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Research article

Glycine betaine functionalized graphene oxide as a new engineering nanoparticle lessens salt stress impacts in sweet basil (*Ocimum basilicum* L.)

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ABSTRACT

Regarding destructive impacts of salinity on different vital processes of plants, many strategies have been developed to alleviate salinity effects. Amongst, nanoparticles (NPs) application has been achieved great attention. For that point, considering positive effects of graphene oxide NPs (GO) and glycine betaine (GB) on different plant processes, GO-GB NPs were primarily synthesized to use GO as a carrier for GB. Then, GO, GB and GO-GB (each in three concentrations; 0, 50 and 100 mg L^{-1}) were applied on sweet basil (*Ocimum basilicum* L.) plants under 0, 50 and 100 mM salinity stress conditions. The results demonstrated that GO-GB NPs could lessen negative effects of salinity by enhancing agronomic traits, photosynthetic pigments, chlorophyll fluorescence parameters, membrane stability index (MSI), proline, phenols, antioxidant enzymes activities and dominant constituents of essential oils and decreasing MDA and H_2O_2. These positive effects were more considerable at its lower dose (50 mg L^{-1}) introducing it as the best treatment to ameliorate sweet basil performance especially essential oil compounds under salt stress. GO application at its higher dose (100 mg L^{-1}) demonstrated toxicity by negative impacts on the measured parameters. In conclusion, the positive response of sweet basil to GO-GB NPs under non-stress and salt stress conditions cause to consider the NPs as potential novel plant growth promoting and stress protecting agent with innovative outlooks for its use in agriculture.

1. Introduction

Ocimum basilicum L. (sweet basil), an aromatic herb and probably the most important species of Lamiaceae family, has antioxidant properties (Jayasinghe et al., 2003). Sweet basil has various uses in industries like pharmaceutical, food, sanitary and fragrance productions (Raja, 2012). Methyl chavicol, linalool, citral, eugenol, cineol, geranium, camphor and methyl cinnamate are key constituents of sweet basil essential oil (Simon et al., 1990). Consequently, sweet basil could be considered as one of the main medicinal/aromatic plants (Simon et al., 1990).

Amongst all environmental stresses, the most restraining cause for plants distribution in habitats is salt stress (Tang et al., 2015). Salinity is an important global problem with destructive impacts on plants (Isayenkov and Maathuis, 2019) leading to different biochemical and metabolic changes in plants through induced oxidative stress that disturbs metabolism, growth, performance and productivity of plants (Xiong and Zhu, 2002). Regarding negative impacts of salinity, some chemicals (e.g., Gohari et al., 2020a; Ahanger et al., 2020, Kaya et al., 2020; Noreen et al., 2020) and nanoparticles (NPs) (e.g., Ye et al., 2019; Gohari et al., 2020b, c; Shah et al., 2020) have been applied to modulate these impacts as novel approaches. Consequently, nanotechnology offers different profits in this regard (Ioannou et al., 2020) through confidential impacts of NPs on plant growth and development and additionally in plant tolerance to abiotic stresses as previously confirmed (e.g., Tripathi et al., 2017; Vishwakarma et al., 2018; Rastogi et al., 2019 a,b; Ahmad and Akhtar, 2019). NPs enhance plant tolerance via ROS detoxification (Rico et al., 2015), protecting photosynthesis process (Khan et al., 2017) and antioxidant enzyme-like effect that reduce osmotic and oxidative stresses (Rico et al., 2015; Khan et al., 2017).

Graphene oxide (GO), a water-soluble derivative of graphene with 2-D structure, has distinctive properties (Wang et al., 2014) that could be applied in agricultural sector especially for plants under stress (Safikhan et al., 2018). Beneficial impacts of GO applications on growth and

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development of faba bean, wheat, tomato, maize and apple were previously reported by Anjum et al. (2014), Hu et al. (2014), Zhang et al. (2015), Ren et al. (2016) and Li et al. (2018), respectively. Graphene application resulted in enhanced phenols, flavonoids, ascorbic acid, glutathione, photosynthetic pigments and activity of APX, GPX, CAT and PAL enzymes introducing its positive role in plant facing stress conditions (González-García et al., 2019). In addition, GO positive roles on plants under salt stress were recorded (Yao et al., 2018; Safikhan et al., 2018). GO increased growth and biomass, chlorophyll content, photosystem efficiency, performance index, membrane stability index, proline, soluble carbohydrate content and cell water potential through enhancing the net concentration of solutes in cells of milk thistle plant under salinity condition (Safikhan et al., 2018). However, there were some reports on ecotoxicological and phytotoxicological effects of graphene oxide in environment and different plant species (Ghorbanpour et al., 2018; Yin et al., 2020; Weng et al., 2020).

Glycine betaine (GB), a free amino acid and compatible solute, has various key functions in cellular osmotic adjustment, maintaining cell organelles (e.g., mitochondria, chloroplast), improving water-use efficiency (Tisarum et al., 2020) and enzyme and membrane integrity (Annunziata et al., 2019). GB is a non-toxic compound even at high doses (Ahmad et al., 2013; Xu et al., 2018). Importantly, GB has protective actions against different stresses like salinity and ameliorates their destructive effects (Ahmad et al., 2013; Roychoudhury and Banerjee, 2016; Sofy et al., 2020). GB content increases under stress condition and acts as osmolyte, ROS quencher and gene expression inducer (Ahmad et al., 2013; Xu et al., 2018). GB enhances photosynthetic rate (Li et al., 2019), protects various enzymes activities involved in stress protection (e.g., Rubisco, malate dehydrogenase particularly under salt stress) or ROS detoxification (enzymes in ascorbate-glutathione cycle) and refolding proteins and increasing their thermal stability. Consequently, exogenous application of GB is considered as an easy and successful method to cope with stress conditions (Xu et al., 2018; Annunziata et al., 2019; Tisarum et al., 2020).

Given the facts of encouraging influences of GO and GB on plant growth and physiological parameters under non-stress and stress conditions, their collaboration in the nano-structure "GO-GB" might enhance these positive effects. GO-GB NPs might cause better efficiency of GB particularly at its lower doses and using GO as a carrier for it. Therefore, GO-GB NPs were synthesized in advance and then applied on sweet basil to lessen salinity effects, to the best of our knowledge as the first report.

2. Materials and methods

All chemicals such as graphite powder, $KMnO_4$, N,N′-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP) and glycine betaine were purchased from Sigma-Aldrich.

FT-IR spectrum was recorded by using a PerkinElmer 781 spectrometer. SEM image was collected by MIRA3 TESCAN microscope.

2.1. Experimental site, plant materials, applied treatments and sampling time

The experiment was conducted in the research greenhouse of the Faculty of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran (longitude 47°71′ E, latitude 37°42′ N, altitude 1115 m). The study was done as factorial experiment using a completely randomized design (CRD) in three replications. Sweet basil (*Ocimum basilicum* L.) seeds were purchased from Pakan Bazr Company (Isfahan, Iran). Seeds surface sterilization was done with 1% (v/v) sodium hypochlorite (NaOCl) for 5 min, washed three times with distilled water and finally soaked in distilled water for 10 min. Seeds were subsequently wetted with tap water and let to germinate for a week. After seedling emergence, five plants were transplanted into each 5-kg pot containing a mixture of coco peat and medium grain perlite in a ratio of 3:1. Then, planted pots irrigated with one-quarter Hoagland solution. Three weeks after sowing, salinity stress was applied daily in uniform seedlings (eight-leaf stage) at three concentrations (0, 50 and 100 mM NaCl), in combination with quarter-strength Hoagland solution, and continued up to plant harvest (prolonged stress). The treatments including graphene oxide (GO), glycine betaine (GB) and Glycine betaine functionalized graphene oxide nano particle (GO-GB) each in three concentrations (0, 50 and 100 mg L^{-1}) were applied three weeks after imposing salinity stress application through spraying method four times with 48 h intervals. Control plants were irrigated daily with quarter-strength Hoagland solution until harvest at the same manner and treated with 0 mM NaCl and 0 mg L^{-1} GO or GB or GO-GB. We used to apply tween 20% with our compounds in order to fix them to the leaf surface and help to penetrability when they are applied in foliar spraying. All measurements were performed at the harvest stage. Three technical replications were set for each measurement of the parameters.

2.2. Preparation of GO and functionalization of GO by glycine betaine (GO-GB) synthesis

A modified Hummers' method was utilized to synthesis of GO from natural graphite powder (Marcano et al., 2010). Typically, in a 250 mL round bottom flask containing 70 mL H_2SO_4/HNO_3 solution (9/1 v/v), 0.5 g of graphite powder and 3 g of $KMnO_4$ were added and stirred at 50 °C for 12 h. Then the reaction mixture was cooled to room temperature and transferred into the 70 mL ice with 5 mL H_2O_2 (30%). Obtained product was separated by centrifuge (8000 rpm for 30 min) and washed with DI water and HCl (0.1 N) repeatedly. The resulted GO was freeze-dried overnight and kept in a sealed container for using in the next step.

In order to attach glycine betaine (GB), synthesized GO (100 mg) was dispersed in 5 mL ethanol throughout ultra-sonication for 20 min and then DCC (300 mg) and DMAP (30 mg) were poured to the reaction mixture. In the next step, appropriate amount of glycine betaine was added to the above solution and the reaction was carried out at 80 °C for 48 h. After completion the reaction, GO-GB was separated by centrifuge (8000 rpm) and washed well with water and ethanol.

2.3. Agronomic traits assay

Agronomical parameters including plant height, inflorescence length, inflorescence dry and fresh weights, node number, leaf number, leaf fresh and dry weights and shoot fresh and dry weights were assayed. Three plant's leaf and shoot samples were individually weighed for their fresh weights and then kept in the oven (70 °C, 72 h) for dry weights measurements at the harvest stage.

2.4. Photosynthetic pigments assay (chlorophyll a, b and carotenoids)

The young and fully expended leaves (0.2 g) were extracted in 0.5 mL acetone (3% v/v) and then centrifuged (10,000 rpm, 10 min) and the absorption of the obtained supernatant was recorded at 645 nm (chlorophyll (Chl) *b*), 663 nm (Chl *a*) and 470 nm (carotenoids) by UV–Vis spectrophotometry (UV-1800 Shimadzu, Japan). Then Chl *a*, *b* and carotenoids contents were calculated through the equations (Sharma et al., 2012).

Chl a = (19/3 * A663 – 0/86 *A645) V/100W

Chl b = (19/3*A645–3/6*A663) V/100W

Carotenoids = 100(A470)–3/27(mg chl *b*) /227

Note: V= Solution volume of the filtrate, A = Light absorption in wavelengths 663, 645 and 470 nm and W= Sample fresh weight (g).

2.5. Chlorophyll fluorescence and SPAD assay

A dual-pam-100 chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany) was used to measure chlorophyll fluorescence parameters including $^{Fv}/_{Fo}$, $^{Fv}/_{Fm}$, Y (NO) and Y (II) in the young and fully expended leaves. The measurement was done after the plants adaption in the dark for 20 min (Maxwell and Johnson, 2000). Three technical replications were set for each measurement of the parameters.

Five randomly selected leaves of each pot were used to determine SPAD values (leaf chlorophyll concentrations) via a SPAD-meter (502 Plus Chlorophyll Meter, Japan) (Ling et al., 2011).

2.6. Membrane stability index (MSI), malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) assay

MSI was assayed through the protocol described by Sairam et al. (1997) and calculated according to the equation:

$$MSI = [1\text{-}(C1/C2)] \times 100$$

where C1 is EL content after being exposed at 40 °C and C2 is EL content after being exposed at 100 °C.

Leaves (0.1 g) were homogenized with 2.5 mL acetic acid (10% w/v) solution. After centrifuging (15,000 rpm, 20 min), the same volume of the extract and thiobarbituric acid (0.5% w/v) in trichloroacetic acid (TCA) (20%) were transferred to the test tube for 30 min at 96 °C. Lastly, after incubating extracts at 0 °C for 5 min, they centrifuged (10,000 rpm, 5 min) and their absorbances were recorded at 532 and 600 nm by the spectrophotometer. MDA content was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ using the following equations:

$$MDA\ (nmol\ g^{-1}\ FW) = [(A532\text{-}A600) \times V \times 1000/\epsilon] \times W$$

Note: ϵ = the specific extinction coefficient, V = the volume of crushing medium, W = the leaf FW, A 600 = absorbance at 600 nm and A 532 = the absorbance at 532 nm (Stewart and Bewley, 1980).

To determine H_2O_2 content, 0.2 g leaves were mixed with 5 mL trichloroacetic acid (0.1% w/v) in an ice bath. After centrifuging (12,000 rpm, 15 min) and obtaining the supernatant, to 0.5 mL potassium phosphate buffer (pH 6.8, 10 mM) and 1 mL potassium iodide (1 M), 0.5 mL supernatant was added and the mixture absorbance was recorded at 390 nm H_2O_2 content was calculated by standard calibration curve previously made by various H_2O_2 concentrations and expressed as μmol g^{-1} FW (Sinha et al., 2005).

2.7. Proline quantification and total phenolic compounds

Proline quantification.

Ninhydrin method was used to assay proline content of leaf samples. Leaf samples (0.5 g) were homogenized in 10 mL of 3% aqueous sulfosalicylic acid and placed in an ice bath. After centrifuging the mixture (1000 rpm, 4 °C), 2 mL ninhydrin acid and 2 mL glacial acetic acid were added to 2 mL obtained supernatant and finely mixed and incubated at 100 °C for 1 h. The reaction was stopped in an ice bath and finally 4 mL toluene was added and mixed vigorously (20 s). The mixture absorbance was recorded at 520 nm using a spectrophotometer. Different concentration of L-proline was used for standard curve and final calculation of proline values (Marín Velázquez et al., 2009).

Folin-Ciocalteu method was used to assay total phenolics. After digesting leaf samples (0.1 g) with 95% ethanol (5 mL), the mixture was kept in dark for 24 h. Then, 1 mL 95% ethanol and 3 mL distilled water were added to 1 mL of supernatant. Next step was adding 0.5 mL 50% Folin-Ciocalteu solution and 1 mL 5% sodium bicarbonate. After 1 h in the dark, the absorbance was recorded at 725 nm using a spectrophotometer. The absorbance values were converted to total phenols and expressed as mg chlorogenic acid (CA) g^{-1} FW. Different concentrations of chlorogenic acid were used as standards (Xu et al., 2010).

2.8. Assay of antioxidant enzymes activity

Antioxidant enzymes activities were assayed through young and fully expanded leaves. All steps of enzyme extraction were carried out at 4 °C as follows: leaves (0.5 g) were homogenized with potassium phosphate buffer (pH 6.8, 100 mM) containing 1% polyvinylpyrrolidone (PVP) and EDTA (4 mM) using magnetic stirrer for 10 min. Later centrifuging (6000 rpm, 20 min), the supernatant was collected to evaluate ascorbate peroxidase (APX), superoxide dismutase (SOD) and guaiacol peroxidase (GP) enzymes activities based on the same procedures described by Gohari et al. (2020b).

2.9. Essential oil extraction and profiling

Essential oil of sweet basil was obtained air-dried powdered aerial parts (50 g) using the hydrodistillation technique and heated by heating jacket at 100 °C for 2 h in an all-glass Clevenger-type apparatus, according to the procedures outlined in the European pharmacopeia. The collected crude essential oil was dried over anhydrous sodium sulfate and then stored in sealed glass vials. Obtained samples were evaluated for its components by gas chromatography/mass spectrometry (GC/MS) instrument (Agilent 6890N GC and Agilent 5973 mass selective detector operating in the electron ionization mode) according to Hussain et al. (2008).

2.10. Statistical analysis

All obtained data analysis was performed by SAS software and the means of each treatment (with three technical replications) were analyzed by Duncan's multiple range test at the 95% level of probability (SAS Institute Inc., ver. 9.1, Cary, NC, USA).

3. Results and discussion

3.1. GO-GB synthesis and characterization

The GO-GB was fabricated by the reaction between GO and GB via two steps illustrated in Fig. 1. At first, strong oxidation, Hummers' method, of bare graphite powder was carried out and various carboxylic acid, hydroxy and epoxy functional groups were introduced between carbon layers of graphite. In the second step, DCC chemistry (Siriviriyanun et al., 2015) led to the formation of ester bond via condensation reaction between the carboxy group of glycine betaine and hydroxy groups on the GO surface. As-prepared GO-GB was characterized using FT-IR, SEM and EDX analyses.

The existence of various functional groups as well as the formation of ester group were confirmed using FT-IR technique. As it could be seen from Fig. 2. A, the FT-IR spectrum showed some important characteristic bands of GO including epoxy C–O (1228 cm^{-1}), hydroxy –OH (3428 cm^{-1}), aromatic C═C (1626 cm^{-1}) and carboxy C–O (1415 cm^{-1}). Two new characteristic bands at 2850 cm^{-1} and 1570 cm^{-1} appeared in the spectrum of GO-GB, related to the $–CH_3$ stretching vibration of GB and ester C═O of GO-GB respectively, indicating that GB has been successfully grafted onto GO surface. The surface morphology and elemental composition of synthesized GO-GB were determined by SEM and EDX analyses, respectively. From Fig. 2. B, a clear sheet like structure could be seen for GO-GB sample. Moreover, SEM image showed that the initial layered structure of GO remains intact even after functionalization by glycine molecules. This surface morphology is in a good agreement with previous report (Eftekhari et al., 2018; Amini et al., 2018). The EDX result is exhibited in Fig. 2. C, which proves the presence of C, O and N elements in the structure of GO-GB. The content of C, O and N are 69.25%, 14.94% and 14.71%, respectively.

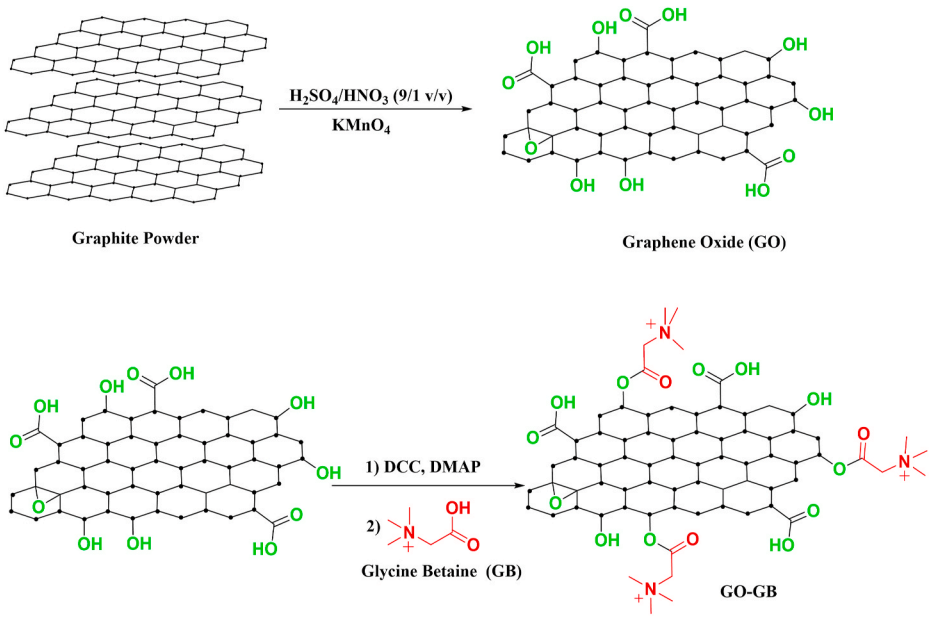

Fig. 1. Schematic reaction pathway for GO-GB synthesis. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide.

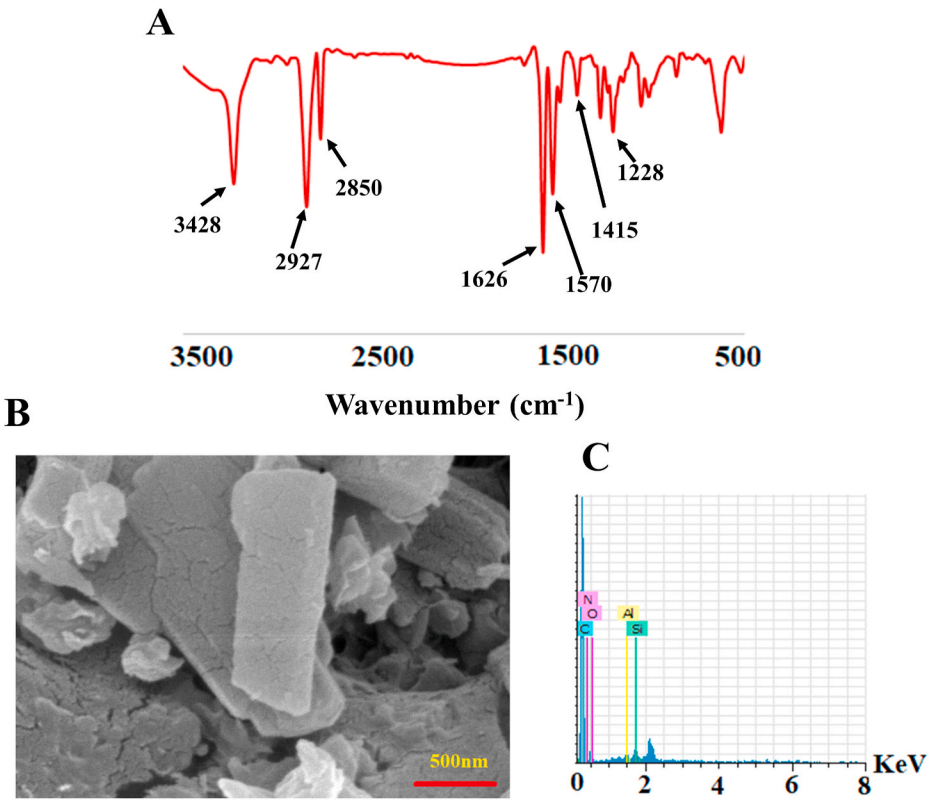

Fig. 2. FT-IR spectrum (A), SEM image (B) and EDX profile (C) of synthesized GO-GB NPs.

3.2. Agronomical traits

Regarding all agronomic traits, salinity caused reduction in all traits. Under non-stress condition, except 100 mg L^{-1} GO, the other treatments had no effect or increased the values of the traits in comparison with the control. Mostly, the treatments caused enhancement in the traits values, not including 100 mg L^{-1} GO with similar or reduced values as compared to sweet basil under 50 and 100 mM salinity conditions with any treatment. Considering non-stress and both stress conditions, GO-GB NPs at 50 mg L^{-1} generally acted as the best treatment. The negative effect of GO at 100 mg L^{-1} concentration could be attributed to its toxic effect (Table 1).

Significant reduction in agronomic traits of sweet basil under salinity stress was previously reported (Gohari et al., 2020a, c). Safikhan et al. (2018) informed that GO caused a significant enhancement in plant height, growth and total biomass under non-stress and particularly salinity conditions. Likewise, González-García et al. (2019) report positive effect of graphene on growth due to improving water and nutrient

Table 1
Effect of different concentrations of glycine betaine functionalized graphene oxide nano particles on morphological parameters of *O. basilicum* L. under salinity stress. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. Different letters indicate significantly different values at $p < 0.05$.

NaCl (mM)	Treatments	Plant Height (cm)	Inflorescence Length (cm)	Inflorescence FW (g)	Inflorescence DW (g)	Node Number	Leaf Number	Leaf FW (g)	Leaf DW (g)	Shoot FW (g)	Shoot DW (g)
0	**No Treatment**	46.7^{b}	8.17^{b}	7.13^{bc}	1.03^{c}	11.5^{bc}	56.7^{cd}	11.6^{c}	0.87^{c}	12.52^{c}	1.280^{c}
	GB 50 mg L^{-1}	45.0^{b}	6.33^{cd}	7.46^{bc}	2.36^{ab}	12.2^{b}	62.3^{bc}	12.8^{bc}	0.80^{c}	13.56^{b}	1.397^{bc}
	GB 100 mg L^{-1}	47.7^{ab}	8.07^{b}	10.34^{a}	2.84^{a}	14.5^{ab}	67.0^{b}	13.1^{b}	1.11^{ab}	14.84^{a}	1.812^{ab}
	GO 50 mg L^{-1}	47.7^{ab}	6.16^{cd}	6.33^{c}	1.34^{bc}	11.3^{bc}	62.5^{bc}	12.1^{c}	1.00^{b}	11.16^{cd}	1.341^{bc}
	GO 100 mg L^{-1}	43.3^{bc}	5.13^{d}	4.47^{de}	0.87^{d}	8.66^{d}	54.4^{cd}	11.4^{cd}	0.66^{d}	9.00^{d}	1.019^{cd}
	GO-GB 50 mg L^{-1}	48.7^{a}	11.33^{a}	8.80^{b}	2.26^{b}	14.7^{a}	71.7^{a}	14.8^{a}	1.09^{b}	14.91^{a}	1.927^{a}
	GO-GB 100 mg L^{-1}	47.2^{ab}	9.66^{ab}	7.74^{bc}	1.43^{bc}	13.2^{ab}	67.3^{b}	15.2^{a}	1.55^{a}	14.14^{ab}	2.014^{a}
50	**No Treatment**	41.3^{c}	5.16^{d}	4.39^{de}	0.95^{cd}	8.00^{de}	38.3^{g}	8.79^{e}	0.70^{d}	8.46^{de}	1.11^{c}
	GB 50 mg L^{-1}	43.5^{bc}	6.16^{cd}	4.31^{de}	0.83^{d}	8.66^{d}	36.2^{gh}	11.7^{cd}	0.67^{d}	9.38^{d}	1.34^{bc}
	GB100 mg L^{-1}	46.7^{ab}	7.03^{c}	5.43^{cd}	1.36^{bc}	10.7^{c}	46.5^{e}	12.7^{bc}	0.87^{c}	11.5^{cd}	1.58^{b}
	GO 50 mg L^{-1}	39.8^{de}	6.52^{cd}	4.65^{de}	0.66^{e}	8.25^{d}	42.3^{f}	10.7^{d}	0.68^{d}	9.5^{d}	1.09^{cd}
	GO 100 mg L^{-1}	35.3^{f}	5.13^{d}	3.76^{e}	0.94^{cd}	11.0^{bc}	39.4^{g}	8.27^{e}	0.47^{e}	7.2^{e}	0.86^{de}
	GO-GB 50 mg L^{-1}	46.8^{ab}	9.14^{ab}	5.07^{d}	1.52^{bc}	12.7^{ab}	52.2^{d}	13.2^{b}	0.82^{c}	12.1^{c}	1.14^{c}
	GO-GB 100 mg L^{-1}	44.7^{bc}	11.0^{a}	4.67^{de}	1.67^{bc}	10.3^{c}	53.1^{d}	12.3^{bc}	0.88^{c}	12.6^{c}	1.11^{c}
100	**No Treatment**	34.0^{g}	3.67^{e}	2.5^{f}	0.52^{g}	5.7^{f}	21.0^{i}	6.27^{f}	0.32^{f}	4.05^{g}	0.49^{f}
	GB 50 mg L^{-1}	37.2^{e}	6.14^{cd}	2.84^{ef}	0.63^{ef}	7.33^{e}	29.8^{h}	8.53^{e}	0.59^{de}	5.75^{f}	0.74^{e}
	GB 100 mg L^{-1}	42.8^{c}	7.83^{bc}	4.01^{de}	1.00^{c}	8.66^{d}	35.5^{gh}	10.9^{d}	0.73^{cd}	6.87^{ef}	0.97^{d}
	GO 50 mg L^{-1}	40.7^{d}	6.16^{cd}	3.23^{ef}	1.81^{bc}	7.25^{e}	33.7^{gh}	7.66^{ef}	0.64^{d}	6.01^{f}	0.77^{e}
	GO 100 mg L^{-1}	38.5^{de}	4.93^{d}	1.44^{g}	0.61^{f}	5.66^{f}	27.5^{h}	6.33^{f}	0.22^{g}	4.06^{g}	0.57^{f}
	GO-GB 50 mg L^{-1}	43.3^{bc}	8.07^{b}	4.11^{de}	1.03^{c}	8.75^{d}	40.6^{fg}	11.7^{cd}	0.74^{cd}	7.49^{e}	0.92^{d}
	GO-GB 100 mg L^{-1}	40.7^{d}	9.94^{ab}	3.20^{ef}	0.80^{de}	6.00^{f}	41.3^{f}	10.5^{d}	0.87^{c}	6.93^{ef}	0.89^{de}

absorption, acting as elicitors in the regulation of plant growth, activating the biosynthesis of indole acetic acid and abscisic acid, promoting the expression of marker genes of cell division and elongation of the cell wall and increasing the activity of SOD, GP, CAT and APX enzymes leading to proteins accumulation and finally improved growth. Application of GB improved growth and yield of rice under drought stress (Tisarum et al., 2020) and maize under salinity stress (Ren et al., 2016). In addition, GB enhanced height, fresh and dry weights of *Oryza sativa* (Yao et al., 2018) and fresh weight of *Phaseolus vulgaris* (Osman and Salim, 2016) all under salinity condition. In the current study, the positive impacts of GB, GO-GB NPs and GO (at lower dose) on agronomic traits could be attributed to their encouraging effects on growth that could be doubled in GO-GB NPs. Better absorption of water and macro- and micro-nutrients particularly under salt stress might be probable reasons for their positive effects. The negative effect of GO at higher dose (demonstrating toxic effect) was confirmed additionally by Wang et al. (2014).

3.3. Chl a, b and carotenoids

Salinity significantly reduced chl *a*, *b* and carotenoids contents. The best results for all photosynthetic pigments were recorded at 50 mg L^{-1} GO-GB NPs treated sweet basil under non-stress and both stress conditions (Fig. 3). Generally, 100 mg L^{-1} GO caused toxic effect through decreasing the pigments content or ineffectiveness under non-stress and stress conditions.

Salinity enhances toxic ions which result in breakdown and reduction of photosynthetic pigments as recorded in different plants (Gohari et al., 2020a, c; Akhter et al., 2020). Possible reasons for the reduction could be through ROS damages to them through induced oxidative stress by salinity (Gohari et al., 2020a). Photosynthetic pigments were positively influenced by some NPs (in a dose-dependent manner) (Gohari et al., 2020a,b). GO treatment caused an increase in photosynthetic pigments of rice and milk thistle under abiotic stress condition (Yao et al., 2018; Safikhan et al., 2018) possibly due to the stimulating action on light absorption caused by the penetration ability of carbon-based NPs into chloroplast membranes and increase in the number and size of chloroplasts (González-García et al., 2019). Enhancement in agronomic traits especially leaf number and chl biosynthesis might be considered as reasons for increase in chl by the treatments with positive effect under salinity. GB impact on maintain chloroplast could be the other reason for improving photosynthetic pigments of sweet basil under salinity condition. In addition, positive effect of GO-GB NPs might be referred to enhanced effect of GB and GO in the synthesized nanostructure. The negative effect of some NPs at higher doses (here 100 mg L^{-1} GO) could be described through decrease in biosynthesis of

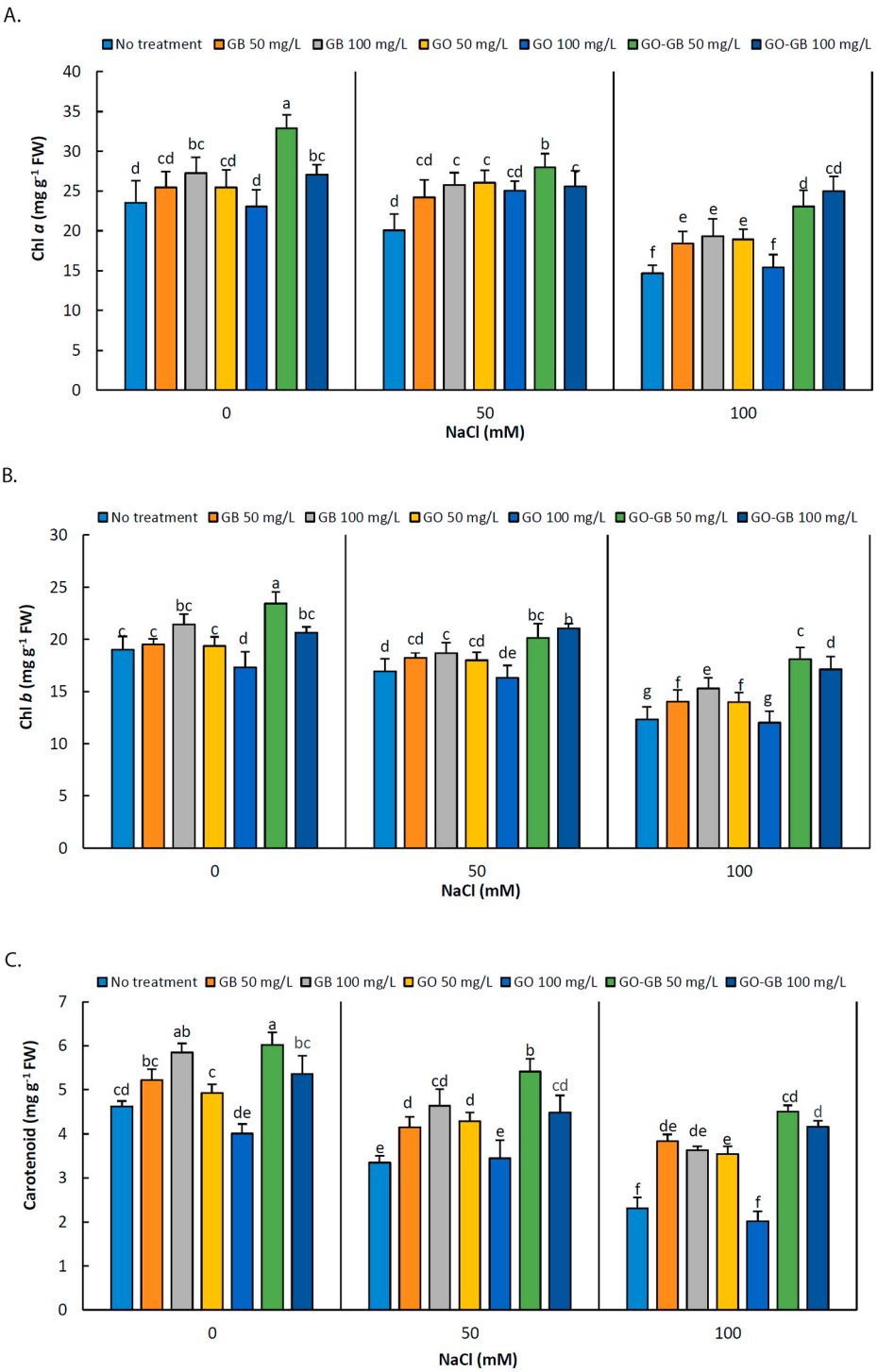

Fig. 3. Effect of different concentrations of glycine betaine functionalized graphene oxide nano particles on photosynthesis pigments, Chl *a* (A), Chl *b* (B), and carotenoids (C) of *O. basilicum* L. under salinity stress. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. Different letters indicate significantly different values at $p < 0.05$.

photosynthetic pigments as reported by Tang et al. (2015) and Gohari et al. (2020c).

3.4. Chlorophyll fluorescence and SPAD

Salinity had negative impacts on chlorophyll fluorescence parameters and SPAD. Y (II) values increased by application of 50 mg L^{-1} GO, 100 mg L^{-1} GB and 50 mg L^{-1} GO-GB NPs under 50 mM salinity and all treatments under 100 mM salinity (Table 2). GO-GB (50 and 100 mg L^{-1}) and GO and GB at 100 mg L^{-1} concentrations increased SPAD value under 50 mM salinity. Under 100 mM salinity, except 100 mg L^{-1} GO, the other treatments increased SPAD values (Table S2). Most treatments were effective in $^{Fv}/_{Fm}$ enhancement under salinity conditions, exception is for 100 mg L^{-1} GO with poisonous effect (Table 2). The negative effect of GO at high dose demonstrated its toxic effect. In general, the best results mostly were recorded in 50 mg L^{-1} GO-GB.

Gohari et al. (2020a,b) and Sharma et al. (2019) reported positive effects of some NPs on these parameters of plants (including sweet basil)

Table 2
Effect of different concentrations of glycine betaine functionalized graphene oxide nano particles on SPAD and chlorophyll fluorescence parameters of *O. basilicum* L. under salinity stress. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. Different letters indicate significantly different values at $p < 0.05$.

NaCl (mM)	Treatments	SPAD	Y(II)	Y(NO)	Fv	Fo	Fm	Fv/Fo	Fv/fm
0	**No Treatment**	32.9^{bc}	0.77^{c}	0.43^{f}	2.77^{bc}	1.44^{cd}	3.34^{cd}	1.92^{de}	0.80^{d}
	GB 50 mg L^{-1}	33.8^{b}	0.77^{c}	0.41^{fg}	2.98^{bc}	1.15^{de}	3.17^{de}	2.59^{c}	0.96^{c}
	GB 100 mg L^{-1}	34.8^{ab}	0.83^{ab}	0.40^{g}	3.35^{ab}	0.83^{fg}	3.01^{e}	4.01^{b}	1.11b
	GO 50 mg L^{-1}	31.0^{c}	0.73^{d}	0.42^{fg}	3.05^{b}	1.07^{e}	3.27^{d}	2.85^{bc}	0.94^{c}
	GO 100 mg L^{-1}	32.8^{bc}	0.71^{fg}	0.49^{e}	2.34^{c}	1.48^{cd}	3.45^{c}	1.59^{ef}	0.68^{e}
	GO-GB 50 mg L^{-1}	35.3^{a}	0.87^{a}	0.40^{g}	3.97^{a}	0.80^{g}	3.16^{de}	4.93^{a}	1.24^{a}
	GO-GB 100 mg L^{-1}	33.7^{b}	0.77^{c}	0.43^{f}	3.50^{ab}	0.94^{ef}	2.43^{f}	3.56^{ab}	0.39^{h}
50	**No Treatment**	27.5^{e}	0.72^{de}	0.57^{cd}	2.04^{d}	1.75^{b}	3.54^{bc}	1.17^{fg}	0.57^{ef}
	GB 50 mg L^{-1}	28.0^{de}	0.72^{de}	0.57^{c}	2.12^{cd}	1.56^{c}	3.03^{e}	1.36^{ef}	0.51^{f}
	GB 100 mg L^{-1}	30.2^{cd}	0.82^{b}	0.53^{de}	2.65^{bc}	1.07^{e}	2.98^{ef}	2.47^{cd}	0.89^{cd}
	GO 50 mg L^{-1}	28.0^{e}	0.76^{cd}	0.57^{cd}	2.30^{c}	1.45^{cd}	3.33^{cd}	1.59^{ef}	0.65^{de}
	GO 100 mg L^{-1}	30.0^{d}	0.71^{de}	0.63^{ab}	2.00^{d}	1.69^{bc}	3.68^{b}	1.18^{fg}	0.54^{ef}
	GO-GB 50 mg L^{-1}	31.5^{c}	0.83^{ab}	0.52^{e}	2.85^{bc}	0.98^{ef}	2.99^{ef}	2.89^{bc}	0.95^{bc}
	GO-GB 100 mg L^{-1}	32.5^{bc}	0.74^{d}	0.55^{d}	3.01^{b}	0.96^{ef}	3.00^{e}	3.12^{c}	1.00^{bc}
100	**No Treatment**	22.0^{g}	0.64^{f}	0.65^{ab}	1.40^{ef}	1.97^{a}	3.82^{a}	0.71^{gh}	0.36^{h}
	GB 50 mg L^{-1}	24.3^{f}	0.72^{de}	0.59^{bc}	1.50^{e}	1.76^{b}	3.44^{c}	0.85^{g}	0.44^{g}
	GB 100 mg L^{-1}	28.0^{e}	0.78^{c}	0.60^{bc}	2.16^{cd}	1.33d	3.37^{cd}	1.62^{e}	0.64^{e}
	GO 50 mg L^{-1}	23.9^{f}	0.76^{cd}	0.62^{ab}	2.01^{d}	1.58^{c}	3.68^{b}	1.28^{f}	0.54^{ef}
	GO 100 mg L^{-1}	21.0^{g}	0.70^{e}	0.67^{a}	0.92^{f}	1.83^{ab}	3.89^{a}	0.51^{i}	0.24^{i}
	GO-GB 50 mg L^{-1}	28.1^{de}	0.78^{c}	0.60^{bc}	2.08^{d}	0.90^{f}	3.02^{e}	2.31^{cd}	0.69^{de}
	GO-GB 100 mg L^{-1}	27.9^{e}	0.71^{de}	0.58^{c}	1.91^{de}	0.95^{ef}	3.22^{d}	2.00^{d}	0.59^{ef}

under salinity stress condition mostly in a dose-dependent manner. In addition, GO application on milk thistle under salt stress caused enhancement in maximum quantum efficiency of PS II and performance index (Safikhan et al., 2018). Tisarum et al. (2020) reported positive effect of GB application on chlorophyll fluorescence parameters of rice under drought stress probably through GB role in chloroplast maintenance. These findings were mostly in agreement with our results and could define the enhanced effect of GO-GB NPs on chlorophyll fluorescence parameters and SPAD. In all probability, GO-GB NPs might affect the electron transport and energy pathways. Higher dose of MWCNTs-COOH (Gohari et al., 2020c) and Ag NPs (Sharma et al., 2019) caused toxic effect as observed at 100 mg L^{-1} GO in current study.

3.5. MSI, MDA and H_2O_2

Salinity caused significant reduction in MSI value. Only GO-GB NPs application at both concentrations increased MSI value under 50 mM salinity condition (Fig. 4A). Salinity increased MDA and H_2O_2 contents. The lowest contents of MDA and H_2O_2 under non-stress and both stress conditions were recorded at 50 mg L^{-1} GO-GB NPs application. Significant decrease in MSI by 100 mg L^{-1} GO treatment or ineffectiveness of the treatment on MDA and H_2O_2 contents under non-stress and stress conditions could be described through its toxic impact (Fig. 4B and C).

MDA, as a marker for stress, determines damages to cell membrane and lipids. In fact, MDA demonstrates lipid peroxidation degree (Gohari et al., 2019; Ahanger et al., 2020). Graphene quantum dots application at low doses decreased MDA content (Feng et al., 2019). Iron-NPs caused reduction in MDA of grape under salinity (Mozafari et al., 2018). Reduction in MDA after GB application was reported (Roychoudhury and Banerjee, 2016) particularly in maize under drought stress due to decrease in ROS (Anjum et al., 2012). There are several reports regarding decrease in MDA of plants under salinity after GB treatment (Hu et al., 2012; Malekzadeh et al., 2015; Yildirim et al., 2015). The possible reason could be considered through GB roles in different vital plant processes for growth and development (e.g., photosynthesis, enzymes functions, protein structures, gene expression) most importantly preserving membrane integrity (Fan et al., 2012) that causes decrease in stress effects and therefore decline in MDA and enhanced resistance to stress. On top, MDA results could explain MSI results since decrease in MDA was the same as preserving membrane integrity or MSI. Safikhan et al. (2018) reported increased MSI in milk thistle plant under salinity probably through modifications of lipid structure of plasma membranes. Considering GB impact on membrane integrity, positive effect of GO-GB NPs on MSI could be expectable. Although H_2O_2 play crucial role in plant defense mechanism, increase in its concentration caused toxic impacts and consequently damages to biological membranes due to induced oxidative stress. H_2O_2 is scavenged by APX and then GP and CAT (Gohari et al., 2019; Noreen et al., 2020). Increase in H_2O_2 content of plant under salinity was previously reported. Reduction in H_2O_2 content of plant under salinity after NPs application was previously reported (Mozafari et al., 2018; Gohari et al., 2020b). High concentration of GO resulted in H_2O_2 increase under non-stress and salt stress (Wang et al., 2014) in accordance with our results considering 100 mg L^{-1}GO, as toxic dose. Yildirim et al. (2015) reported decrease in H_2O_2 of lettuce under salinity after GB application. NPs and GB could reduce induced oxidative stress by salinity and then after decrease H_2O_2. Increase in antioxidant enzymes activities and phenolic compounds in the current study could cause in ROS quenching and lead to decrease in H_2O_2.

3.6. Proline and total phenolic compounds

Salinity enhanced the content of proline and phenolic compounds. Considering proline, the best results were achieved by 50 mg L^{-1} GO-GB NPs under both salinity stress conditions. GO at 100 mg L^{-1} concentration had adverse effects under non-stress and 50 mM salinity representing its toxicity (Fig. 5A). Except 50 mg L^{-1} GO and 50 mg L^{-1} GB with no effect, the other treatments increased phenolic content under both salinity conditions. The highest contents were recorded at 50 and 100 mg L^{-1} GO-GB NPs treatments under non-stress and stress conditions. Interestingly, no toxic effect of GO at high concentration was recorded in this regard (Fig. 5B).

Proline is an antioxidant and signaling molecule that accumulates under stress condition. In fact, proline accumulation in plants under stress leads to enhanced resistance (Hayat et al., 2012) through modulating the osmotic pressure of cells. Increase in proline of plants under salinity was reported (e.g., Mozafari et al., 2018). Safikhan et al. (2018) and Anjum et al. (2014) confirmed increase in proline of plants under salinity after GO application. This positive effect was also reported by other NPs mostly under stress condition (Mozafari et al., 2018; Feng et al., 2019). GB application additionally enhanced proline content of plants under salinity condition (Malekzadeh et al., 2015; Yao et al., 2018). Probably, decline in proline oxidation and enhancement in its

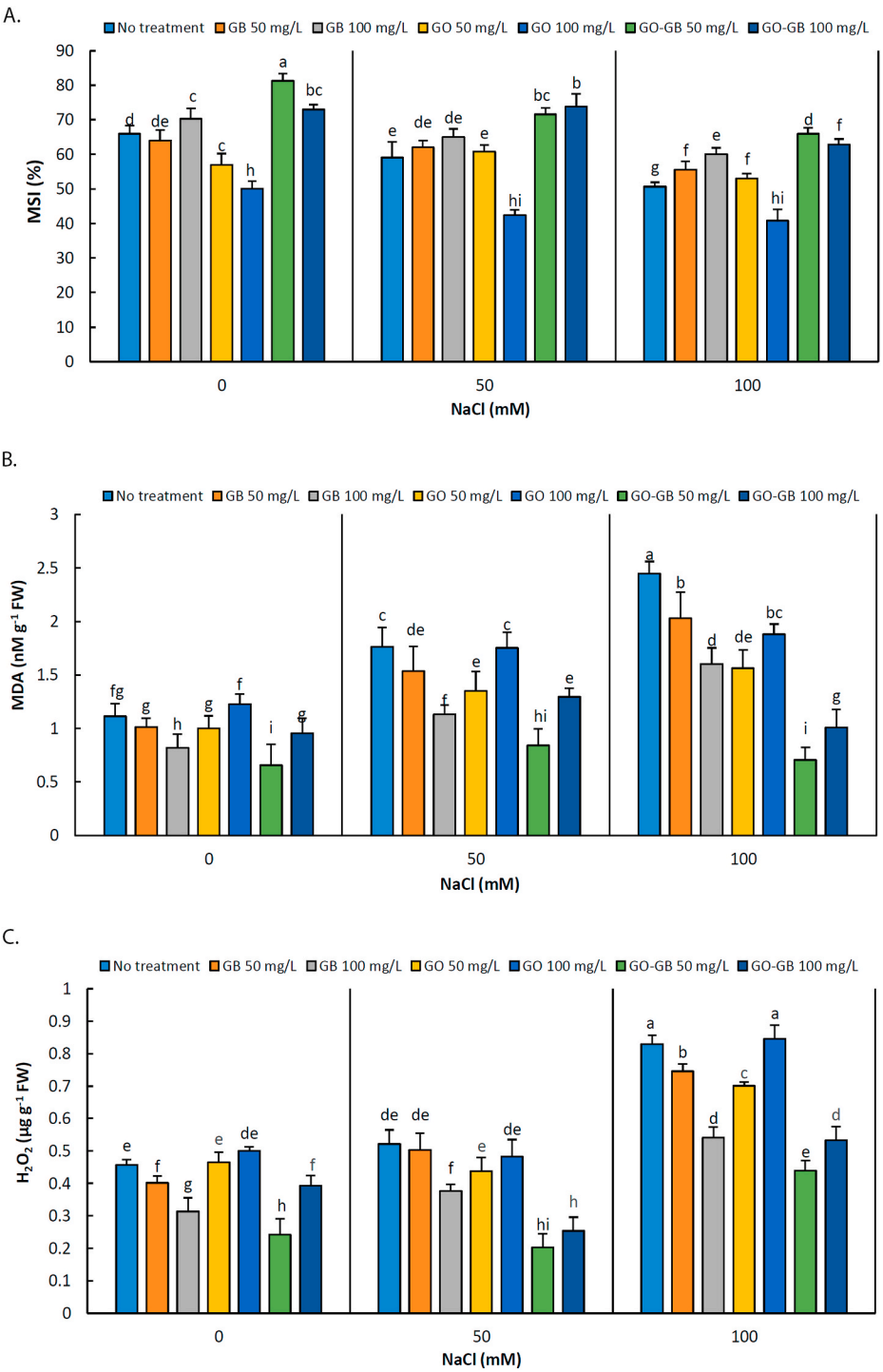

Fig. 4. Effect of different concentrations of glycine betaine functionalized graphene oxide nano particles on membrane stability index (A), malondialdehyde content (B), and hydrogen peroxide content (C) of *O. basilicum* L. under salinity stress. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. Different letters indicate significantly different values at $p < 0.05$.

biosynthesis might be considered as reasons for the encouraging impacts of treatments with positive effect. Additionally, enhancement in proline especially by applying GO-GB NPs could describe decrease in H_2O_2, MDA and even higher MSI especially under salinity through proline positive effects in this regard.

Commonly, stress conditions cause increase in phenolic compounds formerly confirmed especially under salt stress (Ashraf et al., 2010; Gohari et al., 2020c). Feng et al. (2019) reported increased phenolics at low concentration of QDs. In addition, low concentration of MWCNTs-COOH enhanced sweet basil phenolics under salinity condition (Gohari et al., 2020c). Graphene application enhanced phenols and flavonoids of tomato under oxidative stress perhaps through NPs-induced slight oxidative stress and overexpression of the genes involved in stress signaling in plants (González-García et al., 2019). This enhancement by NPs and GB application might be considered as a line of antioxidant defense against oxidative stress imposed by NaCl. Another possible reason for increase in phenolics might be their role in phenolics biosynthesis or preventing phenolic degradation probably by effect on

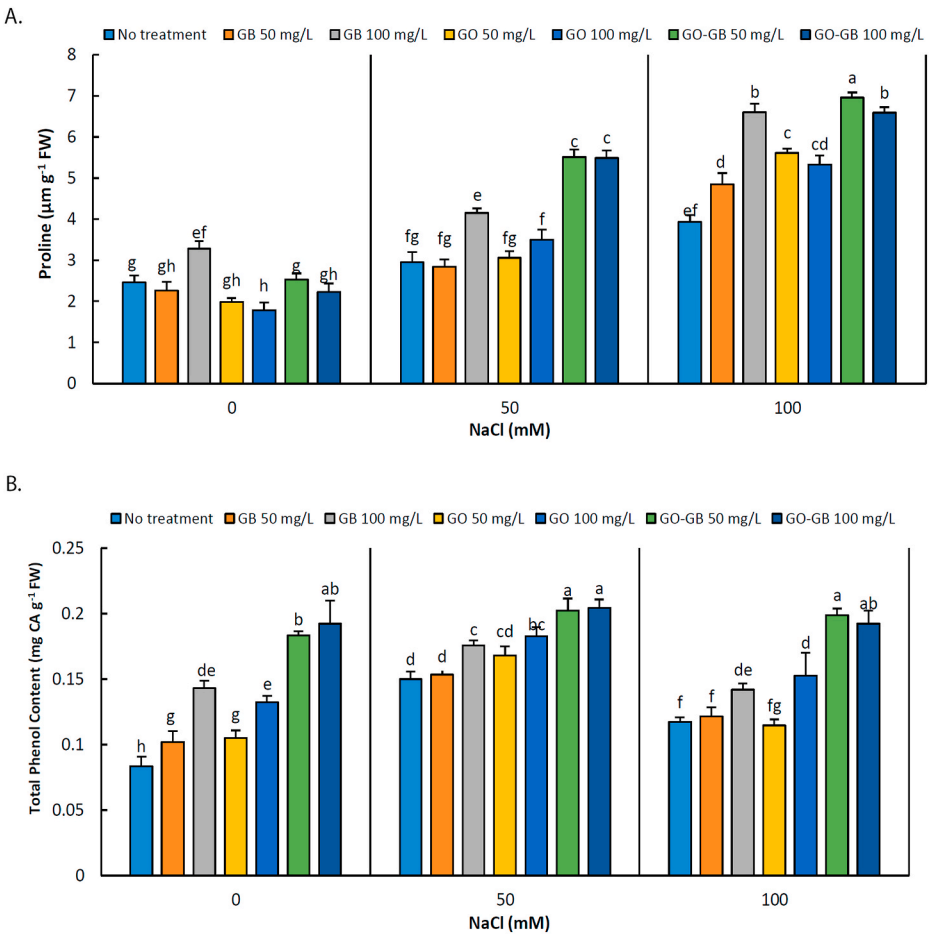

Fig. 5. Effect of different concentrations of glycine betaine functionalized graphene oxide nano particles on proline (A), and total phenolic compound (B) of *O. basilicum* L. under salinity stress. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. Different letters indicate significantly different values at $p < 0.05$.

their genes or enzymes. Finally, this enhancement in their content causes improved plant performance under salinity.

3.7. Antioxidant enzymes activities (APX, SOD, GP)

All measured enzymes activities were improved after imposing salinity. Regarding APX, GB and GO (at 100 mg L^{-1} concentrations) and 50 and 100 mg L^{-1} GO-GB NPs improved the enzyme activity under 50 mM salinity. Under 100 mM salinity, 100 mg L^{-1} GB and 50 and 100 mg L^{-1} GO-GB NPs increased APX activity. The highest activity was observed at 100 mg L^{-1} GO-GB NPs treatment under non-stress and stress conditions (Fig. 6A). GO, GB and GO-GB NPs at 100 mg L^{-1} concentrations enhanced SOD activity under 50 mM salinity while only 100 mg L^{-1} GB and 50 mg L^{-1} GO-GB NPs had this effect under 100 mM salinity (Fig. 6B). Under 50 mM salinity, except GO treatments, the other treatments enhanced GP activity. GB at 100 mg L^{-1} and GO-GB NPs at 50 and 100 mg L^{-1} increased GP activity under 100 mM salinity. The highest GP activity was recorded at 50 mg L^{-1} GO-GB NPs under non-stress and stress conditions (Fig. 6C).

Salinity leads to oxidative stress mostly by ROS generation and accumulation in plant cells (Rasool et al., 2013). Consequently, APX, SOD and GP, as the studied antioxidant enzymes, detoxify ROS and free radicals resulting in reduced stress impacts. Therefore, these enzymes are key parts of essential strategy for enhanced tolerance to stress condition (Reddy et al., 2015; Chandna et al., 2013). Increase in APX, SOD and GP activities was noticed in plants (including sweet basil) after imposing salinity (Gohari et al., 2020a, b, c) in line with the current findings. Graphene application increased APX and GP of tomato under oxidative stress (González-García et al., 2019). Toxic effect of high dose of GO through reduction in SOD and CAT activities and increase in ROS of plant under salinity was previously reported as observed in some terms in the current study at 100 mg L^{-1} GO. The probable reason for this toxic effect might be alternations in gene expression related to response to stress (Wang et al., 2014). GB improved SOD, CAT and POD activities. Malekzadeh (2015) reported increase in CAT, APX and decrease in ROS after GB application on plant under salinity condition. Similar positive effect in enhanced antioxidant enzymes of plants under salinity by GB application additionally reported (Hu et al., 2012). GB regulates antioxidant defense system (Tisarum et al., 2020) via inducing expression of various genes especially those mitigates stress condition (Ahmad et al., 2013). Therefore, increase in measured antioxidant enzymes activities especially by GO-GB treatment could ameliorate the negative impacts of salinity representing enhanced positive effect of GB and GO in the combined nano-structure and might be explained through signified impact of them. Since GB could be used with other stress protecting materials (Ahmad et al., 2013) its combination with GO, that totally lead to better results, could be expectable. It is worth stating that since plant response and tolerance to stress conditions are complex, the mechanisms or reasons beyond improved measured factors under non-stress and stress conditions by the application of the current treatments (GO and GO-GB) additionally needs more investigations.

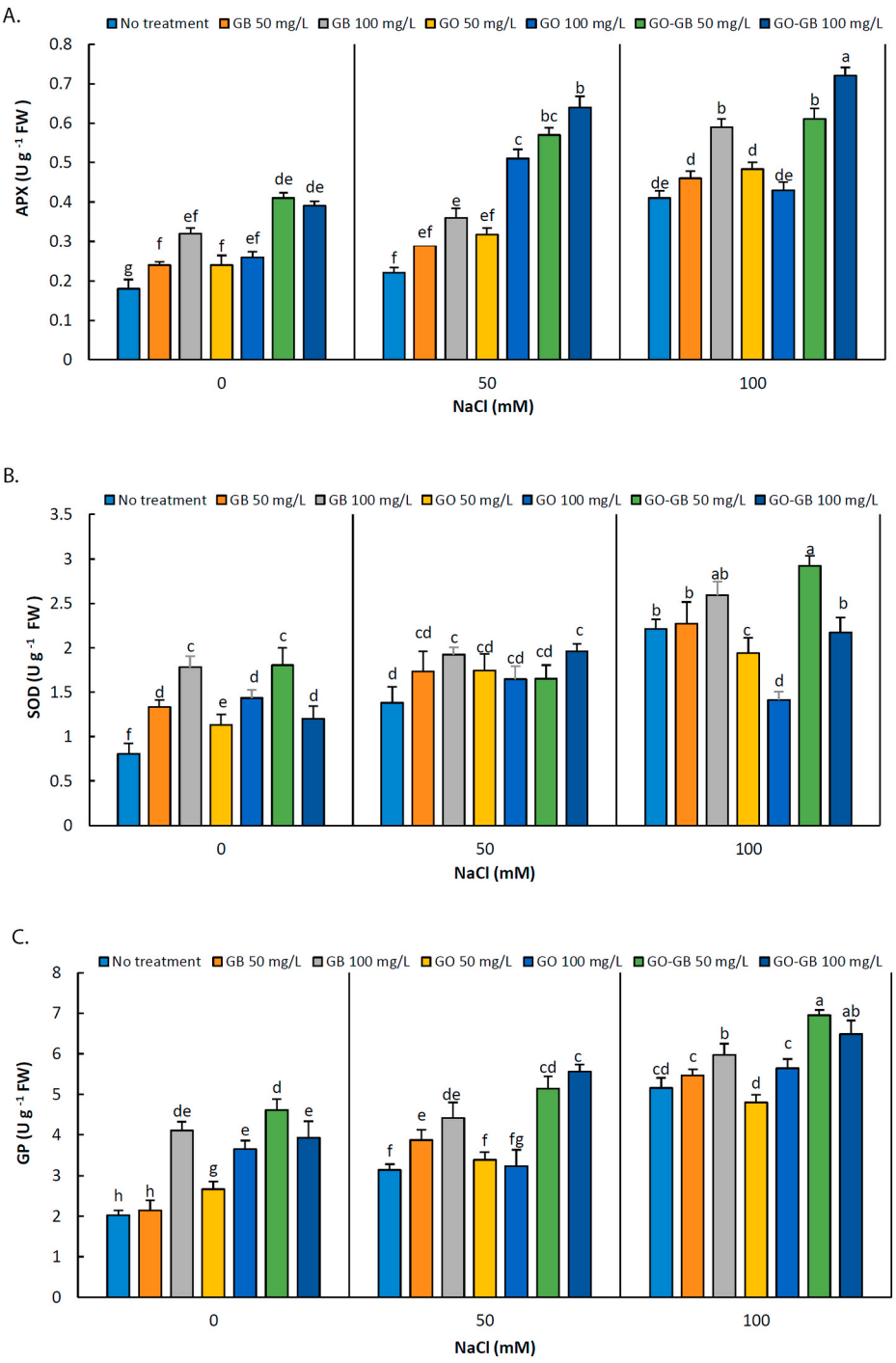

Fig. 6. Effect of different concentrations of glycine betaine functionalized graphene oxide nano particles on antioxidant enzyme activity; ascorbate peroxidase (A), superoxide dismutase (B), and guaiacol peroxidase (C) of *O. basilicum* L. under salinity stress. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. Different letters indicate significantly different values at $p < 0.05$.

3.8. Essential oil

GC/MS analysis of sweet basil essential oil revealed 37 components with higher values of estragole, methyl chavicol, germacrene D and linalool as dominant components (Table 3). Salinity enhanced methyl chavicol, germacrene D and linalool. Under salinity conditions, GB treatments customarily resulted in increased dominant constituents. GO at 50 mg L^{-1} concentration increased methyl chavicol, esteragole and linalool under non-stress and both salinity condition. Mostly, the best results were obtained by 100 mg L^{-1} GB and 50 mg L^{-1} GO-GB NPs and the worst ones were recorded at 100 mg L^{-1} GO.

The response of medicinal and aromatic plants to abiotic stress are different from other crops. Different agro-climatic conditions cause significant differences in the production and accumulation of secondary metabolites in medicinal plants (Jan et al., 2020). The chemical constituents of medicinal and aromatic plants are directly influenced by the environmental conditions (Jan et al., 2018; Jan and Abbas, 2018) confirmed by contradictory effect of salinity on sweet basil constituents (Gohari et al., 2020a, c). Therefore, salinity could change content and components of sweet basil (Aziz et al., 2008). Probable reason for

Table 3
Effect of different concentrations of glycine betaine functionalized graphene oxide nano particles on essential oil composition of *O. basilicum* L. under salinity stress. S0, S1, and S2 were 0, 50, and 100 mM NaCl, respectively. GO: graphene oxide; GB: glycine betaine; GO-GB: glycine betaine functionalized graphene oxide. RI values represent retention indices determined on GC/MS capillary column.

	Compounds	RI	No Treatment			GB 50 mg L^{-1}			GB 100 mg L^{-1}			GO 50 mg L^{-1}			GO 100 mg L^{-1}			GO-GB 50 mg L^{-1}			GO-GB 100 mg L^{-1}		
			S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
1	α-Pinene	932	–	0.08	0.17	0.16	0.15	0.16	0.15	0.13	0.14	0.13	0.12	0.122	0.12	0.12	0.12	–	0.08	–	0.16	0.15	0.16
2	**Sabinene**	969	0.8	1.02	1.07	1.9	1.04	1.01	1.19	1.35	1.25	0.81	1.09	1.02	0.08	–	–	1.73	**1.98**	1.62	0.18	0.35	–
3	β-Pinene	974	0.08	0.06	0.12	0.14	0.16	0.15	0.13	0.10	0.10	0.10	0.11	0.104	0.114	0.12	0.14	–	0.06	0.12	0.14	0.16	0.15
4	Terpinene-4-ol	1117	0.24	0.70	0.60	0.70	0.92	0.63	1.02	–	1.21	0.89	1.24	1.358	1.089	2.04	–	1.69	0.74	1.72	0.72	0.60	0.70
5	Borneol	1169	0.22	0.21	0.19	0.24	0.21	5.15	–	0.22	0.20	6.21	–	–	0.273	–	–	0.27	–	0.22	0.21	0.19	0.24
6	α-Terpinoel	1189	0.03	0.22	0.21	0.19	0.24	0.21	0.15	–	0.22	0.20	0.21	–	–	0.073	–	0.31	0.27	0.36	0.64	0.21	–
7	**Methyl chavicol**	1196	22.1	25.2	25.1	**24.4**	**25.7**	**26.9**	**39.18**	**37.54**	**41.1**	38.0	36.2	40.1	35.3	36.4	34.7	49.1	49.5	**49.8**	21.2	25.8	21.0
8	Myrcene	1352	–	0.082	–	0.06	0.12	0.09	0.05	0.01	–	0.02	0.05	0.03	0.05	0.07	0.11	–	–	–	0.05	0.12	0.09
9	1,8-**Cineole**	1356	1.5	1.94	1.37	1.34	1.81	1.93	**2.14**	1.96	1.81	0.51	1.05	1.25	0.65	0.75	–	1.52	1.94	1.37	0.74	1.01	1.01
10	Ocimene-cis	1362	0.13	0.06	–	–	0.05	–	0.14	0.29	–	0.58	1.16	0.87	0.65	0.44	–	0.13	0.06	–	0.02	0.05	–
11	α-Ylangene	1375	0.01	0.40	0.40	0.45	0.47	0.43	0.62	–	0.52	0.601	1.03	0.83	0.96	0.98	1.36	0.93	0.62	0.90	0.40	0.40	0.45
12	α-Cedrene	1412	0.06	0.29	0.25	0.22	0.28	0.25	0.32	–	0.32	0.36	0.40	0.39	0.35	0.41	–	0.61	0.34	0.51	0.28	0.25	0.22
13	(E)-β-Farnesene	1457	0.08	0.13	0.40	0.20	0.20	0.187	0.20	0.07	0.09	0.05	–	0.02	0.07	0.12	0.22	–	0.10	0.20	–	0.10	0.13
14	α-Bulnesene	1510	0.40	0.40	0.45	0.47	0.43	0.62	–	0.52	0.60	1.03	0.83	0.96	0.98	1.36	0.9	0.62	0.90	0.40	0.39	0.4	0.47
15	**Linalool**	1513	4.18	5.30	5.42	6.36	7.30	7.32	7.31	7.30	**8.32**	5.28	6.25	7.01	5.27	5.26	2.26	8.08	6.30	7.42	7.35	6.29	5.32
16	**Camphor**	1525	1.18	1.09	1.10	1.98	1.32	1.36	1.90	1.89	**2.35**	1.02	1.11	0.97	–	0.33	–	1.98	1.80	1.53	1.08	0.98	0.52
17	γ-Cadinene	1534	0.78	0.01	0.52	–	0.04	0.99	0.22	0.89	0.78	0.39	0.69	–	0.60	0.52	0.38	–	0.88	0.57	0.68	0.62	0.12
18	Δ-Cadinene	1539	1.48	1.41	2.02	1.91	1.81	1.96	1.90	1.86	2.04	1.68	1.73	1.51	1.39	1.28	1.05	1.80	1.41	2.02	1.91	1.89	1.86
19	Caryophyllene oxid	1583	1.16	0.80	0.73	0.84	0.68	1.28	–	1.09	1.44	1.70	1.63	1.23	1.50	2.39	–	1.07	1.67	1.09	1.44	1.70	1.09
20	**Estragole**	1618	36.7	31.0	33.4	44.4	45.4	39.9	55.5	56.0	64.9	37.1	39.3	38.2	35.3	31.5	28.7	56.7	60.0	**63.4**	34.4	45.4	44.9
21	β-Sinensal	1625	0.62	0.56	0.49	0.40	0.20	0.44	0.44	0.47	0.29	0.44	0.95	0.79	0.80	0.73	0.84	0.62	0.36	0.49	0.79	0.10	0.45
22	Geraniol	1627	0.10	0.10	0.02	0.05	0.03	0.05	0.07	–	–	0.03	–	–	0.08	0.16	0.33	0.10	0.11	0.10	0.10	–	–
23	**Geranial citral**	1702	2.06	2.18	1.98	2.39	1.97	2.61	2.36	2.87	3.01	1.97	2.01	1.65	1.07	1.23	0.45	3.74	4.02	**5.123**	2.36	3.28	2.09
24	α-Copaene	1776	0.03	–	–	0.04	0.09	0.06	0.03	0.20	0.19	0.20	0.07	–	0.08	–	–	0.07	0.06	–	0.04	–	–
25	Geranyl acetate	1789	–	0.10	0.20	0.15	0.10	0.13	0.10	0.07	0.09	0.05	–	0.02	0.07	0.12	0.22	–	0.13	0.20	0.15	0.10	0.13
26	β-Cubebene	1801	–	–	–	0.10	0.20	0.15	0.09	0.03	–	0.06	0.12	0.09	0.07	0.05	–	–	0.09	–	0.18	0.20	0.15
27	β-Elemene	1806	–	0.25	–	0.11	0.21	0.16	0.16	0.16	0.22	0.11	–	0.05	0.08	0.10	0.14	–	0.08	–	0.10	0.21	0.16
28	Methyl Eugenol	1843	1.98	1.07	1.16	0.90	0.65	0.78	0.73	0.69	0.87	0.51	0.15	0.33	0.38	0.44	0.55	35.0	18.1	1.16	0.90	0.65	0.78
29	β-Caryophyllene	1848	1.85	0.67	0.49	0.45	0.47	0.43	0.21	–	–	–	0.26	–	0.06	0.12	0.24	0.85	1.67	0.49	0.44	0.41	0.43
30	α-Bergamotane	1854	0.35	0.26	0.16	0.08	–	0.04	0.25	0.46	0.44	0.49	0.54	0.51	0.38	0.25	–	0.35	0.26	0.163	0.08	–	0.04
31	Naphtalene methoxy	1883	0.01	–	–	0.09	0.18	0.13	0.14	0.16	0.17	0.15	0.12	0.13	0.12	0.11	0.09	–	–	–	0.08	0.18	0.13
32	α-Humulene	1889	0.12	0.14	0.29	0.28	0.28	0.29	0.27	0.26	0.29	0.23	0.17	0.20	0.19	0.17	0.15	–	0.14	0.217	0.23	0.27	0.28
33	**Germacrene D**	1926	4.62	5.56	5.49	5.80	6.10	6.45	6.41	7.37	7.15	3.58	4.01	3.79	2.80	3.02	2.84	8.62	8.56	**9.094**	4.79	4.10	5.01
34	Bicyclo Germacrene	1927	0.17	0.08	0.16	0.19	0.15	0.17	0.13	0.11	0.19	0.06	–	0.04	0.03	0.02	–	–	0.09	0.187	0.11	0.15	0.17
35	Germacrene A	1929	0.02	–	0.09	–	0.05	–	0.01	0.02	–	0.05	0.10	0.07	0.05	0.04	–	–	0.06	–	–	0.02	–
36	α-Amorphene	1960	0.24	–	–	0.07	0.14	0.16	0.20	0.11	0.14	0.07	–	0.03	0.08	0.04	–	–	–	–	0.07	0.14	0.11
37	Spathulenol	2046	0.10	0.12	0.24	0.21	0.17	0.19	0.15	0.12	0.12	0.11	0.14	0.11	0.10	0.1	0.10	–	0.12	0.24	0.20	0.18	0.19

salinity effect on components could be modifications in enzymes activities involved in their biosynthesis under salinity stress (Gohari et al., 2020c). Positive effect of NPs application on essential oils of plants (including sweet basil) under non-stress and salinity conditions was previously recorded by Gohari et al. (2020a, c) in line with our results especially encouraging impacts of GO and GO-GB NPs at lower doses. NPs could probably act as an elicitor for essential oil production through induction of various cellular signal transduction pathways that lead to transcriptional modifications and enzyme activation that could alter essential oil content and components (Ebadollahi et al., 2019; Gohari et al., 2020c). Besides enhancement in enzymes activities involved in the biosynthesis of the corresponding components, substrates availability might be considered as the other main reason for encouraging effect of NPs in this regard (Nemati et al., 2018). However, the real mechanism of NPs action on essential oil content and components is not yet clarified.

4. Conclusion

Given that GO and GB, individually, have promising effects on different plants processes, the idea of their combination in a nanostructure "GO-GB" caused to its synthesizing for doubled positive effects. The application of GO-GB NPs was successful at alleviating destructive effects of salinity. The positive impacts of GO-GB NPs were demonstrated by higher agronomic traits, photosynthetic pigments, chlorophyll fluorescence parameters, membrane stability index, proline, phenols, antioxidant enzymes activities and dominant constituents of essential oils under both non-stress and stress conditions. In addition, GO-GB NPs decreased MDA and H_2O_2 demonstrating its helpful properties in preserving membrane lipids and detoxifying ROS. Moreover, 100 mg L^{-1} GO caused negative effects under non-stress and stress conditions introducing its toxicity symptoms. Mostly, no toxicity symptom of higher dose of GO-GB NPs might be related to positive effects of GB that could suppress toxic effect of GO; this could be concluded through GB encouraging effects at both concentrations on most measured parameters. To be brief, the positive response of sweet basil to GO-GB NPs introduces NPs application as a supportive approach in plant production mainly under stressful conditions. This positive effect is more considerable by given attention to plant essential oil especially under stressful condition with importance in the cosmetic and pharmaceutical industries.

Abbreviations

NPs, nanoparticles; GO, graphene oxide; GB, glycine betaine; GO-GB, glycine betaine functionalized graphene oxide; Chl, chlorophyll; MSI, membrane stability index; H2O2, Hydrogen peroxide; MDA, malondialdehyde; GP, guaiacol peroxidase; APX, ascorbate peroxidase; SOD, superoxide dismutase.

CRediT authorship contribution statement

Ali Shakouri Ganjavi: performed greenhouse experiments, biochemical and essential oil studies. **Mehdi Oraei:** Formal analysis, designed the experimental setup, analyzed data and results. **Gholamreza Gohari:** Writing – review & editing, Formal analysis, designed the experimental setup, analyzed data and results, wrote the manuscript. **Ali Akbari:** Writing – review & editing, Formal analysis, designed the experimental setup, synthesized nanomaterial, analyzed data and results, wrote the manuscript. **Ali Faramarzi:** performed greenhouse experiments, biochemical and essential oil studies.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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