



# Mollifying Salt Depression on *Anethum graveolens* L. by the Foliar Prescription of Nano-Zn, KNO<sub>3</sub>, Methanol, and Graphene Oxide

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## Abstract

The aim of the study was to assay the salinity impacts on *Anethum graveolens* by the foliar application of nano-Zn, KNO<sub>3</sub>, methanol, and graphene oxide in hope to mitigate the stressor side effects. NaCl salinity (0, 50, and 100 mM) and foliar spray with graphene oxide, methanol, KNO<sub>3</sub>, and nano-zinc were examined to evaluate the growth and physiological responses, antioxidant enzyme activity, and the essential oil content and constituents of *Anethum graveolens*. The results revealed that salinity × foliar combinations significantly affected N, P, and Na content of plants. The top recorded data for K<sup>+</sup>/Na<sup>+</sup>, catalase activity, and chlorophyll a content belonged to control plants. Control and 50-mM salinity treatments attained the highest aerial part dry weights, total chlorophylls, and essential oil yield. One hundred-millimolar salinity induced the greatest malondialdehyde, H<sub>2</sub>O<sub>2</sub>, and proline content. Foliar treatment of methanol, KNO<sub>3</sub>, and nano-zinc added up K<sup>+</sup> content. GC/MS analysis revealed the occurrence of 21 constituents in the oil. Dill-apiol (24.06–88.5%) was the major constituent; NaCl<sub>100mM</sub> × methanol treatment attained the highest dill-apiol content. In conclusion, *Anethum graveolens* was tolerable to 50-mM salinity without remarkable loss in the growth characteristics and yield. Moreover, foliar treatment of KNO<sub>3</sub> and nano-zinc partially ameliorated the adverse side effects of salinity.

**Keywords** *Anethum graveolens* · Nutrient content · Essential oil · Proline content

## 1 Introduction

Dill (*Anethum graveolens*) is a commercial medicinal and aromatic plant of Apiaceae family. Dill is rich in aromatic constituents and under common growth for fresh, culinary, and essential oil (EO) use. The EO possesses antimicrobial and antifungal properties. Furthermore, dill products have a long history of use as flavorings in the food, and as aromatizer in the hygienic and cosmetic industries (Biesiada et al.

2019). The seeds are containing high quantity of carvone, thujone, di-hydrocarvone, apiole, d-pinene, iso-myrcetene, limonene, and carvacrol (Said-Al Ahl and Omer 2016).

Growing environments greatly influence the biosynthesis and accumulation of secondary metabolite in medicinal and aromatic plants (Tsamaidi et al. 2017).

The salinity of the irrigation water is a serious environmental problem that checks the plant's normal growth, development, and productivity. Salinity impacts the plants either by reducing the rhizosphere osmotic potential, ionic imbalances, reducing the nutrient absorption, oxidative damage via AOS (activated oxygen species) and, cell membrane damage, or by the huge decline in the photosynthetic potential of plants (Tsamaidi et al. 2017). The yield reduction by the direct and indirect side effects of salinity is the dominant reason for the prominent economic losses in the salinity-prone soils. So, there is an emergent necessity to combat the salinity defects on the plant's growth, quality, and productivity.

Using some compounds as the foliar application is a reliable procedure to reduce the salinity damages and to reach

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suitable yield components. The growth and productivity of  $C_3$  plants improve with ethanol and methanol foliar treatment in the main part due to the enhanced  $CO_2$  concentration and the combination with ribulose-1-5-biphosphate which is the regulatory key enzyme in carbon metabolism. Methanol as foliar spray acts as a hydrocarbonic compound, binds the hydrophobic membrane-anchored proteins, activates several enzymes, and enhances photosynthesis and eventually the yield (Gout et al. 2000; Vojodi Mehrabani et al., 2019).

Zinc is a major micronutrient with pivotal roles in several enzymes' structure and activity, plant growth, cell division, membrane stability, auxin biosynthesis, and photosynthesis, and also has functions in the RNA and DNA biosynthesis (Marschner 2012). Under salinity stress, Zn treatment alleviates ion toxicity by preventing Na and/or Cl uptake or translocation (Alpaslan et al. 1999). In rosemary plants grown under salinity, nano-zinc treatment elevated the essential oil content (Vojodi Mehrabani et al. 2018). Chrysargyris et al. (2018) reported that foliar K and Zn application under salinity improved the plant fresh yield, essential oil content, and antioxidant capacity of *Lavandula angustifolia*. Furthermore, in the research conducted by Tsamaidi et al. (2017), they have noted that salinity had no significant adverse effect on chlorophyll, carotenoids, phenolics, and ascorbic acid content, but at the same time, increased  $\alpha$ -phellandrene accumulation in plants.

Potassium plays crucial actions in plant growth, maintaining cell turgor, membrane potential, enzyme activity, protein metabolism, phloem transport, and osmotic potential maintenance (Marschner 2012). Due to the similar electrical charge of  $Na^+$  and  $K^+$ , sodium ions can compete with  $K^+$  under NaCl salinity stress, and negatively affect physiological processes related to  $K^+$ . Under salinity, nutrition with  $KNO_3$  plays an important role in reducing the negative effects of salinity stress on the plant (Munns and Tester 2008). In *Coriandrum sativum*, the foliar application of  $KNO_3$  reduced ion leakage, protein, and chlorophyll content, and contrarily increased proline and  $Na^+$  content (Elhindi et al., 2016 a).

Methanol as a carbonic compound is easily absorbed by plants. Methanol has been confirmed as a nontoxic compound to encourage plant growth. Methanol foliar spray promotes the metabolic pathways ( $CO_2$  fixation and pectin metabolism in the cell wall) and plant defense mechanisms (Dorokhov et al. 2018). Under saline-sodic conditions, methanol foliar application increased the plants' tolerance versus salinity depression (Valizadeh Kamran et al. 2019).

Graphene oxide is a carbonic two-dimensional compound which has recently been discovered and widely employed in several industries. Graphene oxide exhibits excellent characteristics due to its unique combination of crystallographic, electronic, and chemical structure (Deng and Berry 2016). Graphene oxide has been tried in the agriculture to smooth the effects of several environmental stresses. Under salinity

stress, this compound has pivotal roles in cell water balance maintenance, as a carrier for several compounds inside cells (translocation of amino acids), enhanced chlorophylls content and photosynthesis efficiency, improved proline and soluble carbohydrate content and hence, improves the growth under stressful environments (Deng and Berry 2016; Pandey et al. 2018; Safikhan et al. 2018). Safikhan et al. (2018) demonstrated that under salinity-imposed conditions, graphene oxide treatment (0.01% carbon oxide nano-element) ameliorated cell water potential, enhanced the plants' viability, and increased the yield. A similar result was reported by Mahmoud and Abdelhameed (2021). They showed that graphene oxide application under salinity improved *Pennisetum glaucum* growth, total protein content, free radical scavenging potential (protection of intercellular macromolecules), and photosynthetic pigment content of the plant. Li et al. (2018) reported that graphene oxide had a role in controlling the gene expression levels during protein synthesis. In total, graphene oxide by modulating the plants' morphological and biophysical traits causes more tolerance under salinity stress (Mahmoud and Abdelhameed, 2021).

Iran is facing great and widespread climatic variations. The significant rainfall reductions, heterogeneous precipitation patterns, and environmental problems owing to the vast drying of rivers and lakes have drastically imposed the salinity depression effects on the neighboring environments and plants. These all profoundly impact the agricultural system and the rural populations' life and income. Considering, exploring the novel compounds and procedures to combat salinity deteriorative actions with no or very low side environmental effects are of huge importance. In the present study, we aimed to evaluate the foliar application of carbonic compounds (graphene oxide and methanol), nano-zinc, and  $KNO_3$  on the growth potential and productivity of dill under saline-sodic stressful conditions.

## 2 Materials and Methods

### 2.1 Plant Material and Experimental Setup

To study the effects of NaCl salinity (0, 50, and 100 mM) and foliar application of graphene oxide (GO), methanol, and  $KNO_3$  and nano-zinc on the growth and some physiological traits of dill, a factorial experiment based on RCRD with three replications was arranged at the Research Greenhouse of Azerbaijan Shahid Madani University, Tabriz, Iran, during 2019. *Anethum graveolens* seeds were planted in 5-l pots containing medium-sized perlite in an open soilless culture system. Light intensity was about  $450 \mu mol m^{-2} s^{-1}$ , temperature regime: 25 and 20°C at day and night, and the relative humidity was 65%. The plants were acclimatized for

20 days with the modified Hoagland's nutrient solution (EC of  $2.1 \text{ mS cm}^{-1}$ ;  $\text{pH}=5.8$ ).

The salinity levels began with  $25 \text{ mM}$  and were gradually increased to reach the final level by weekly intervals. To avoid the salinity shock, the pots were washed with tap water once a week. Thereafter, plants were grown for an additional period of 9 weeks. The treatments were applied by foliar spraying. Solutions were including  $\text{dH}_2\text{O}$  (control); nano-zinc oxide,  $3 \text{ mg l}^{-1}$  (Vojodi Mehrabani et al. 2018); methanol (20%) (Valizadeh Kamran et al. 2019);  $\text{KNO}_3$ ,  $300 \text{ mg l}^{-1}$  (Chrysargyris et al. 2017); and graphene oxide ( $0.05 \text{ g l}^{-1}$ ) (Begum et al. 2011). Nano-ZnO was supplied by the US-Nano Company (US Research Nano Materials, TX, USA).

The first foliar treatment was coincident with the salinity running up. They were repeated 2 weeks later. Seven weeks after the second foliar treatment, plant samples were taken for the trait measurement. The optimal pH of NS was 5.8 and was recorded every other day and adjusted accordingly by using  $\text{H}_2\text{SO}_4$  (5% v/v).

## 2.2 Reagents and Materials for the Synthesis of Graphene Oxide

All chemicals used were of analytical grade.  $\text{NaNO}_3$ ,  $\text{KMnO}_4$ , and graphite powder (50 meshes) were acquired from Merck (Germany). Ethanol was purchased from Hamon Teb Markazi (Zarandieh, Iran).

### 2.2.1 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  $\text{Cu-K}\alpha$  radiation source ( $1.54 \text{ \AA}$ ) between  $8$  and  $80^\circ$  generated at  $40 \text{ kV}$  and  $35 \text{ mA}$  at room temperature. In addition, the morphology of the GO was observed under a scanning electron microscope (SEM, model MIRA3, Tescan, Czech Republic).

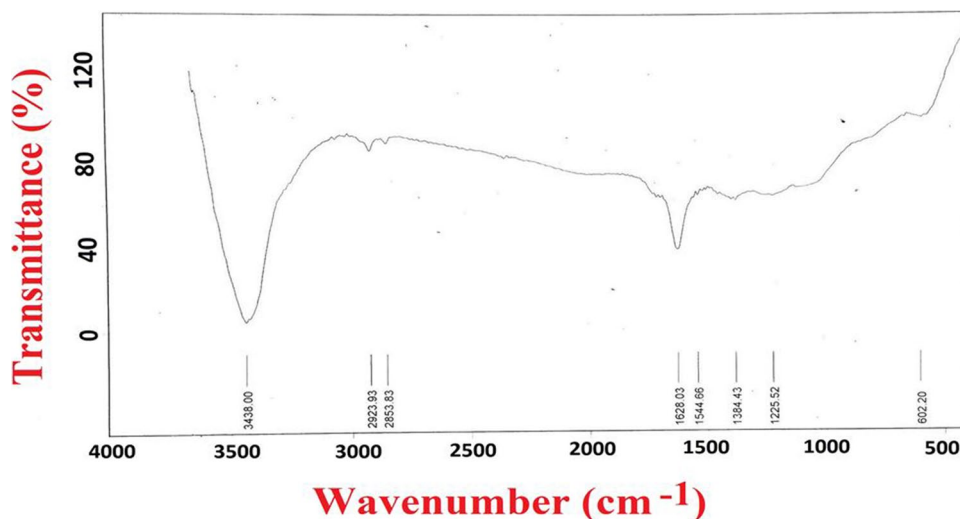
## 2.3 Synthesis of Graphene Oxide

Graphene oxide was prepared from graphite powders using the modified Hummers' method. In brief,  $10 \text{ g}$  of graphite powder and  $5 \text{ g}$  of  $\text{NaNO}_3$  were put in  $230 \text{ mL}$  of cont.  $\text{H}_2\text{SO}_4$  and  $\text{KMnO}_4$  ( $30 \text{ g}$ ) were then gradually added to the solution with stirring and cooling to prevent overheating and explosion. After adding  $\text{KMnO}_4$ , the solution was allowed to stand overnight at room temperature. The next day, the solution was poured into  $500 \text{ ml H}_2\text{O}$  heated at about  $100^\circ\text{C}$ , and the reaction was allowed to progress for a certain period of time. Finally, the graphite oxide formed was washed with ethanol and then dried at  $65^\circ\text{C}$  for  $24 \text{ h}$  in an oven (Nakajima et al. 1988). Later, the graphite oxide is subjected to ultrasound irradiation for  $2 \text{ h}$  to obtain the GO.

## 2.4 Characterization of GO

The FTIR spectrum of GO (Fig. 1) shows a broad peak around  $3400 \text{ cm}^{-1}$  in the high-frequency area corresponding to the stretching vibration of OH groups of water molecules adsorbed on graphene oxide. The peaks at  $2923 \text{ cm}^{-1}$  and  $2853 \text{ cm}^{-1}$  are representing the symmetric and anti-symmetric stretching vibrations of  $\text{CH}_2$ , while the presence of the peak observed in the medium frequency area, at  $1628 \text{ cm}^{-1}$ , can be attributed to the stretching vibration of  $\text{C}=\text{C}$  and  $\text{C}=\text{O}$  of carboxylic acid groups present at the edges of graphene oxide (Shahriary and Athawale 2014). Finally, the peak at  $1384 \text{ cm}^{-1}$  is corresponding to the stretching vibration of  $\text{C}-\text{O}$  of carboxylic acid. The presence of these

**Fig. 1** FTIR spectrum of graphene oxide



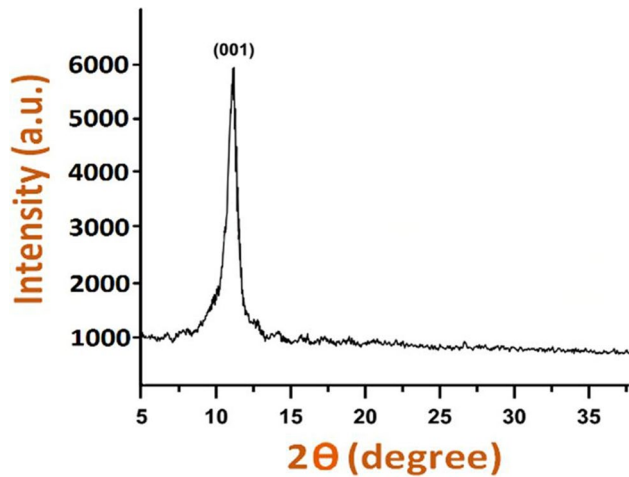


Fig. 2 XRD pattern of graphene oxide

oxygen-containing groups reveals that the graphene has been oxidized (Fig. 2).

## 2.5 Fresh and Dry Weight (Biomass)

Dry weight was calculated following oven drying of 40°C until constant weight.

## 2.6 Relative Water Content (RWC)

Leaf samples (0.5 g) were incubated in 100 ml of distilled water for 4 h. The turgid weight of leaf samples was measured. The leaf samples were packed in butter paper bags and oven-dried at 70°C for 48 h. The RWC was determined by the following formula (Xu et al. 2006):

$$\text{RWC} = \frac{[(\text{Fresh Wt.} - \text{Dry Wt.}) / (\text{Turgid Wt.} - \text{Dry Wt.})] \times 100}$$

## 2.7 Essential Oil Extraction

The essential oils were extracted from 15-g dry plant material by water distillation during 3 h using a Clevenger-type apparatus and the oils were dried over anhydrous sodium sulfate. Oils were kept in amber vials until GC/MS analysis (Figure 3).

## 2.8 Mineral Analysis

Dry leaf samples (0.3 g) were acid-digested (2 N HCl) and analyzed for nutrient content as described by Chrysargyris et al. (2018). The content of Na and K was quantified by the flame photometric method (Corning, 410, England). N content was measured by the Kjeldahl method. The content of Zn, Ca, Mg, and Fe was quantified by atomic absorption spectroscopy (Shimadzu, AA6300, Japan) as previously

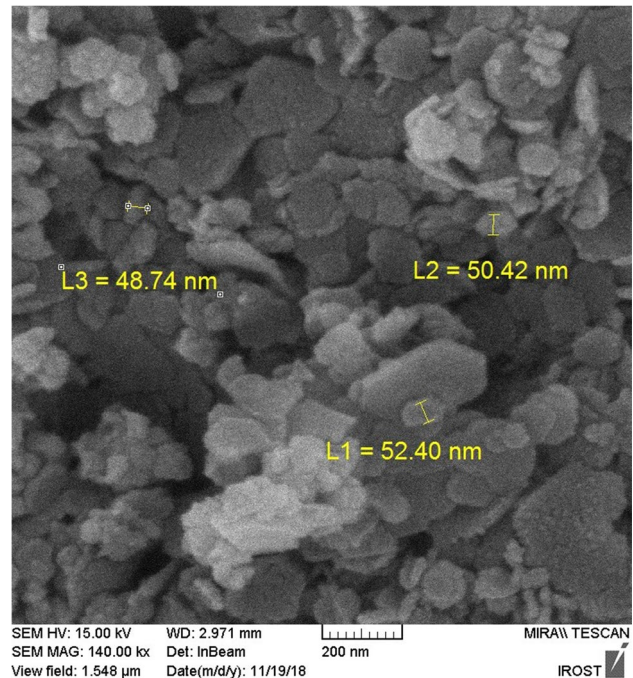


Fig. 3 SEM image of graphene oxide. The SEM image of synthesized GO is given in the figure. From the figure, it can be observed that graphene oxide has a layered structure, which affords ultra-thin and homogeneous graphene oxide films. Also, considering to SEM image of synthesized GO, the size of GO flakes was about 50 nm

described by Honarjoo et al. (2013). P was assayed by Vanadate Molybdate method.

## 2.9 Chlorophyll Content

Chlorophylls a and b, and total chlorophyll content were quantified by the method of Prochazkova et al. (2001).

## 2.10 Total Soluble Solids (TSS)

TSS was quantified by a hand refractometer (Erma, Tokyo, Japan), and the data were reported as °Brix.

## 2.11 Proline Content

Proline content was assayed according to the method of acid ninhydrin and toluene at 520 nm, as described by Fedina et al. (2006).

## 2.12 Hydrogen Peroxide and Lipid Peroxidation

The content of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was assessed as described previously (Chrysargyris et al. 2019) and lipid peroxidation was measured according to Azevedo Neto et al. (2006). Lipid peroxidation was expressed in terms of malondialdehyde (MDA) content.

### 2.13 Catalase Activity

Catalase enzyme activity was determined according to the methods of Sairam et al. (2002).

### 2.14 GC-MS Analysis

A gas chromatography (GC) system (Agilent Technologies, 7890B) equipped with a flame ionization detector (FID) and coupled to a mass spectrometry (MSD) detector (Agilent Technologies, 5977A) was employed to identify the oil constituents. An HP-Innowax column (Agilent 19091N-116: 60-m×0.320-mm internal diameter and 0.25-µm film thickness) was used for the separation of the compounds. Samples were analyzed with the column held initially at 70°C after injection with 5-min hold time, then increased to 160°C with a 3°C min<sup>-1</sup> heating ramp. Finally, the temperature was raised to 250°C with 6°C min<sup>-1</sup> heating ramp with 5-min hold time by using helium (99.99% purity) as carrier gas at 1.3 mL min<sup>-1</sup> flow rate with 1-µl injection volume (20-µL essential oil was dissolved in 1 mL n-Hexane) The solvent delay time was 8.20 min. The injection was performed in split mode (40:1). Detector, injector, and ion source temperatures were 270°C, 250°C, and 270°C, respectively. MS scan range was (m/z): 35–450 atomic mass units (AMU) under electron impact (EI) ionization of 70 eV.

Retention indices calculated against n-alkanes (C7-C30/ Sigma-Aldrich) on HP-Innowax column by GC/FID system (Agilent Technologies, 7890B) under the same conditions. The constituents were identified by comparison of retention indices and mass spectra by computer library search database of US National Institute of Standards and Technology (NIST), Wiley libraries, other published mass spectra (1), and available data from the literature (Adams 2007).

### 2.15 Data Analysis

The data were analyzed by SPSS (ver. 15). The tables were drawn by Excel and measurements were compared by LSD test at P ≤ 0.05 and P ≤ 0.01.

## 3 Results

### 3.1 Aerial Part Yield (Dry Biomass)

Aerial part yield was influenced by the independent effects of treatments (Table 1). The least data belonged to control non-sprayed plants. All foliar application treatments improved the aerial part yield (Table 3). No-saline treatment and 50-mM salinity increased this treat. One hundred-millimolar salinity drastically declined aerial part dry biomass (Table 2).

**Table 1** Effects of salinity (0, 50, and 100 mM NaCl) and foliar treatments (no foliar, nano-Zn, KNO<sub>3</sub>, and graphene oxide) on *Anethum graveolens* plant growth and physiological responses

Significance	Aerial part dry weight	Total soluble solid content	Relative water content	Total chlorophyll content	Chlorophyll a	Chlorophyll b	Proline content	MDA content	H <sub>2</sub> O <sub>2</sub> content	Catalase activity	Essential oil content	Essential oil yield
Replication	**	**	*	*	ns	ns	ns	ns	**	**	ns	**
Salinity (S)	**	ns	**	**	**	**	**	**	**	**	**	**
Foliar (F)	**	**	*	**	ns	ns	ns	ns	ns	ns	ns	*
S × F	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns

ns, non-significant; \*Significant difference at P ≤ 5%; \*\*Significant difference at P ≤ 1%

**Table 2** Mean comparisons for the effects of NaCl salinity on growth and physiological responses of *Anethum graveolens* plants

NaCl salinity (mM)	Aerial part dry weight (g m <sup>-2</sup> )	Relative water content (%)	Chlorophyll a content (mg g <sup>-1</sup> FWt)	Total chlorophyll content (mg g <sup>-1</sup> FWt)	Proline content (µg g <sup>-1</sup> FWt)	MDA content (nmol g <sup>-1</sup> FWt)	H <sub>2</sub> O <sub>2</sub> content (µmol g <sup>-1</sup> fw)	Catalase activity (units mg <sup>-1</sup> protein)	K content (g kg <sup>-1</sup> )	Ca content (g kg <sup>-1</sup> )	Fe content (mg kg <sup>-1</sup> )	Mg content (g kg <sup>-1</sup> )	K/Na	Essential oil yield (ml m <sup>-2</sup> )	Essential oil content (%)
0	367 ± 25 <sup>a</sup>	89.7 ± 0.5 <sup>a</sup>	1.4 ± 0.05 <sup>b</sup>	2.04 ± 0.08 <sup>a</sup>	38.5 ± 1.1 <sup>c</sup>	47.1 ± 0.5 <sup>c</sup>	1.04 ± 0.05 <sup>b</sup>	45.7 ± 1.3 <sup>a</sup>	11.5 ± 0.7 <sup>a</sup>	3.1 ± 0.15 <sup>a</sup>	30 ± 1.6 <sup>a</sup>	1.09 ± 0.03 <sup>a</sup>	6.8 ± 0.9 <sup>a</sup>	0.43 ± 0.002 <sup>a</sup>	0.116 ± 0.002 <sup>a</sup>
50	351 ± 24 <sup>a</sup>	81.9 ± 1.1 <sup>b</sup>	0.95 ± 0.04 <sup>b</sup>	1.30 ± 0.06 <sup>a</sup>	65.6 ± 1.9 <sup>b</sup>	60.2 ± 1.6 <sup>b</sup>	1.30 ± 0.09 <sup>b</sup>	35.6 ± 1.4 <sup>b</sup>	9.3 ± 0.6 <sup>b</sup>	2.4 ± 0.11 <sup>b</sup>	29 ± 1.0 <sup>a</sup>	0.87 ± 0.04 <sup>b</sup>	3.06 ± 0.6 <sup>b</sup>	0.41 ± 0.003 <sup>a</sup>	0.112 ± 0.003 <sup>a</sup>
100	249 ± 9.0 <sup>b</sup>	72.4 ± 1.0 <sup>c</sup>	0.75 ± 0.03 <sup>c</sup>	1.08 ± 0.05 <sup>c</sup>	114.7 ± 2.2 <sup>a</sup>	65.6 ± 1.2 <sup>a</sup>	1.68 ± 0.08 <sup>a</sup>	27.1 ± 1.1 <sup>c</sup>	8.7 ± 0.5 <sup>c</sup>	2.08 ± 0.10 <sup>b</sup>	24 ± 1.1 <sup>b</sup>	0.64 ± 0.05 <sup>c</sup>	0.92 ± 0.06 <sup>c</sup>	0.25 ± 0.002 <sup>b</sup>	0.107 ± 0.002 <sup>b</sup>

Significant differences among treatments are indicated by the different Latin letters

**Table 3** Mean comparisons for the effects of foliar applications (control, 0.05 g L<sup>-1</sup> graphene oxide, 3 g L<sup>-1</sup> nano-Zn, 300 mg L<sup>-1</sup> KNO<sub>3</sub>, and 20 % methanol) on the growth, physiological responses, and elemental content of *Anethum graveolens* plants

Foliar application	Aerial part dry weight (m <sup>-2</sup> )	Total soluble solid content (°Brix)	Relative water content (%)	Chlorophyll a content (mg g <sup>-1</sup> FWt)	Total chlorophyll content (mg g <sup>-1</sup> FWt)	Total chlorophyll content (mg g <sup>-1</sup> FWt)	K content (g kg <sup>-1</sup> )	Ca content (g kg <sup>-1</sup> )	Fe content (mg kg <sup>-1</sup> )	Mg content (g kg <sup>-1</sup> )	Zn content (mg kg <sup>-1</sup> )	Essential oil yield (ml m <sup>-2</sup> )
Control	268 ± 31 <sup>b</sup>	0.89 ± 0.11 <sup>b</sup>	78.1 ± 2.9 <sup>b</sup>	0.88 ± 0.09 <sup>b</sup>	1.2 ± 0.14 <sup>d</sup>	8.6 ± 0.50 <sup>b</sup>	2.8 ± 0.18 <sup>a</sup>	31.5 ± 1.4 <sup>a</sup>	0.95 ± 0.06 <sup>a</sup>	3.8 ± 0.19 <sup>b</sup>	0.287 ± 0.004 <sup>a</sup>	
Graphene oxide	308 ± 29 <sup>ab</sup>	1.05 ± 0.15 <sup>b</sup>	80.2 ± 2.6 <sup>ab</sup>	0.94 ± 0.08 <sup>b</sup>	1.3 ± 0.14 <sup>cd</sup>	8.6 ± 0.71 <sup>b</sup>	2.7 ± 0.18 <sup>a</sup>	30.7 ± 1.3 <sup>a</sup>	0.97 ± 0.06 <sup>a</sup>	4.4 ± 0.26 <sup>b</sup>	0.348 ± 0.003 <sup>ab</sup>	
Methanol	330 ± 30 <sup>ab</sup>	1.1 ± 0.13 <sup>b</sup>	82.3 ± 2.0 <sup>a</sup>	0.98 ± 0.08 <sup>b</sup>	1.4 ± 0.14 <sup>bc</sup>	13.2 ± 0.87 <sup>a</sup>	2.8 ± 0.15 <sup>a</sup>	29.6 ± 1.0 <sup>a</sup>	0.89 ± 0.06 <sup>ab</sup>	4.4 ± 0.26 <sup>b</sup>	0.401 ± 0.003 <sup>a</sup>	
KNO <sub>3</sub>	346 ± 34 <sup>a</sup>	1.6 ± 0.20 <sup>a</sup>	83.8 ± 2.5 <sup>a</sup>	1.2 ± 0.12 <sup>a</sup>	1.7 ± 0.18 <sup>a</sup>	11.2 ± 0.66 <sup>ab</sup>	2.4 ± 0.22 <sup>a</sup>	23.3 ± 1.0 <sup>b</sup>	0.75 ± 0.08 <sup>b</sup>	3.7 ± 0.28 <sup>b</sup>	0.414 ± 0.003 <sup>a</sup>	
Nano-Zn	356 ± 25 <sup>a</sup>	1.5 ± 0.21 <sup>a</sup>	83.1 ± 2.7 <sup>a</sup>	1.1 ± 0.11 <sup>a</sup>	1.5 ± 0.12 <sup>ab</sup>	9.2 ± 0.78 <sup>ab</sup>	1.9 ± 0.18 <sup>b</sup>	21.8 ± 1.0 <sup>b</sup>	0.80 ± 0.10 <sup>ab</sup>	9.9 ± 1.1 <sup>a</sup>	0.383 ± 0.002 <sup>a</sup>	

Significant differences among treatments are indicated by the different Latin letters

### 3.2 TSS Content

The trait was responsive to the foliar treatments (Table 1).  $\text{KNO}_3$  (1.6 °Brix) and nano-Zn foliar spray (1.5 °Brix) increased TSS content more than other treatments (Table 3).

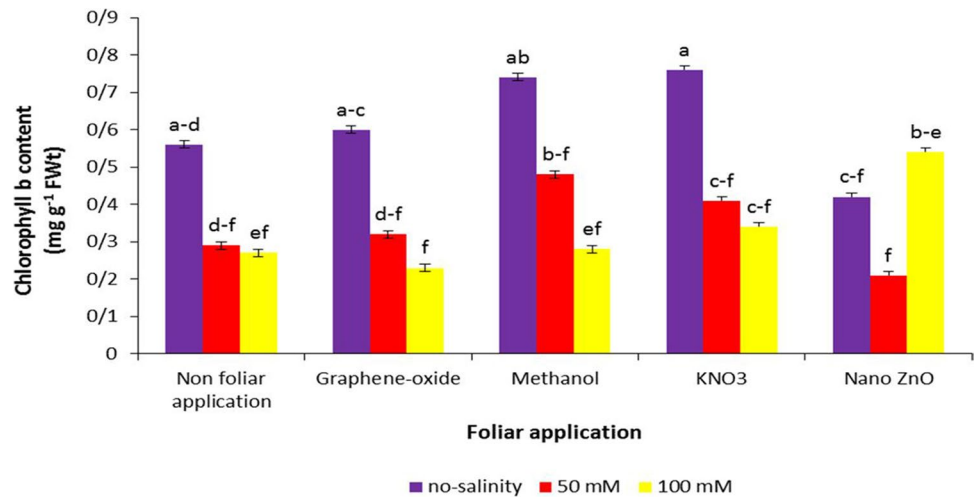
### 3.3 RWC

RWC was influenced by the independent effects of treatments (Table 1). All foliar treatments improved RWC (Table 3). Salinity, in contrast, reduced RWC. The highest RWC belonged to control (no-saline), and the least data was recorded for 100-mM salinity treatment (Table 2).

### 3.4 Chlorophylls a and b, and Total Chlorophyll

Chlorophyll a and total chlorophyll content was influenced by nano-Zn and  $\text{KNO}_3$  (Table 3). Salinity reduced chlorophyll a and total chlorophyll content, and the top chlorophyll a content was recorded for control (Table 2). In contrast, chlorophyll b was responsive to the treatment interactions, and the top data belonged to no-saline  $\times$  non-foliar application and no-saline  $\times$  GO, methanol, and  $\text{KNO}_3$  foliar treatments (Fig. 4).

**Fig. 4** Interaction effect of salinity levels (0, 50, and 100 mM NaCl) and foliar applications (no foliar, nano-Zn,  $\text{KNO}_3$ , methanol, and graphene oxide) on chlorophyll b content of *Anethum graveolens*. Significant differences among treatments are indicated by different Latin letters



**Table 4** ANOVA for the effect of salinity (0, 50, and 100 mM NaCl) and foliar applications (no foliar, nano-Zn,  $\text{KNO}_3$ , and graphene oxide) on *Anethum graveolens* plant elemental content

Significance	N	P	K	Ca	Mg	Fe	Mn	Na	Zn	K/Na
Replication	*	**	ns	ns	ns	**	*	**	ns	*
Salinity (S)	**	**	**	**	**	**	**	**	ns	**
Foliar (F)	**	**	**	**	*	*	*	*	**	**
S $\times$ F	*	*	ns	ns	ns	ns	*	*	ns	*

Significant differences among treatments are indicated by the different Latin letters

### 3.5 Proline Content

Salinity stress increased proline content. With a salinity of 100 mM, proline content in plant tissue was increased (114.7  $\mu\text{g g}^{-1}$  FWt) and the least proline content (38.5  $\mu\text{g g}^{-1}$  FWt) was devoted to control no-saline treatment (Table 2).

### 3.6 MDA and $\text{H}_2\text{O}_2$ Content

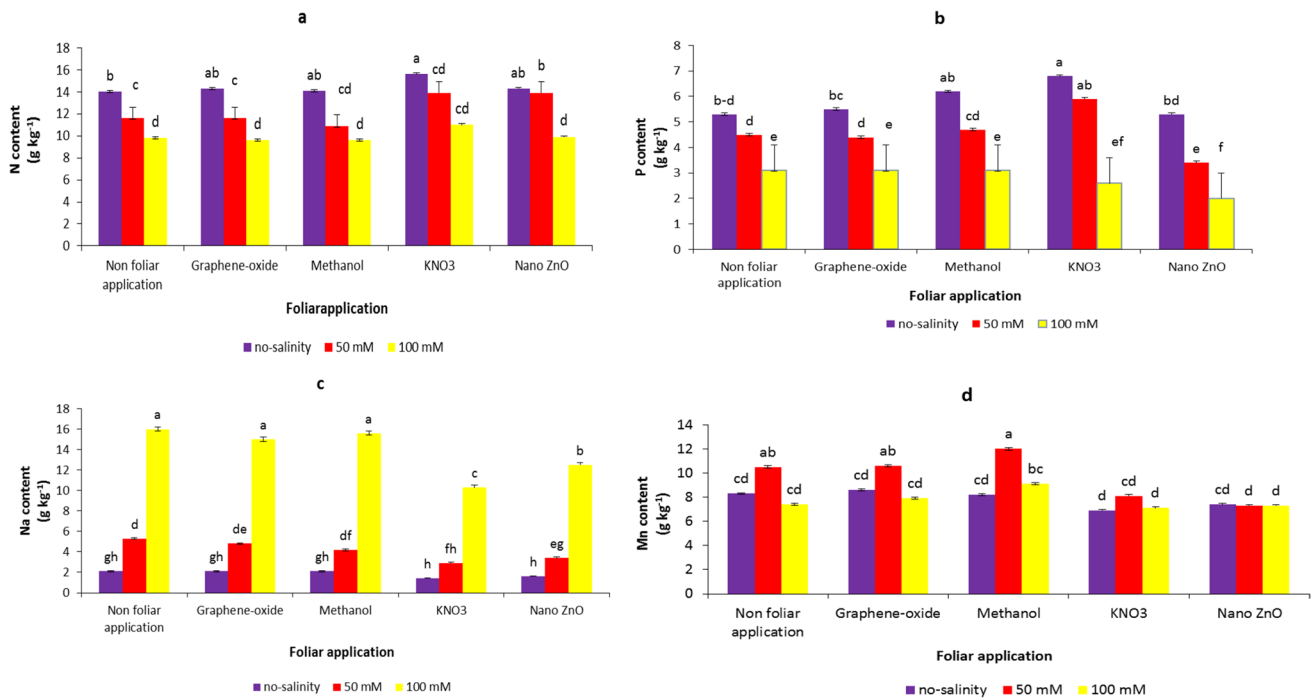
These traits were responded to the salinity raise with their high recorded amounts belonged to 100-mM salinity level (Table 2).

### 3.7 CAT Activity

The highest CAT activity was recorded for control plants. With adding up salinity levels to 100 mM, CAT activity was 40% decreased compared to control (Table 2).

### 3.8 Mineral Content

N, P, Mn, and Na were impacted by the treatment's interactions (Table 4). Foliar application  $\times$  no-salinity improved nitrogen content of plants (Fig. 5a). Foliar treatment with methanol and  $\text{KNO}_3$  under the normal no-saline condition and 50 mM



**Fig. 5** Interaction effect of salinity levels (0, 50, and 100 mM NaCl) and foliar applications (no foliar, nano-Zn, KNO<sub>3</sub>, methanol, and graphene oxide) on elemental content of *Anethum graveolens* plants. Significant differences among treatments are indicated by different Latin letters

NaCl × KNO<sub>3</sub> added up P content of plants (Fig. 5b). For Na, 100-mM salinity × no foliar and 100-mM salinity with graphene oxide and methanol foliar sprays attained the highest amounts (Fig. 5c). The sole 50-mM salinity and foliar sprayed with graphene oxide and methanol attained the top Mn content (Fig. 5d). K, Fe, Ca, and Mg content of plant tissues was influenced by the independent effects of salinity and foliar applications (Table 4).

The highest Fe content belonged to no-saline and 50-mM salinity (Table 2). With the salinity of 100 mM, Fe content decreased compared to control (20%) (Table 2). Furthermore, salinity declined Ca and Mg content of plants (Table 2). Moreover, Fe attained the highest data with no-foliar treatment and foliar sprayed with GO and methanol (Table 3). No-foliar treatment, as well as foliar GO, methanol, and KNO<sub>3</sub> application amended the plant's Ca<sup>2+</sup> content (Table 3). The least Mg amount was traced with the plants foliar sprayed with KNO<sub>3</sub> (Table 3). Foliar application of KNO<sub>3</sub>, methanol, and nano-Zn improved K content of plant samples (Table 3). K/Na ratio was responsive to the salinity, and the highest ratio was recorded with normal no-saline conditions. With salinity increase, the ratio was declined correspondingly (Table 2).

### 3.9 Essential Oil Content, Yield, and Oil Constituents

The oil content was influenced by salinity, and the highest oil content belonged to no-saline control and 50-mM

salinity treatment (Table 2). The oil yield was affected by the individual effects of salinity and foliar treatments (Table 1). Foliar treatments ameliorated essential oil yield (Table 3). Fifty-millimolar salinity and control ones attained the top oil yield (Table 2) GC/MS analysis revealed the occurrence of 21 constituents in the oil (Table 5). Dill-apiol (24.06–88.05%) was the major constituent; 100-mM salinity × methanol foliar treatment was the combination having the highest dill apiol content. Anethol (0.33–26.99) was the other constituent; its content was greatly influenced by the treatments. The maximum anethol content belonged to 50-mM salinity × no-foliar spray. The top amount of l-phellandrene (3.09–36.4) was detected in no-salinity × KNO<sub>3</sub> spray. Estragol (0–35.04%) was very variably distributed within the treatments. The highest amount of this compound belonged to 50-mM salinity × KNO<sub>3</sub> foliar spray. o-Cymene (0–6.87%) had the highest percentage with 100 mM × KNO<sub>3</sub> treatment (Table 5).

## 4 Discussion

Salinity adversely impacts the growth and physiological responses of plants in the main part via the hyper-accumulation Cl<sup>-</sup> and Na<sup>+</sup> ions, and the direct and indirect footprints on the photosynthesis rate, ion homeostasis, and water balance (Safikhani et al. 2018).



**Table 5** Essential oil composition of *Anethum graveolens* plants exposed to the salinity and foliar application of nano-Zn, KNO<sub>3</sub>, methanol, and graphene oxide

Compound	RT	RI	Percent of compounds (%)																
			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15		
α-Pinene	8.756	1029	0.216	0	0	0	1.48	0	0	0	0	0.812	0	0	0	0	0	1.315	
β-Pinene	10.884	1119	0	0	0	0	0.434	0	0	0	0	0	0	0	0	0	0	0.411	
β-Myrcene	12.361	1166	0	0	0	0	0.385	0	0	0	0	0	0	0	0	0	0	0.352	
<b>l-Phellandrene</b>	<b>12.555</b>	<b>1172</b>	<b>10.927</b>	<b>7.174</b>	<b>5.915</b>	<b>11.97</b>	<b>36.436</b>	<b>4.639</b>	<b>3.957</b>	<b>4.786</b>	<b>3.156</b>	<b>24.648</b>	<b>8.189</b>	<b>3.451</b>	<b>3.091</b>	<b>4.703</b>	<b>30.226</b>		
dl-Limonene	13.688	1207	1.025	0.75	1.141	1.622	2.453	1.453	0.676	0.727	1.108	2.089	0.92	0.53	0	0	0	2.294	
β-Phellandrene	14.083	1218	2.167	1.552	1.355	2.212	5.263	0.862	0.701	0.784	0	3.544	1.253	0.598	0	0	0	4.886	
cis-OCimene	14.856	1238	0	0	0	0	0	0	0	0	0	1.303	0	0	0	0	0	0.309	
β-cis-OCimene	15.525	1256	0	0	0	0	0	0	0	0	0	0.858	0	0	0	0	0	0.169	
<b>o-Cymene</b>	<b>16.389</b>	<b>1278</b>	<b>4.006</b>	<b>2.89</b>	<b>2.502</b>	<b>3.257</b>	<b>6.718</b>	<b>1.447</b>	<b>1.117</b>	<b>1.271</b>	<b>0</b>	<b>3.734</b>	<b>1.323</b>	<b>0.915</b>	<b>0</b>	<b>1.204</b>	<b>6.871</b>		
D-Fenchone	21.607	1408	0	0	0	0	0	0.98	0.301	0	0	0	0	0	0	0	0	0	
Fenchyl acetate	25.407	1501	0	0	0	0	0	0	0.216	0	0	0	0	0	0	0	0	0	
Dill ether	26.334	1524	1.505	1.634	1.896	2.05	2.086	1.085	0.986	1.006	0.589	1.351	0.834	0.739	0	0	0	1.411	
(-)-cis-Carane	28.108	1569	0.482	0	0.552	0	0	0	0	0	0	0	0	0	0	0	0	0	
Estragole	32.307	1678	0	0	0	0	2.048	0.791	0.252	0	0	35.047	0	0	0	0	12.306		
Germacrene D	33.812	1717	3.627	3.078	3.38	3.208	0.979	1.903	1.827	1.469	2.121	0.783	1.952	1.658	2.203	1.708	0.839		
α-Phellandrene epoxide	37.371	1810	1.161	1	1.289	1.138	0.736	0	0.6	0	0	0	0.542	0	0	0	0.533		
<b>Anethole</b>	<b>38.321</b>	<b>1832</b>	<b>2.408</b>	<b>1.827</b>	<b>6.433</b>	<b>11.503</b>	<b>0.331</b>	<b>26.993</b>	<b>10.032</b>	<b>7.955</b>	<b>9.164</b>	<b>1.204</b>	<b>4.839</b>	<b>3.382</b>	<b>4.23</b>	<b>9.59</b>	<b>0.362</b>		
Methyl eugenol	45.382	2017	0	0	0	0	0	0	0	0	0	0.567	0	0	0	0	0	0.256	
Thymol	50.447	2217	0.785	0.855	0.735	0.696	0.417	2.204	1.343	0.915	1.177	0	0.814	0.867	1.645	1.852	0.364		
<b>Dill-apiole</b>	<b>53.399</b>	<b>2371</b>	<b>70.749</b>	<b>78.061</b>	<b>73.691</b>	<b>61.792</b>	<b>39.532</b>	<b>56.495</b>	<b>75.821</b>	<b>80.198</b>	<b>81.003</b>	<b>24.06</b>	<b>78.611</b>	<b>86.963</b>	<b>88.054</b>	<b>76.653</b>	<b>36.686</b>		
Apiol	55.516	2497	0.942	1.178	1.111	0.55	0.701	1.148	2.17	0.887	1.68	0	0.722	0.896	0.776	4.29	0.409		
Total		100	100	99.999	100	99.998	99.999	100	99.999	99.998	99.998	100	99.999	99.999	99.999	100	99.999		

T1, NaCl<sub>0</sub> × no spray; T2, NaCl<sub>0</sub> × graphene oxide; T3, NaCl<sub>0</sub> × methanol; T4, NaCl<sub>0</sub> × nano-Zn; T5, NaCl<sub>0</sub> × KNO<sub>3</sub>; T6, NaCl<sub>50</sub> × no spray; T7, NaCl<sub>50</sub> × graphene oxide; T8, NaCl<sub>50</sub> × methanol; T9, NaCl<sub>50</sub> × nano-Zn; T10, NaCl<sub>50</sub> × KNO<sub>3</sub>; T11, NaCl<sub>100</sub> × no spray; T12, NaCl<sub>100</sub> × graphene oxide; T13, NaCl<sub>100</sub> × methanol; T14, NaCl<sub>100</sub> × nano-Zn; T15, NaCl<sub>100</sub> × KNO<sub>3</sub>

Foliar nourishment with nano-Fe and Zn improves photosynthetic potential and also diminishes the toxic effects of  $\text{Na}^+$  inside cells and hence, ameliorates the growth potential and productivity of plants (Hassanpouraghdam and Vojodi Mehrabani, 2019). In coriander, salinity negatively influenced yield, but  $\text{KNO}_3$  foliar treatment reduced the adverse salinity depressions. Seemingly,  $\text{K}^+$  foliar treatment hinders  $\text{Na}^+$  translocation and accumulation from the roots towards the aerial parts and hence, keeps the photosynthesis potential of plants while reducing  $\text{Na}^+$  toxic behaviors (Elhindi et al. 2016 a). GO treatment improves the photosynthetic apparatus activity, elevates soluble sugar content, and stabilizes cell membranes especially under saline-sodic conditions, and thereupon recovers the growth and productivity potential (Pandey et al. 2018; Safikhan et al. 2018). In line, Valizadeh Kamran et al. (2019) reported that in lavender, the dry weight, chlorophylls, phenolics, flavonoids, and  $\text{K}^+$  content of plants were influenced by methanol treatments under salinity. Methanol is feasibly metabolized by the plants since the compound easily participates in photosynthesis and, in carbohydrates and amino acid biosynthetic pathways and, hereinto improves the growth and yield-related traits (Yazdi Far et al. 2015). Zn holds dominant actions in the activity of key enzymes in carbohydrates metabolism, i.e., carbonic anhydrase, ribulose 1,5-bisphosphate carboxylases/oxidases, and fructose 1,6 biphosphate (Marschner 2012). Furthermore, Amjad et al. (2014) reported that tomato foliar treatment with potassium under salinity increased the plant carbohydrate content. Potassium has functional roles in water translocation, carbohydrate metabolism, stomatal action, cell growth, enzyme activity, osmotic regulation, water equilibrium, and cell membrane stability (Kaya et al. 2007).

Salinity in coriander reduced the RWC and cell growth. However,  $\text{KNO}_3$  foliar treatment re-directed the RWC and mitigated the salinity effects (Elhindi et al. 2016 a). GO application in *Silybum marianum* increased cell water potential via the intensified metabolism and agglomeration of compatible solutes inside cells (Safikhan et al. 2018). Pandey et al. (2018) demonstrated that the elevated expression of aquaporins in salinity-exposed plants foliar sprayed with GO was the reason for the improved water relations and hence, the enhanced growth and yield responses.

The increase in chlorophyll content by the  $\text{KNO}_3$  foliar spray has been reported in coriander under salinity (Elhindi et al. 2016 a). Salinity interferes in the absorption and translocation of Fe and Mg, thereby, hinders their role in chlorophyll biosynthesis and hence greatly declines the growth, quality, and yield attributes (Hassanpouraghdam and Vojodi Mehrabani, 2019). K keeps essential functions in pH equilibrium during photosynthesis (photosystem II), water balance, cell and tissue turgidity, stomatal opening behavior, carbohydrate metabolism, and accumulation, enzymatic activity, and eventually in regular photosynthesis process

(Marschner 2012). Zn is another micro-nutrient that plays crucial functions in the activity of several enzymes involved in carbohydrate metabolism, keeps cell membrane integrity, and smoothens the side effects of ROS radicals (Hafeez et al. 2013). Safikhan et al. (2018) noted that salinity added up proline content in milk thistle. Proline accumulation is a reliable physiological stressor's tolerance marker in the cells (Grattan and Grieve 1998). Furthermore, proline availability inhibits the toxic ion absorption, maintains cell membrane integrity, and hereby helps in cell survival, and even fortifies the cells and photosynthetic tissues' intrinsic physiological duties (Grattan and Grieve 1998).

Safikhan et al. (2018) in *Silybum marianum* and Chrysoargyris et al. (2018) in lavender reported that salinity multiplied MDA and  $\text{H}_2\text{O}_2$  accumulation in plants. Under salinity, MDA is over-generated by the cell membrane deterioration (Liang et al. 2018). Damages on cell membranes via the ROS molecules are the dominant side effect of salinity depression which denature the cell membrane-anchored proteins and phospholipid bilayers and wherefore, interfere with the normal metabolism and function of cells.

Dong et al. (2015) reported the adverse effects of salinity on CAT activity in cotton plants. CAT is predominantly synthesized in peroxisome and glyoxylic acid-circulating bodies. This enzyme is a key scavenger of  $\text{H}_2\text{O}_2$  molecules produced by light transpiration (Willekens et al. 1994). CAT converts  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  and oxygen (Kang and Saltveit 2002). Any increase in the CAT, peroxidase, and ascorbate activities under salinity declines the cell membranes' deterioration and hence, improves the plants' withstand versus environmental stress factors (Willekens et al. 1994). Salinity interferes with the nutrient absorption and partitioning inside plants. Substitution of  $\text{Ca}^{2+}$  ions in the cell walls by  $\text{Na}^+$  obstacles the activity of carriers and ionic channels within the root cells and hence, by the partial and/or total blockage of essential nutrients and water absorption and even with the uncontrollable ion distribution and relocation greatly reduce the growth potential of plants (Guo et al. 2015).

Moreover, perturbation in the availability and acquisition of the nutrients hugely diminish the chlorophyll and carotenoid content by the chloroplast's structural breakdown, photosynthetic apparatus demolition, chlorophyll photo-oxidation, and/or by the prevented new chlorophyll biosynthesis, and ultimately detracts the growth and productivity of plants (Neocleous and Vasilakakis 2007). In pelargonium, foliar treatment of methanol and ethanol lessened the salinity defects and improved the absorption of Fe and  $\text{K}^+$  (Vojodi Mehrabani 2019).

$\text{KNO}_3$  foliar treatment of salinity-exposed plants improved their mineral absorption (Elhindi et al. 2016). The elevated  $\text{K}^+$  content under salinity is an authentic tolerance evidence. With the high potassium content, plants are capable of retarding  $\text{Na}^+$  sorption and relocation to the aerial

parts, and hence attain the intensified tolerance versus stress factor (Munns and Tester 2008).

Potassium has a primordial function in several metabolic processes, i.e., stomatal actions, carbohydrate partitioning, cell growth, osmotic regulation, cell pH equilibrium, water balance, and membrane stability (Kaya et al. 2007). In wheat plants, salinity impeded the acquisition of N, K, Ca, and P. However, nano-Zn and Fe treatment improved the absorption of the nutrients and resumed plants' normal growth (El-Fouly et al. 2011). Furthermore, nano-Zn owing to the very high specific area, the enhanced mobility, and high reaction potential improved the metabolism of IAA and by the accelerated new root generation enhanced the mineral nutrient absorption and further translocation (Pessaraki 2016). Foliar Zn application under saline-sodic conditions reduced Na absorption and improved membrane stability. Moreover, chlorophyll biosynthesis was stimulated leading to the lessened salinity depression (Tufail et al. 2017).

Essential oil biosynthesis is under the control of genetic and epigenetic stimuli. Essential oils have diverse roles in the protection of plants against pests, diseases, and environmental stressors (Chrysargyris et al. 2018; Elhindi et al., 2016a). Salinity adversely affects the primary and subsequently the secondary metabolism and the active metabolite profile of plants (Chrysargyris et al. 2019). In pelargonium, methanol foliar treatment ameliorated the oil content of plant (Vojodi Mehrabani 2019). Seemingly, foliar application of alcoholic compounds fortifies the tolerance mechanism of plants by the accelerated biosynthesis of phenolics and terpenoid constituents (Gout et al., 2000; Valizadeh Kamran et al. 2019). In *Ocimum basilicum*, salinity increased linalool and eugenol content of the plant (Elhindi et al., 2016b). Moreover, salinity  $\times$  foliar Fe and Zn spray increased basil linalool content (Said-Al Ahl and Mohmoud, 2010). Furthermore, no-saline  $\times$  Fe and Zn foliar spray ameliorated methyl-chavicol content in basil plants (Said-Al Ahl and Mohmoud 2010). Zn has a predominant function in photosynthesis and glucose formation. Glucose serves as an energy source and carbon skeleton for the terpenoid biosynthesis. So, Zn application more possibly improves the oil content and composition (Hafeez et al. 2013; Vojodi Mehrabani et al. 2018). (Pandey et al. (2018) reported that GO treatment reduced  $\text{Na}^+$  absorption from the growing medium and improved the plants' growth and metabolism under stressful harsh conditions.

## 5 Conclusions

The results revealed that salinity adversely affected the growth and physiological responses of dill plants. However, foliar application of the compounds experienced in the present experiment increased the aerial part biomass

and essential oil yield. Foliar methanol and  $\text{KNO}_3$  application raised the chlorophyll content of plants. Nano-Zn and  $\text{KNO}_3$  foliar spray enhanced the total soluble solids content of plants as well. With salinity added up, proline and malondialdehyde content was increased. Foliar treatments and no-saline conditions raised the nitrogen content of plants.  $\text{KNO}_3$ , nano-Zn, and methanol under normal-no-saline conditions improved the plant's phosphorus content. Dill-apiol (24.06–88.5%) was the major essential oil constituent;  $\text{NaCl}_{100\text{mM}} \times$  methanol treatment attained the highest dill-apiol content. Foliar treatments partially reduced the adverse side effects of salinity, and the response was treatment dependent. Overall, the foliar treatments had partial advantages in the amelioration of salinity defects, and the results could be of interest to the research sections for more detailed studies, but need further in-depth assays with a wide range of salinity and foliar treatments to be advisable for the extension section and pioneer farmers.

**Availability of Data and Material** All-new research data were presented in this contribution.

**Code Availability** Not applicable.

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## Declarations

**Conflict of Interest** The authors declare no competing interests.

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