100	Selenium	$265 \pm 0.14 f$	3.9 ± 0.00f-h	$0.11 \pm 0.47 d$	73 ± 0.47f
100	Nano-Fe	$244 \pm 0.12g$	4 ± 0.00fg	0.11 ± 0.81cd	73 ± 0.47ef

Significant differences among treatments are indicated by the different Latin letters.

Selenium application led to a 13% increase in plant dry weight compared to graphene under the salinity of 50 and 100 mM (Table 2).

## 3.3. Plant height

The independent effects of treatments affected plant height (Table 1). Salinity stress decreased the height of plants. The

top plant height was observed in the treatment without salt stress. By increasing the salinity to 50 and 100 mM, the height of the plant decreased by 11% and 30% compared to the control (Table 3). Foliar spray with iron and selenium nanoparticles improved plant height compared to the control and graphene oxide treatments (Table 4).

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Salinity	Plant height Total soluble solids		Chlorophyll a content	Chlorophyll b content	Catalase activity	
(mM)	(cm)	content (°Brix)	$(mg g^{-1} FW)$	$(mg g^{-1} FW)$	(unit mg <sup>-1</sup> protein min <sup>-1</sup> )	
0	$34 \pm 0.08a$	$1.4 \pm 0.12a$	$1.2 \pm 0.14a$	0.78 ± 0.12a	$41 \pm 0.04a$	
50	30 ± 0.30b	$1.2 \pm 0.08b$	$0.94 \pm 0.12b$	0.61 ± 0.16a	39 ± 0.09a	
100	24 ± 0.26c	1.0 ± .09c	$0.75 \pm 0.30c$	0.39 ± 0.18b	$28 \pm 0.04b$	

 Table 3. Mean comparisons for the effects of salinity on some physiological traits of Ocimum basilicum.

Significant differences among treatments are indicated by the different Latin letters.

Table 4. Mean compressions for the effect of foliar application on some physiological traits of Ocimum basilicum plants.

Foliar application	Plant height (cm)	Total soluble solids content (°Brix)	Chlorophyll a content (mg g <sup>-1</sup> FW)	Chlorophyll b content (mg g <sup>-1</sup> FW)	Catalase activity (unit mg <sup>-1</sup> protein min <sup>-1</sup> )
No-foliar	$25 \pm 0.07c$	$1.0 \pm 0.07c$	0.7 ± 0.22c	0.54 ± 0.18a	33 ± 0.23b
Graphene oxide	29 ± 0.09b	1.1 ± 0.19bc	$0.9 \pm 0.14b$	0.56 ± 0.33a	37 ± 0.35a
Selenium	30 ± 0.21ab	$1.2 \pm 0.08b$	$1.0 \pm 0.08b$	0.59 ± 0.14a	38 ± 0.19a
Nano-Fe	32 ± 0.18a	1.4 ± 0.00a	1.2 ± 0.10a	0.70 ± 0.18a	35 ± 0.27ab

Significant differences among treatments are indicated by the different Latin letters.

# 3.4. Essential oil content

Foliar iron nanoparticles application under no salt stress and 50 mM salt stress increased the essential oil content of plants. The least essential oil content was obtained with no foliar treatment  $\times$  salinity stress of 100 mM. From a statistical point of view, no difference was observed between foliar spraying with graphene and iron nanoparticles in the essential oil content under 100 mM salinity stress (Table 2).

## 3.5. Total soluble solids content (TSS)

TSS content was the highest under no-stress conditions. By increasing the salinity stress to 50 and 100 mM, TSS decreased (Table 3). There was no difference in TSS content between no-foliar treatment and foliar spraying with graphene nanoparticles. Selenium treatment increased the content of soluble solids by 10% compared to graphene treatment. However, the highest TSS content was observed in the foliar treatment with iron nanoparticles, which showed an increase of up to 28% compared to the control (Table 4).

# 3.6. Relative water content (RWC)

The highest RWC was recorded under no salinity stress, no foliar spraying, no stress  $\times$  foliar graphene oxide, selenium, and iron use. Foliar graphene and iron nanoparticles use caused a 5% increase in RWC compared to the control. In the salinity of 50 and 100 mM  $\times$  without foliar applications, the RWC of the leaves decreased. Under the stress mentioned

above, foliar treatments effectively increased the relative water content of leaves. In 100 mM salinity, the foliar application of selenium and iron nanoparticles increased the RWC by 18% compared to the treatment without foliar application at the same salinity level (Table 2).

# 3.7. Chlorophyll content

The independent effects of experimental treatments influenced chlorophyll a and b content in basil (Table 1). The highest chlorophyll a content was observed in no-saline conditions. Under no-saline and 50 mM salinity, chlorophyll b content increased, while 100 mM salt stress decreased the content of chlorophyll a and b (Table 3). No difference in chlorophyll b content was observed between foliar treatments. However, a difference was observed between the foliar treatments for chlorophyll a content. The highest chlorophyll a content was recorded in the foliar treatment with iron nanoparticles. For chlorophyll a content, no difference was observed between selenium and graphene foliar treatments, and the lowest chlorophyll a content was recorded for the conditions without foliar application (Table 4).

## 3.8. Hydrogen peroxide content

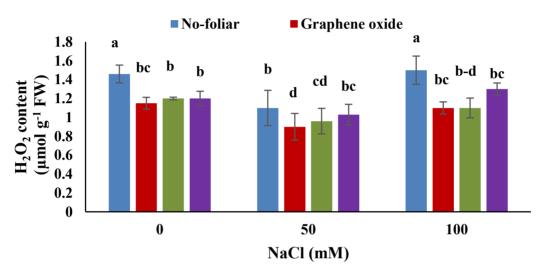
The interaction effects of salinity and foliar application influenced  $H_2O_2$  content of the plant (Table 5). The higher hydrogen peroxide content was traced under no salt stress and, 100 mM stress without foliar spraying. The foliar treatments at all salinity stress levels reduced the hydrogen peroxide content (Figure 5).

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Significant	H <sub>2</sub> O <sub>2</sub> content	MDA content	Catalase activity	Proline content
Salinity (S)	**	**	**	**
Foliar (F)	**	**	**	**
S×F	**	**	ns	**

Table 5. Table 5. ANOVA for the effects of salinity and foliar applications on some physiological traits of Ocimum basilicum.

Significant effects for the main factors and their interaction are indicated with asterisk: \* p < 0.05; \*\* p < 0.01; ns: nonsignificant.



**Figure 5.** Mean comparisons for the interaction effects of salinity and foliar applications on H<sub>2</sub>O<sub>2</sub> content of *Ocimum basilicum*. Significant differences among treatments are indicated by the different Latin letters.

## 3.9. Malondialdehyde content

Malondialdehyde content was increased by adding up the salinity stress to 100 mM under no foliar treatment. Foliar treatment with all three compounds under 50 and 100 mM salinity stress positively reduced malondialdehyde content. Foliar graphene oxide, selenium, and iron nanoparticles treatments caused 10%, 15%, and 22% decrease in malondialdehyde content under 100 mM salinity stress, respectively (Figure 6).

## 3.10. Catalase activity

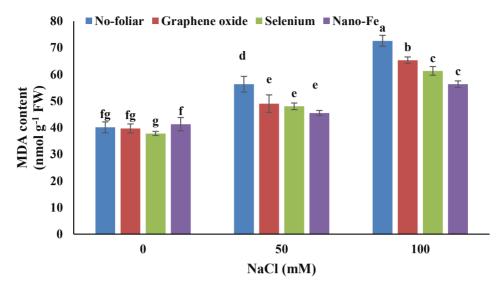
By the salinity stress of up to 100 mM, the catalase activity decreased in basil leaves. There was no difference in catalase activity between the treatments without salt stress and 50 mM salt stress (Table 3). The minor catalase activity was observed in the condition without foliar spraying. All three foliar applications increased catalase activity. Although, there was no statistical difference in the catalase activity between the three foliar treatments, it seems that the effect of selenium treatment on the activity of this enzyme was higher than that of the others (Table 4).

## 3.10. Proline content

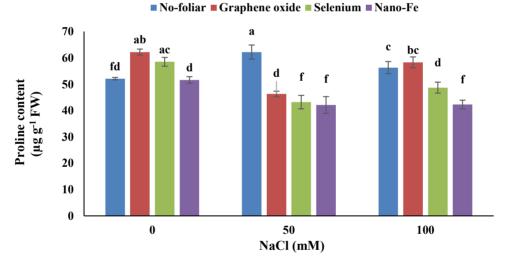
Graphene oxide and selenium × without salinity stress and, 50 mM salinity × without foliar application increased the proline content of the plant. Under 50 mM salt stress, the foliar treatments decreased proline content. However, in the treatment of 100 mM salinity × graphene oxide, proline content increased by 4% compared to the treatment without foliar spraying. Foliar selenium and iron treatment × 100 mM salinity stress decreased proline content up to 13% and 25% compared to 100 mM salinity × without foliar spraying (Figure 7).

## 3.11. Nitrogen and potassium content

The nitrogen and K content of plants was influenced by the independent effects of experimental treatment (Table 6). Salinity stress decreased nitrogen and potassium content. The highest content of both elements was recorded under no salt stress conditions. By the salinity stress of 100 mM, nitrogen and potassium content decreased up to 37% and 35% compared to the control, respectively (Table 7). The foliar spraying positively affected the plant's nitrogen



**Figure 6.** Mean comparisons for the interaction effects of salinity and foliar applications on MDA content of *Ocimum basilicum*. Significant differences among treatments are indicated by the different Latin letters.



**Figure 7.** Mean comparisons for the interaction effects of salinity and foliar applications on proline content of *Ocimum basilicum*. Significant differences among treatments are indicated by the different Latin letters.

Table 6. ANOVA for the effects of salinity and foliar applications on Ocimum basilicum elemental content.

Significant	N	Р	K	Ca	Mg	Na	Na/K	Fe	Zn	Mn
Salinity (S)	**	**	**	**	**	**	**	**	**	**
Foliar (F)	**	**	**	**	**	**	**	**	**	**
S×F	ns	**	ns	ns	ns	**	**	**	ns	ns

Significant effects for the main factors and their interaction are indicated with asterisk: \* p < 0.05; \*\* p < 0.01; ns: nonsignificant

and potassium content. The lowest potassium content was observed in the control treatment. No difference in potassium content was recorded between foliar graphene oxide and selenium treatment (Table 8). In contrast, foliar use of iron nanoparticles caused a 13% decrease in potassium content compared to the other two treatments. Foliar iron nanoparticles treatment led to a 25% increase in nitrogen content compared to the control. Also, foliar iron application caused a 7% and 10% increase in nitrogen content compared to selenium and graphene oxide treatment (Table 8).

## 3.12. Phosphorus content

Phosphorus content was affected by the interactions of salinity and foliar treatments (Table 6). The highest phosphorus content was observed in no salinity × graphene oxide and iron nanoparticles. By the salinity of 50 and 100 mM, the phosphorus content of the plants was reduced. However, the foliar application with iron, graphene oxide, and selenium decreased the adverse effects of the stress and enhanced the phosphorus content. Foliar application of graphene oxide reduced phosphorus content in plants compared to selenium and iron. The least phosphorus content was measured in 100 mM salinity × iron. In the salinity of 100 mM, foliar treatment with graphene oxide caused a 42% increase in phosphorus content compared to 100 mM salinity × iron foliar spraying (Figure 8).

## 3.13. Calcium and magnesium content

The salt stress of 50 and 100 mM decreased the calcium and magnesium content of plants. No salinity conditions

increased the content of the mentioned elements (Table 7). The foliar treatments with graphene and selenium increased magnesium and calcium content. Regarding calcium content, no difference was observed between the foliar iron treatment and the control (Table 8).

## 3.14. Iron content

The foliar iron nanoparticles treatment under no salinity influenced the iron content of the plant (37% increase in iron content compared to the control). By increasing the salinity stress up to 50 and 100 mM under no foliar treatments, the iron content of the plant decreased, and the lowest iron content was observed in 100 mM salinity × without foliar application. Under 50 and 100 mM salinity, foliar treatment with iron increased the iron content to 42 and 32 mg kg<sup>-1</sup> of dry weight, indicating the treatment's effectiveness in improving the iron content compared to the selenium and graphene oxide treatments (Figure 9).

## 3.15. Manganese and zinc content

No salinity and 50 mM salinity stress increased the manganese content compared to 100 mM salinity (Table 7). The foliar treatments enhanced manganese content compared to the control (Table 8). Salinity stress of 50 and 100 mM decreased zinc content in the plant. The highest zinc content was observed in the control treatment (Table 7). Foliar treatment with graphene oxide and selenium increased zinc content compared to the control and foliar iron nanoparticle use.

Table 7. Mean compression for salinity effect on Ocimum basilicum elemental content.

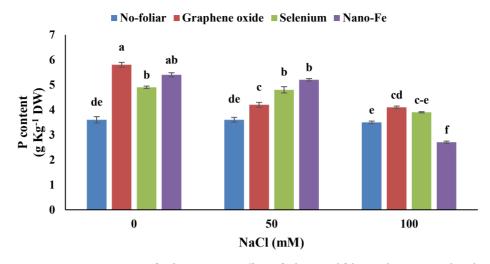
Salinity	N content (g kg <sup>-1</sup> )	K content (g kg <sup>-1</sup> )	Ca content (g kg <sup>-1</sup> )	Mg content (g kg <sup>-1</sup> )	Mn content (mg kg <sup>-1</sup> )	Zn content (mg kg <sup>-1</sup> )
0	13.3 ± <b>0.18a</b>	12.9 ± <b>0.19a</b>	3.7 ± <b>0.28</b> a	1.4 ± <b>0.1</b> a	7.8 ± <b>0.06</b> a	4.2 ± 0.08a
50	10.8 ± <b>0.11</b> b	9.9 ± 0.09b	$2.2\pm \textbf{0.14}b$	1.2 ± <b>0.4</b> b	7.4 ± <b>0.07</b> a	3.5 ± 0.01b
100	8.4 ± <b>0.12</b> c	8.1 ± <b>0.15</b> c	2.2 ± 0.11b	0.89 ± <b>0.09</b> c	5.8 ± <b>0.08</b> b	3.07 ± 0.05b

Significant differences among treatments are indicated by the different Latin letters.

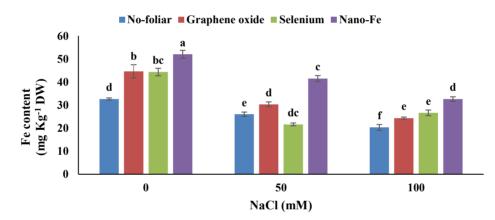
Table 8. Mean comparisons for the effect of foliar application on elemental content of Ocimum basilicum plants.

Foliar application	N content (g kg <sup>-1</sup> )	K content (g kg <sup>-1</sup> )	Ca content (g kg <sup>-1</sup> )	Mg content (g kg <sup>-1</sup> )	Mn content (mg kg <sup>-1</sup> )	Zn content (mg kg <sup>-1</sup> )
No-foliar	9.1 ± <b>0.011</b> b	9.2 ± <b>0.02</b> b	2.3 ± <b>0.11</b> b	1.1 ± <b>0.07</b> b	6.0 ± <b>0.09</b> b	3.5 ± <b>0.07</b> b
Graphene oxide	10.6 ± <b>0.07</b> a	11.2 ± <b>0.07</b> a	2.9 ± <b>0.13</b> a	1.3 ± <b>0.09</b> a	7.3 ± <b>0.18</b> a	4.2 ± <b>0.06</b> a
Selenium	11.3 ± <b>0.08</b> a	11 ± <b>0.00</b> a	3.3 ± <b>0.17</b> a	1.4 ± <b>0.04</b> a	7.7 ± <b>0.14</b> a	4.2 ± 0,18a
Nano-Fe	12.1 ± <b>0.07</b> a	9.8 ± <b>0.06</b> ab	2.3 ± <b>0.11</b> b	0.9 ± <b>0.00</b> c	7.0 ± <b>0.08</b> a	2.4 ± <b>0.02</b> c

Significant differences among treatments are indicated by the different Latin letters.



**Figure 8.** Mean comparisons for the interaction effects of salinity and foliar applications on phosphorus content of *Ocimum basilicum*. Significant differences among treatments are indicated by the different Latin letters



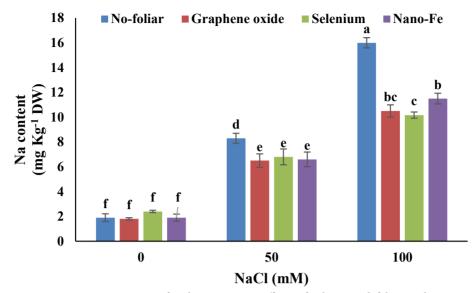
**Figure 9.** Mean comparisons for the interaction effects of salinity and foliar applications on Fe content of *Ocimum basilicum*. Significant differences among treatments are indicated by the different Latin letters.

## 3.16. Sodium content

With adding of the salinity stress, the sodium content of the plant increased, and the highest sodium content was observed in 100 mM salinity  $\times$  without foliar spraying. Under 50 and 100 mM salinity, foliar treatment with all three foliar treatments reduced the sodium content of plants. On the other hand, 100 mM salinity  $\times$  foliar application of selenium reduced sodium content by 37% compared to the treatment without foliar application at the same level of salinity stress. No significant difference was observed in sodium content between foliar treatments with selenium and graphene. However, the selenium foliar application caused a 12% decrease in sodium content compared to the iron nanoparticle application. Moreover, no difference was observed for the trait between foliar treatments with iron, selenium, and graphene at 50 mM salinity stress (Figure 10).

## 3.17. Sodium to potassium ratio

The highest sodium-to-potassium ratio was recorded in the treatment without foliar spraying  $\times$  salinity stress of 100 mM. Under 50 and 100 mM salinity, foliar treatment with all three compounds declined the sodium-to-potassium ratio. Under 50 mM salinity, there was no difference between foliar treatments. However, in 100 mM salinity, the iron treatment decreased the sodium-to-potassium ratio compared to the graphene oxide treatment (Figure 11).



**Figure 10.** Mean comparisons for the interaction effects of salinity and foliar applications on Na content of *Ocimum basilicum*. Significant differences among treatments are indicated by the different Latin letters.

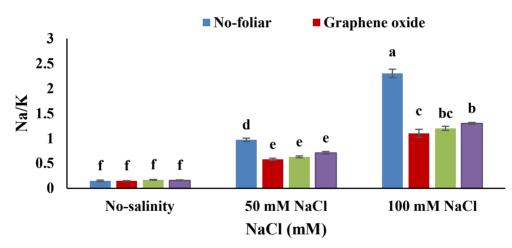


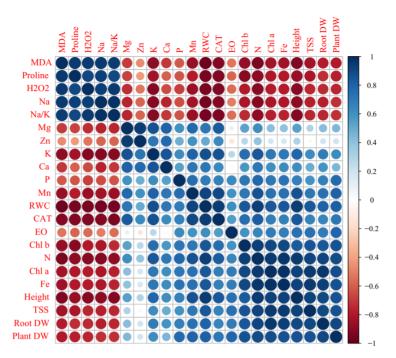
Figure 11. Mean comparisons for the interaction effects of salinity and foliar applications on Na content of *Ocimum basilicum*. Significant differences among treatments are indicated by the different Latin letters.

#### 3.18. Multivariate analysis

Based on Pearson's correlations for the growth traits, nutritional contents, and physiological attributes; MDA, proline,  $H_2O_2$ , Na, and Na/K positively correlated with each other and negatively correlated with macro- and microelements, RWC, height, CAT activity, TSS, plant dry weight (DW), and root dry weight (DW). As a result, a significant positive correlation was determined among

macro- and microelements, Chl a, Chl b, height, TSS, plant DW, and root DW. Essential oil content was positively related to plant and root DW, TSS, height, Fe, N, and Chl a (Figure 12).

Heat map analysis based on the response of basil plants to the salinity stress and foliar graphene oxide, Fe, and Se use revealed that the traits including Na, Na/K, MDA, proline, and  $H_2O_2$  had a positive response to



**Figure 12.** Heat map of Pearson's correlation analysis for the growth, nutritional, and physiological traits of Ocimum basilicum under salinity stress × nano-Fe, Se, and graphene oxide foliar application. Heat map representing mMalondialdehyde (MDA), pProline content,  $H_2O_2$  content, Na, Na/K, macro- and microelements, relative water content (RWC), catalase activity (CAT), plant and root dry weight (DW), Chlorophyllchlorophyll a (Chl a), Chlorophyllchlorophyll b (Chl b), height, and total soluble solids (TSS).

salinity stress and also were negatively related to the foliar applications. However, some attributes such as macro- and microelements content, RWC, height, CAT activity, TSS, plant DW, and root DW showed a negative response to the salinity stress and in contrast a positive response to the foliar applications (Figure 13). Cluster analysis and dendrograms in the heat map (Figure 13a) revealed three main clusters in the evaluated attributes of the basil plant under saline conditions × Fe, Se, and graphene oxide foliar treatments. Cluster I consisted of Na, Na/K, MDA, proline, and H<sub>2</sub>O<sub>2</sub>; cluster II contained: Mg, K, Ca, and Zn content; and cluster III included: N, P, Mn, and Fe content, catalase activity (CAT), EO content, Chl a, Chl b, height, TSS, plant DW, and root DW (Figure 13 a). In general, cluster analysis of heat maps for salinity stress and the foliar treatments indicated two clusters. Cluster I contained: the basil plants subjected to Se and graphene oxide under 0 and 50 mM NaCl and the control plants. Cluster II had all plants treated with Fe  $\times$  without salinity stress, the plants under 50 mM  $NaCl \times no$  foliar application and Fe treatment, and finally, the plants under 100 mM NaCl × no foliar application, Se, Fe, and graphene oxide application (Figure 13a).

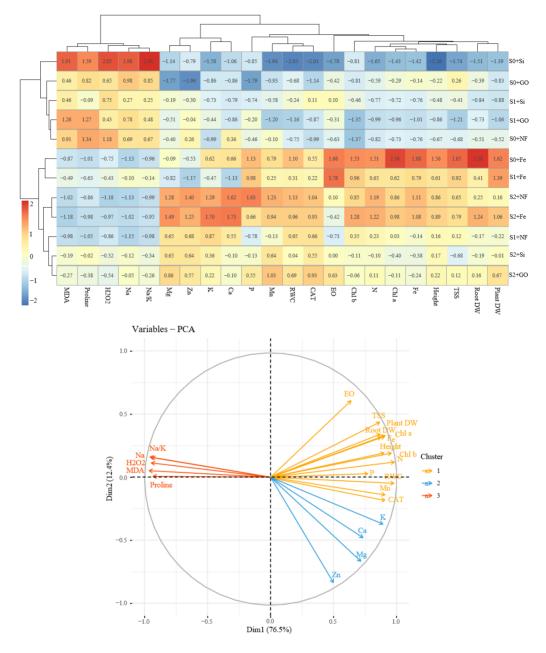
Moreover, the principal component analysis (PCA) of the evaluated variables in this experiment proved the heat map cluster analysis in which traits were ordered in three groups. The PCA and the proportion of total variance revealed that the two PCA described up to 88.9% of the total variation. The first PCA was the most influential, with a variance of 76.5% and the second PCA explained 27.2% of the total variance (Figure 13b).

#### 4. Discussion

The results showed that by increasing the salinity stress to 100 mM, the foliar treatments had no significant effect on mitigating the salinity stress. The highest plant yield was obtained without salt stress and with salt stress of 50 mM  $\times$  foliar application of iron nanoparticles. Salt stress has a destructive effect on the plant's growth and physiological and biochemical responses. The main reasons for reducing plant growth and performance due to salinity stress are ionic toxicity and ion imbalances (Munns and Tester, 2008).

Meanwhile, using mineral elements as nanoparticles plays a vital role in reducing the adverse effects of biotic and abiotic stressors. The results on grapes showed that

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**Figure 13.** Heat map (a) and the principal component analysis (b) for the growth, nutritional, and physiological traits of *Ocimum basilicum* under salinity stress × nano-Fe, Se, and graphene oxide foliar application. Heat map representing mMalondialdehyde (MDA), pProline content,  $H_2O_2$  content, Na, Na/K, macro- and microelements, relative water content (RWC), catalase activity (CAT), plant and root dry weight (DW), Chlorophyllchlorophyll a (Chl a), Chlorophyllchlorophyll b (Chl b), height, and total soluble solids (TSS). S0, S1, and S2 refer to 0, 50, and 100 mM NaCl, and also GO, Fe, and Se.

refer to graphene oxide, iron, and selenium foliar application, respectively.

the soil-based application of graphene oxide and iron foliar use raised the plant's chlorophyll content and dry weight (Aazami et al., 2022). An enhancement in plant yield due to foliar selenium use has been reported in *Cannabis sativa* L under salt stress (Guerriero et al., 2021). Similar results regarding the increase in plant dry weight due to the application of iron nanoparticles were reported in mint (Askary et al., 2017) and *Linum usitatissimum* L. (Singh et al., 2021) under salinity stress. Iron treatment under salinity stress is vital in increasing photosynthetic pigments content and plant yield (Askary et al., 2017). The tiny size and high specific surface area of nanoparticles accelerate their entry into the cell and reduce the effects of salinity stress in the plant. Similar results were reported regarding the increase in plant dry weight and essential oil content due to the application of selenium under salt stress in basil plants (Farouk and Omar, 2020). The production of essential oil depends on genetics and environmental cues. Faced with environmental stressors, plants increase essential oil production to deal with stressful situations. The present study showed an increase in essential oil content in conditions without salt and salt stress of 50 mM × iron foliar use. A study conducted on the Lavandula angustifolia Mill. plant found that iron and zinc nanoparticles foliar application positively affected the essential oil content of plant under salt stress (Talebi et al., 2022). The improvement in photosynthesis potential and the enhanced production of carbon skeletons necessary for the biosynthesis of secondary metabolites are the primary reasons for any increment in essential oil production under treatment with iron nanoparticles.

Our study showed that salinity had a destructive effect on the plant's chlorophyll content; the highest chlorophyll content was observed in no saline × selenium and iron nanoparticle treatment. Salinity stress reduced photosynthetic characteristics such as photosystem II function, net photosynthesis, and other metabolic activities in Linum usitatissimum L. (Singh et al., 2021). The decrease in potassium absorption due to salinity stress reduced the turgor pressure of stomatal cells, and with the stomata closure and reduced photosynthesis potential, plant growth decreased (Germ et al., 2005; Wani et al., 2017). Similar results were reported regarding the increase of photosynthetic pigments content due to the application of iron nanoparticles under salt stress in Linum usitatissimum L. (Singh et al., 2021). The tiny size and particular structure of nanoparticles make their absorption easier by the cell and reduce the adverse effects of stress in the plant.

Moreover, iron plays a fundamental role in the plant's survival by improving several enzymes' activity and the absorption of other nutrients under stress, and by enhancing the content and functionality of photosynthetic pigments (Singh et al., 2021). The reduction in the absorption of elements such as iron, potassium, and calcium as a result of stressleads to a considerable decrease in plant growth, a decline in photosynthesis potential, a defect in stomatal conductance, and a huge decline in carbon dioxide acquisition (Abrar et al., 2020; Aazami et al., 2021). Foliar selenium treatment under stress conditions increased photosynthetic pigments content in basil (Farouk and Omar, 2020). Selenium plays a crucial role in enhancing the biosynthesis of photosynthetic pigments, decreasing the accumulation of sodium and chlorine, enhancing the ratio of potassium to sodium (Farouk and Omar, 2020), maintaining the metastructure of chloroplasts, and reducing ROS levels in the plant, and thus effectively contributes to the survival of plants under stress conditions (Farouk and Omar, 2020).

In our study, salinity stress decreased the relative water content of leaves. It seems that the accumulation of osmolytes due to the application of selenium under stress conditions is one of the reasons for improving the RWC of leaves. The accumulation of osmolytes is a criterion in the amelioration of salinity stress. The sufficient Se supply under stress conditions improves the root development and hence goes to more water absorption and even stimulates the upstream regulation of genes involved in the biosynthesis of aquaporins, which help to maintain the integrity of the cell membranes and contributes to cell durability and normal function under stress conditions in Sorghum bicolor L. (Moench) (Liu et al., 2015). Osmotic regulation in plants under stress conditions is essential in protecting the plant against stress. Several studies have shown that the use of nanoparticles has an important action in the root hydraulic conductance by increasing the expression of plasma-membrane anchored aquaporins and hence has the dominant role in improving water absorption and reducing the effects of oxidative stress on the cell membranes (Zulfiqar and Ashraf, 2021).

Salinity stress causes various nutritional anomalies in the plant, the cause of which may be related to the adverse effects of salinity on the ability to absorb nutrients or may be associated with the effect of salinity in creating competition between several ions for the absorption, transfer, and distribution in different organs of the plant (Munns and Tester, 2008). The mentioned disorders trigger oxidative damage to proteins and fats and disrupt cells' normal metabolism (Hare et al., 1999). The ROS accumulation induced by the stress causes damage to DNA, proteins, and fat in the cell. To deal with environmental stressors, the plant starts the generation of enzymatic and nonenzymatic compounds to withstand stressful conditions (Singh et al., 2021). Increasing the accumulation of low molecular weight and water-soluble osmolytes such as polyamines, proline, and soluble solids is a general strategy to deal with stress in plants (Hare et al., 1999). The coexistence of antioxidant enzymes and nonenzymatic antioxidant compounds significantly reduced the damage caused by ROS in Lavandula angustifolia Mill. (Chrysargyris et al., 2018). Proline plays a vital role in controlling salinity stress by removing oxygen free radicals, reducing cell acidification, and regulating cell osmotic potential and membrane stability (Munns and Tester, 2008; Aazami et al., 2021). Under salt stress conditions, the cell uses proline as a source of carbon, nitrogen, and energy (Munns and Tester, 2008).

Furthermore, the graphene oxide nanoparticles application controlled salinity stress and increased the content of proline, photosynthetic pigments, and dry weight in the plant (Safikhan et al., 2018). The foliar application of elements such as iron, graphene oxide, and Se increases the biosynthesis of sugar compounds in the cell, and by regulating the osmotic potential plays an essential role in reducing stress effects mainly via preventing sodium entry into the cell. Also, the cell uses the produced sugar compounds as an energy source (Zhou et al., 2019; Safikhan et al., 2018). An increase in the soluble solids and proline content was reported due to the application of Se under stress conditions in basil plants (Farouk and Omar, 2020). In a study conducted by Wan et al. (2020), it was found that the application of carbon nanoparticles under salt stress had a dominant role in enhancing the plant weight, soluble solids content, and total protein content.

It seems that the treatments used in the present study played a beneficial role in reducing the content of hydrogen peroxide and malondialdehyde under the stress conditions of 50 and 100 mM. Under salt stress, damage to the cell membranes triggers the nonselective absorption of sodium ions and intensifies the related damage (Askary et al., 2017). Salt stress intensifies lipid peroxidation and increased membrane permeability and ion leakage in cucumbers (Zhu et al., 2004). Reactive oxygen species are byproducts of oxygen that play a chief role in cell signaling and homeostasis. ROS formation induces lipid peroxidation, impairs proteins and DNA, inhibits signal transduction, and interferes with normal cellular function (Mittler, 2002). The results on grapes (Aazami et al., 2021) and linseed (Singh et al., 2021) showed that iron nanoparticle foliar treatment reduced the content of hydrogen peroxide in the plant. Oxidation damage to the cell membranes and proteins is another effect of salt and drought stress which enhances MDA content. Se application under stress conditions reduced the accumulation of MDA in sweet basil due to increasing the stability of the cell membranes (Farouk and Omar, 2020).

Se plays an important role in maintaining cell membrane structure and integrity (Zhu et al., 2004). In the study conducted in basil, it was found that using Se under stress conditions maintained the stability of the cell membranes and reduced the production of MDA (Farouk and Omar, 2020). Foliar treatment of plants with graphene oxide under stress maintains the cell membrane's integrity and fortifies cell proliferation and plant growth (Ruiz et al., 2011; Aazami et al., 2022). Adequate amounts of graphene oxide reduced the destructive effects of hydrogen peroxide and increased the stability of the cell membrane under salt stress conditions (Aazami et al., 2022). The production of osmolytes and proline is essential in reducing MDA overaccumulation (Hare et al., 1999). Similar results were reported regarding the increase in the performance and activity of antioxidant enzymes under salinity stress due to iron nanoparticles treatment in *Dracocephalum moldavica* L. plant (Moradbeygi et al., 2020).

In our study, the highest catalase activity was related to the conditions without salt stress × iron, graphene, and selenium nanoparticles application. Similar results were observed in reducing catalase activity under salinity stress and foliar spraying with iron nanoparticles in mint (Askary et al., 2017). The application of iron nanoparticles had an essential role in the activity of antioxidant enzymes, especially catalase in Linum usitatissimum L. plants (Singh et al., 2021). Selenium plays a vital function in strengthening the cell wall and reducing the harmful effects of hydrogen peroxide, thus helping the plant to survive under stress conditions (Shekari et al., 2017). A similar idea was claimed regarding the increase of catalase activity due to the application of selenium under stress conditions in basil plants (Farouk and Omar, 2020). The application of selenium under salinity stress increases the sodium compartmentation into the plant vacuole. It protects the plant against stress due to the increased activity of antioxidant enzymes (Siddiqui et al., 2020). Se-treated plants attained enhanced enzyme activity (SOD and APX) under salt stress (Kong et al., 2005). SOD is in the first line of antioxidant defense against ROS (catalyzing superoxide to H<sub>2</sub>O<sub>2</sub> and molecular oxygen), and CAT is responsible for scavenging excess ROS. H2O2 activates ZmMPK5 and the expression of ZmCPK11 can increase the expression and activity levels of SOD and APX enzymes in maize (Ding et al., 2013). Carbon nanoparticles (graphene) foliar treatment mitigates the adverse effects of salinity stress in plants through changes in the expression of genes involved in the antioxidant system of salt overly sensitive 1 system (SOS1) (Zhao et al., 2019).

Salinity reduced the content of iron, potassium, nitrogen, manganese, magnesium, and zinc elements. In the present study, those elements' highest content was obtained without salt stress by spraying iron, graphene, and selenium nanoparticles. The soil-based application of graphene oxide and foliar spraying with iron and selenium increased the content of elements in grapes (Aazami et al., 2021). The high availability of sodium and chlorine elements under stress conditions causes disturbances in the absorption of essential nutrients. Due to the similar ionic radius between sodium and potassium, sodium ion enters the cell and destroys the integrity of the cell membranes (Munns and Tester, 2008). The sodium accumulation in the plant's aerial parts reduces the potassium and calcium content due to the antagonistic behavior between sodium, potassium, and calcium. Potassium is one of the essential elements required by plants, which plays an important role in cell growth, osmotic regulation, activity of many cytosolic enzymes, and stomatal conductance (Munns and Tester, 2008). Jiang et al. (2017) showed that selenium use increased the potassium content of cells under

stress conditions. Any potassium availability increases the transfer of water to the aerial part of the rice plant, thus helping to protect the cell against stress (Almeida et al., 2017). In maize, the expression of *ZmNHX1* gene (involved in Na<sup>+</sup> compartmentalization in root vacuoles) was up-regulated by Se at 24 h after salinity treatment and enhanced plant salt tolerance (Jiang et al., 2017).

Iron is an integral part of the biosynthesis of chlorophyll, chloroplast, thylakoid formation, the respiration process, and DNA and RNA biosynthesis (Elhindi et al., 2016). In the study conducted on lavender, it was found that foliar spraying with iron nanoparticles had a positive effect on the iron content of the plant under salt stress. Still, iron foliar application decreased the zinc content of the plant (Talebi et al., 2022). Phosphorus is a fundamental element in photosynthesis. Salt stress reduces photosynthesis in plants by interfering in the P absorption and translocation. Under stress conditions, dihydrogen phosphate absorption decreases due to competition with chlorine, which reduces plant growth and yield (Munns and Tester, 2008).

Moreover, under stress conditions, sodium replaces calcium in the cell wall and affects plant growth and performance by creating an ionic imbalance in wheat cells (Guo et al., 2015). Calcium has highlighted roles in growth, maintaining the integrity of the cell membrane and its function. As a secondary messenger, calcium plays a vital role in cell function. Under salt stress conditions, the use of selenium retains intracellular homeostasis and improves calcium absorption by the plant (Marco Delpino et al., 2019). Reactive oxygen species disturb the cellular signals induced by calcium and reduce the transfer of calcium from the endoplasmic reticulum and Golgi vesicles to the cell wall, thus causing damage to the cell structure. Selenium also has a role in reducing the effects of free radicals. Protein biosynthesis was associated with the oxidative stress response pathway and the calcium

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signaling pathways (Guo et al., 2015; Marco Delpino et al., 2019). Magnesium deficiency under stress conditions causes disturbances in the biosynthesis of photosynthetic pigments and decreases plant performance. Selenium foliar application under such conditions improves the absorption of nutrients and later maintains the stability of the cell membrane (Do-Nascimento et al., 2017). The research conducted in rapeseed showed that the application of carbon nanoparticles under salinity stress improved the absorption of nutrients in the plant by adjusting the ion balance, reducing the ratio of sodium to potassium, and changing the activity of K<sup>+</sup> transporter 1 (KT1) and Na<sup>+</sup>/H<sup>+</sup> exchanger 1 (NHX1) and in this way helped the survival of plant under stressful conditions (Zhao et al., 2019)

#### 5. Conclusion

The results revealed that salinity hurt basil plants' yield and physiological responses. With the salinity of 100 mM, malondialdehyde and sodium content, as well as sodium to potassium ratio were drastically increased. In the plants subjected to no-salinity and 50 mM salinity stress × iron nanoparticle foliar application, the plant's dry weight and essential oil content statistically improved. Furthermore, under control treatment, the foliar use of graphene oxide and selenium enhanced the magnesium, calcium, and zinc content of plants. All three foliar treatments increased the nitrogen and potassium content of plants. Overall, basil can be considered a mild salinity-sensitive plant. Otherwise, to overcome the salinity defects, there is a need to survey a broad range of foliar treatments and maybe some other compounds to reach a tolerance protocol under harsh saline conditions.

#### Funding

This study was funded and supported by the Azarbaijan Shahid Madani University, Iran.

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