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**ORIGINAL PAPER**

# Using nanoparticles to mitigate salinity stress in Mentha species*

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**Abstract**

This study aimed to evaluate the effectiveness of graphene oxide (GO), nanoparticles (NPs), and quercetin (QC) applications in alleviating salinity stress in two Mentha species: *Mentha piperita* (peppermint) and *Mentha spicata* (spearmint). The experiment was conducted in a randomized complete block design with three replications. Foliar applications of GO, NPs, and QC were conducted over three consecutive days at concentrations of 0, 25, and 50 mg $L^{-1}$. Following a 48-hour interval, salinity treatments (0, 50, and 100 mM NaCl) were subsequently imposed through soil irrigation. Phenolic compound contents in leaves were analyzed after treatments, and data were subjected to one-way ANOVA followed by the Duncan's multiple range test ($p<0.05$). The treatments significantly affected the accumulation of phenolic compounds under salt stress. GO application enhanced chlorogenic, hydroxybenzoic, and salicylic acids, while QC increased caffeic, vanillic, and quercetin contents. NP treatment generally promoted the biosynthesis of several phenolic acids, including catechinhydrate, *p*-coumaric, and rutin. In salt stress experiments on *M. spicata* and *M. piperita*, chlorogenic acid content varied among treatments. In *M. spicata*, the highest level was observed in 25 mg $L^{-1}$ GO + 50 mM NaCl (9.22 g $kg^{-1}$), while in *M. piperita*, the maximum was recorded in 25 mg $L^{-1}$ GO + 100 mM NaCl (6.49 g $kg^{-1}$). The lowest levels (0.00 g $kg^{-1}$) occurred in several treatments, including controls. These findings indicate that the use of nanoparticles and bioactive compounds can mitigate the adverse effects of salinity stress by modulating phenolic metabolism in *Mentha species*.

**Keywords:** *Mentha piperita*, *Mentha spicata*, phenolic compounds, abiotic stress, nanotechnology

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# INTRODUCTION

*Mentha* (mint) is a plant genus belonging to the *Lamiaceae* family, which includes approximately 15-20 species (Keifer et al. 2007). Mint is an important commercial aromatic plant due to its aromatic fragrance, refreshing, and pungent taste (Dolzhenko et al. 2010). In particular, peppermint extract is widely used as a nutritional supplement in herbal or alternative medicine (Anyaoku et al. 2023). Topical application or ingestion of peppermint (*Mentha piperita*) extract induces a characteristic cooling effect, primarily due to menthol, its principal bioactive compound. Menthol interacts with the transient receptor potential melastatin 8 (TRPM8) channels located on sensory neurons, thereby eliciting a cold sensation response (Liu et al. 2013). In alternative medicine, mint acts as an antiseptic, antiviral, and stimulant for alleviating conditions such as arthritis, rheumatism, menstrual cramps, and toothache; it also plays an important role in the treatment of the flu, cold, and swelling (Hestmati et al. 2016, Tanu, Harpreet 2016). Its leaves can be consumed fresh or dried, either alone or in herbal infusions combined with other plants (Anyaoku et al. 2023). Moreover, mint is used in the production of ice cream, confectionery, canned food, beverages, chewing gum, toothpaste, soap, shampoos, and skincare products (Salehi et al. 2018, Anyaoku et al. 2023).

As with many plants, the growth and development of mint plants are significantly influenced by environmental factors (Nikolova, Ivancheva 2005, Clark, Menary 2008). Soil salinity, in particular, reduces germination rates and viability in plants, negatively impacting various physiological processes such as morphological characteristics, photosynthesis, and respiration. As a result, it leads to losses in growth, development, and essential oil yield and quality (Rout, Shaw 2001, Parida, Das 2005, Sosa et al. 2005, Aziz et al. 2008*a*, Baghalian et al. 2008). Furthermore, salinity disrupts cell functions in different mint species, slows down plant growth, and reduces photosynthesis by decreasing leaf number, area, plant height, plant weight, and chlorophyll content in the leaves (Aziz et al. 2008*b*, El-Danasoury et al. 2010, Khorasaninejad et al. 2010, Baydar, Çoban 2017, Kuzucu 2021).

Numerous studies have been conducted to minimize the destructive effects of salt stress on plants (Seleiman, 2023, Qian et al. 2024). In this context, the use of nanoparticles (NPs) has gained attention as a potential solution in recent years. It is emphasized that more research is needed on the effects of nanoparticles on aromatic plants (Isayenkov, Maathuis 2019, Ioannou et al. 2020, Mohammadi et al. 2021).

# MATERIALS AND METHODS

## Plant material and growth conditions

This study was conducted in 2022, in the plant production greenhouse of the Department of Horticulture, Faculty of Agriculture, Kilis 7 Aralık University (Kilis, Türkiye). The greenhouse conditions were maintained at a daytime temp. of 34°C, a nighttime temp. of 18°C, and relative humidity ranging from 50% to 60%. Two mint species, *Mentha spicata* L. and *Mentha piperita* L., were used as experimental material. On March 15, seeds were sown in seed trays kept in the greenhouse, and afterwards irrigation was applied every two days to promote uniform germination. When seedlings reached the four-leaf stage, they were transplanted into 3 L plastic pots, with three plants per pot.

Ten days after transplanting, the plants were subjected to salt (NaCl) stress treatments. Foliar applications of quercetin, graphene oxide, and nanoparticles (0, 25, and 50 mM) were performed for three consecutive days. Forty-eight hours after the final foliar application, 200 mL of 50 mM NaCl solution was applied to the soil every two days for one week. Subsequently, different concentrations of NaCl (0, 50, and 100 mM) were administered, and plants were monitored daily until they approached the wilting point, at which time salt applications were terminated. The experiment was arranged in a randomized complete block design with three replications. After harvesting (6 weeks after transplanting), leaves were ground to fine powder and stored in ziplock bags at -20°C until analysis. Phenolic compound profiles of the leaf extracts were subsequently determined.

## Characteristics of the trial soil

The mineral content analysis of samples of the soil used in this study was performed according to the method described by Petropoulos et al. (2018). The samples were dried at 72°C, and 0.5 g of each sample was combusted in a microwave at 200 W for 30 minutes. After combustion, the samples were filtered, transferred to 50 mL tubes, and diluted to 25 mL with distilled water. The concentrations of calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were measured using Atomic Absorption Spectroscopy (AAS). Nitrogen (N) was analyzed using the Kjeldahl method; phosphorus (P) was determined by spectrophotometry, and potassium (K) was measured using Flame Photometry. Electrical conductivity (EC) of the soil was measured as 2.2 dS $m^{-1}$, indicating a moderate salinity level consistent with values reported in similar studies. The results of the physical and chemical analysis of the soil used in the experiment are presented in Table 1.

Table 1

Experimental soil properties

| N | P | K | Ca | Mg | Fe | Cu | Zn | Mn |
|---|---|---|---|---|---|---|---|---|
| (mg $kg^{-1}$) | | | | | | | | |
| 0.361 | 10.24 | 245.73 | 3348.4 | 499.13 | 15.75 | 3.08 | 0.76 | 15.48 |

**Extraction of plant samples**

After harvesting, plant samples were carefully cleaned, subdivided into smaller portions, and dried at room temperature. The dried material was ground into fine powder in a laboratory grinder. One gram of the powdered leaf samples was extracted with a solvent mixture of methanol/chloroform/water (7:2:1, v/v/v) for 48 h using an incubated orbital shaker (OHAUS ISLDO4HDG) at 15°C. Following extraction, the homogenate was centrifuged at 5000 rpm for 15 min, and the supernatant was filtered through Whatman No. 1 filter paper. The extraction procedure was repeated three times with the same plant material to ensure complete recovery of the residues. The combined filtrates were evaporated to dryness using a rotary evaporator (Heidolph 94200, Bioblock Scientific). The vacuum-dried extracts were stored at +4°C until chromatographic analysis for the determination of phenolic compounds.

**Analysis of phenolic compounds by RP-HPLC**

The phenolic compound content of the leaf extracts was analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC) with an Agilent 1260 Infinity system (USA). Phenolic compounds were separated on a C18 reverse-phase HPLC column (110 Å, 5 µm, 4.6 × 250 mm, ACE Generix). The injection volume was set at 10 µL, and the mobile phases consisted of Phase A (0.1% phosphoric acid in water) and Phase B (100% acetonitrile) using a gradient elution system. The column oven temperature was maintained at 30°C, and detection was performed with a diode array detector (DAD). Phenolic compounds were quantified using the external standard method, and identified based on their retention times. The results were expressed as g $kg^{-1}$ of dry weight (DW) to standardize the measurements and eliminate variability caused by differences in leaf moisture content.

**Statistical analysis**

The results were analyzed using one-way analysis of variance (ANOVA) for independent samples with SPSS 18 software, and significant differences among treatment means were determined using the Duncan's Multiple Range Test at a 0.05 probability level ($p<0.05$). Principal Component Analysis (PCA) was performed using PAST software to eliminate dependency structures between variables and to reduce dimensionality. Heat maps were

generated in ClustVis software to visually represent the relationships among treatments.

## RESULTS AND DISCUSSION

### Effect of nanoparticles and stress applications on the phenolic compound content of mint plants

This study investigated the effects of nanoparticle, quercetin, and graphene oxide applications in alleviating salt stress, and focused on the changes in phenolic compound levels in plants. The results indicated species-specific responses to these treatments. Nanoparticle applications generally led to a decrease in the levels of chlorogenic acid and vanillic acid in both species, while increasing the levels of catechin hydrate, rosmarinic acid, ferulic acid, and cinnamic acid. In *Mentha piperita*, significant increases were observed in the levels of caffeic acid, *p*-coumaric acid, chrysin, rutin, hydroxybenzoic acid, hydroxycinnamic acid, naringin, naringenin, *o*-coumaric acid, and rosmarinic acid. On the other hand, *Mentha spicata* exhibited a significant increase in resveratrol levels. When salt treatment was applied along with nanoparticles, it also contributed to an increase in flavonoid levels. Quercetin application resulted in an increase in the levels of caffeic acid, vanillic acid, rutin, quercetin, naringin, *o*-coumaric acid, chrysin, and flavonoids, while decreasing the levels of chlorogenic acid, hydroxybenzoic acid, ferulic acid, and cinnamic acid. Graphene oxide application caused an increase in chlorogenic acid, hydroxybenzoic acid, salicylic acid, resveratrol, hydroxycinnamic acid, naringin, and flavonoid acids, while a decrease was observed in catechin hydrate, vanillic acid, *p*-coumaric acid, rosmarinic acid, ferulic acid, naringin, cinnamic acid, *o*-coumaric acid, and caffeic acid levels.

In the salt stress experiments applied to *M. spicata* and *M. piperita*, changes in chlorogenic acid content were observed. In *M. spicata*, the highest chlorogenic acid level was recorded in the 25 mg $L^{-1}$ GO + 50 mM NaCl treatment (9.22 g $kg^{-1}$ DW), while the lowest level (0.00 g $kg^{-1}$ DW) was observed in the 50 mg $L^{-1}$ QC + 100 mM NaCl and 25 mg $L^{-1}$ NP + 50 mM NaCl treatments. In *M. piperita*, the highest chlorogenic acid content was obtained from the 25 mg $L^{-1}$ GO + 100 mM NaCl treatment (6.49 g $kg^{-1}$ DW), whereas the lowest content (0.00 g $kg^{-1}$ DW) was detected in the control group, 50 mM NaCl, 25 mg $L^{-1}$ QC, 50 mg $L^{-1}$ NP, 25 mg $L^{-1}$ QC + 100 mM NaCl, 50 mg $L^{-1}$ QC + 50 mM NaCl, 50 mg $L^{-1}$ QC + 100 mM NaCl, and 50 mg $L^{-1}$ GO + 50 mM NaCl treatments. In a similar study by Petropoulos et al. (2017), where the effects of salinity on the nutritional value, chemical composition, and bioactive compound content of *Cichorium spinosum* were studied, an increase in phenolic compounds, flavonoids, and antioxidant activity was observed with the rise in salinity, supporting our findings.

According to other data, the highest hydroxycinnamic acid content in *M. spicata* was observed in the 50 mg $L^{-1}$ GO + 100 mM NaCl treatment. The highest naringin content was found in the *M. piperita* species treated with 25 mg $L^{-1}$ QC (78.05 g $kg^{-1}$), while other treatments yielded 0.00 g $kg^{-1}$. Studies on the use of nanoparticles to mitigate salt stress in plants have shown significant increases in plant yield, total soluble sugars, antioxidant enzymes, proline content, and total phenolics (Nasrallah et al. 2022).

Looking at the data obtained for *M. spicata,* the highest rosmarinic acid content was found in the 50 mg $L^{-1}$ NP treatment (406.76 g $kg^{-1}$), while the lowest value was observed in the 25 mg $L^{-1}$ NP + 100 mM NaCl treatment (56.46 g $kg^{-1}$). In *M. piperita*, the highest rosmarinic acid content was observed in the 25 mg $L^{-1}$ NP treatment (255.33 g $kg^{-1}$), and the lowest was found in the control group (3.64 g $kg^{-1}$). Dehghani et al. (2022) found that *Melissa officinalis* under salt stress increased proline, phenolic compounds, rosmarinic acid levels, gene expression, and phenylalanine ammonia-lyase enzyme activity in comparison to *M. officinalis* plants exposed to a drought stress as well as untreated plants.

In *M. spicata*, the highest flavonoid content was found in the 50 mg $L^{-1}$ NP + 100 mM NaCl treatment (4.17 g $kg^{-1}$), while the lowest flavonoid content, including in the 50 mM NaCl group, was 0.00 g $kg^{-1}$. In *M. piperita*, the highest flavonoid content was found in the 25 mg $L^{-1}$ GO + 100 mM NaCl treatment (3.13 g $kg^{-1}$), while the lowest was observed in the control group and thirteen other treatments, where the value was 0.00 g $kg^{-1}$ (Table 2). A study on cantaloupe (*Momordica charantia*) investigated the responses to different NaCl salinity levels (0, 50, and 100 mM) using leaf-applied nanoparticles (Cs-Se NP). In this study, while there was a decrease in growth and yield, increases were observed in morphological, biochemical, and physiological parameters (Sheikhalipour et al. 2021).

**Analysis of phenolic acid compounds**

PCA analysis of the phenolic compound parameters in *Mentha spicata* identified six factors with eigenvalues greater than 1 among the 17 variables analyzed. The first principal component (PC1) was the most significant, explaining 24% of the total variation, followed by PC2 (16%), PC3 (13.1%), PC4 (11.7%), PC5 (7.5%), and PC6 (6.9%). Together, these six components accounted for approximately 79% of the total variation (Table 3).

Principal component analysis (PCA) of phenolic compound properties in *Mentha spicata* revealed four distinct clusters (Figure 1). The first cluster included naringin, flavon, and *o*-coumaric acid, while the second comprised resveratrol, caffeic acid, rosmarinic acid, vanillic acid, chlorogenic acid, and catechin hydrate. The third cluster contained salicylic acid, rutin, quercetin, hydroxybenzoic acid, and hydroxycinnamic acid, whereas the fourth consisted of chrysin, naringenin, ferulic acid, and cinnamic acid. A strong positive

Table 2

Phenolic averages of *Mentha* species under salt stress (g $kg^{-1}$)

| Treatments | Chlorogenic acid | | Catechine hydrate | | Caffeic acid | | Hydroxybenzoic acid | | Vanillic acid | | *p*-Coumaric acid | | Rutin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P |
| Control | 2.06 | 0.00 | 0.00 | 0.00 | 3.49 | 1.75 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.68 | 0.00 |
| 50 mM NaCI | 1.53 | 0.00 | 12.21 | 6.38 | 1.53 | 2.59 | 0.00 | 0.00 | 5.40 | 2.70 | 0.00 | 0.00 | 4.20 | 0.00 |
| 100 mM NaCI | 3.68 | 1.46 | 12.22 | 10.30 | 1.70 | 1.70 | 0.00 | 0.94 | 1.56 | 4.08 | 0.00 | 0.00 | 0.97 | 0.00 |
| 25 mg $L^{-1}$ QC | 0.88 | 0.00 | 8.01 | 5.91 | 1.97 | 1.97 | 0.00 | 0.00 | 2.40 | 1.20 | 0.00 | 0.00 | 1.35 | 0.00 |
| 50 mg $L^{-1}$ QC | 4.75 | 5.25 | 13.73 | 8.19 | 7.50 | 7.50 | 0.84 | 2.15 | 0.00 | 6.48 | 0.00 | 0.00 | 4.86 | 3.35 |
| 25 mg $L^{-1}$ GO | 2.31 | 4.93 | 7.27 | 6.19 | 5.82 | 5.82 | 1.74 | 0.67 | 7.66 | 0.00 | 0.00 | 0.00 | 1.41 | 0.00 |
| 50 mg $L^{-1}$ GO | 4.30 | 2.59 | 4.25 | 9.38 | 3.75 | 3.75 | 2.17 | 2.41 | 0.00 | 1.13 | 0.00 | 0.00 | 0.00 | 3.62 |
| 25 mg $L^{-1}$ NP | 1.64 | 1.99 | 12.28 | 8.24 | 7.56 | 7.56 | 3.34 | 1.32 | 0.00 | 0.00 | 0.00 | 0.84 | 0.00 | 3.37 |
| 50 mg $L^{-1}$ NP | 4.73 | 0.00 | 8.58 | 11.14 | 4.02 | 4.02 | 0.58 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.57 | 4.05 |
| 25 mg $L^{-1}$ QC+50 mM NaCI | 3.99 | 4.85 | 11.63 | 11.77 | 1.13 | 2.97 | 0.64 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.80 | 0.00 |
| 25 mg $L^{-1}$ QC+100 mM NaCI | 0.71 | 0.00 | 2.96 | 4.56 | 0.89 | 0.89 | 1.41 | 2.98 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50 mg $L^{-1}$ QC+50 mM NaCI | 3.98 | 0.00 | 7.93 | 8.87 | 2.28 | 2.28 | 5.79 | 3.74 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50 mg $L^{-1}$ QC+100 mM NaCI | 0.00 | 0.00 | 2.63 | 0.00 | 4.02 | 4.02 | 2.79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.35 |
| 25 mg $L^{-1}$ GO+50 mM NaCI | 9.22 | 1.21 | 10.76 | 10.59 | 1.38 | 1.38 | 4.05 | 0.64 | 0.00 | 0.00 | 0.00 | 0.00 | 3.57 | 0.00 |
| 25 mg $L^{-1}$ GO+100 mM NaCI | 6.49 | 6.49 | 10.94 | 13.12 | 5.18 | 5.18 | 2.44 | 1.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50 mg $L^{-1}$ GO+50 mM NaCI | 2.17 | 0.00 | 5.82 | 7.64 | 3.71 | 3.71 | 2.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.62 | 0.00 |
| 50 mg $L^{-1}$ GO+100 mM NaCI | 3.83 | 4.20 | 12.67 | 9.21 | 4.91 | 4.91 | 2.65 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.47 | 0.00 |
| 25 mg $L^{-1}$ NP+50 mM NaCI | 0.00 | 0.00 | 5.55 | 1.63 | 1.36 | 1.36 | 0.78 | 1.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 25 mg $L^{-1}$ NP+100 mM NaCI | 2.40 | 0.00 | 1.63 | 3.55 | 2.38 | 2.38 | 0.67 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.10 |
| 50 mg $L^{-1}$ NP+50 mM NaCI | 3.57 | 4.67 | 4.46 | 0.00 | 4.06 | 4.06 | 2.54 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50 mg $L^{-1}$ NP+100 mM NaCI | 2.97 | 0.00 | 10.88 | 15.40 | 1.49 | 1.49 | 4.56 | 2.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

M.S.: M. *spicata*, M.P.: M. *piperita*

Cont. of Table 2

Phenolic averages of *Mentha* species under salt stress (g $kg^{-1}$)

| Treatments | Ferulic acid | | Hydroxycinnamic acid | | Naringin | | *o*-Coumaric acid | | Rosmarinic acid | | Salicylic acid | | Resveratrol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P | M.S | M.P |
| Control | 0.84 | 0.00 | 0.92 | 0.00 | 0.00 | 0.00 | 25.25 | 4.03 | 166.41 | 3.64 | 0.00 | 0.00 | 2.89 | 0.00 |
| 50 mM NaCI | 0.63 | 0.00 | 0.00 | 0.00 | 8.67 | 0.00 | 29.74 | 2.59 | 340.73 | 12.84 | 1.63 | 0.00 | 3.84 | 0.00 |
| 100 mM NaCI | 0.58 | 0.00 | 1.57 | 0.00 | 0.00 | 0.00 | 25.21 | 6.70 | 210.33 | 134.56 | 1.88 | 0.00 | 1.76 | 2.50 |
| 25 mg $L^{-1}$ QC | 2.24 | 3.36 | 0.90 | 0.00 | 3.84 | 78.05 | 21.89 | 8.98 | 134.71 | 48.62 | 0.00 | 0.00 | 1.77 | 2.98 |
| 50 mg $L^{-1}$ QC | 1.63 | 2.40 | 4.79 | 0.00 | 0.00 | 0.00 | 5.18 | 6.70 | 311.17 | 220.14 | 1.38 | 0.00 | 4.03 | 4.38 |
| 25 mg $L^{-1}$ GO | 4.07 | 0.59 | 0.75 | 0.00 | 0.00 | 0.98 | 20.41 | 13.60 | 202.30 | 144.05 | 1.23 | 0.00 | 6.95 | 4.44 |
| 50 mg $L^{-1}$ GO | 3.54 | 4.71 | 1.32 | 0.00 | 2.26 | 0.00 | 17.23 | 11.30 | 167.72 | 109.35 | 2.12 | 0.00 | 3.66 | 1.18 |
| 25 mg $L^{-1}$ NP | 0.61 | 1.16 | 0.00 | 0.82 | 1.66 | 4.26 | 25.14 | 21.17 | 274.34 | 255.33 | 3.17 | 0.99 | 5.10 | 5.82 |
| 50 mg $L^{-1}$ NP | 3.06 | 0.00 | 0.00 | 0.00 | 0.00 | 12.25 | 16.89 | 2.82 | 406.76 | 82.30 | 0.00 | 0.00 | 8.96 | 2.77 |
| 25 mg $L^{-1}$ QC+50 mM NaCI | 1.21 | 0.00 | 0.66 | 0.00 | 1.74 | 0.00 | 17.89 | 10.15 | 255.06 | 89.72 | 0.00 | 0.00 | 4.40 | 7.26 |
| 25 mg $L^{-1}$ QC+100 mM NaCI | 4.88 | 0.00 | 0.00 | 3.12 | 0.00 | 0.00 | 16.99 | 2.55 | 98.53 | 22.86 | 0.00 | 0.00 | 4.19 | 1.13 |
| 50 mg $L^{-1}$ QC+50 mM NaCI | 2.31 | 0.00 | 0.92 | 6.68 | 7.27 | 0.00 | 41.76 | 5.51 | 167.75 | 68.81 | 0.81 | 0.00 | 3.47 | 3.46 |
| 50 mg $L^{-1}$ QC+100 mM NaCI | 3.49 | 2.24 | 0.00 | 0.00 | 2.07 | 0.00 | 7.14 | 8.19 | 172.40 | 106.98 | 0.00 | 0.00 | 3.26 | 4.62 |
| 25 mg $L^{-1}$ GO+50 mM NaCI | 0.94 | 0.64 | 0.75 | 6.40 | 0.00 | 0.00 | 12.61 | 7.64 | 276.25 | 59.14 | 0.00 | 3.70 | 2.42 | 29.2 |
| 25 mg $L^{-1}$ GO+100 mM NaCI | 3.66 | 5.48 | 0.83 | 3.86 | 2.89 | 2.41 | 22.07 | 3.21 | 196.39 | 235.44 | 0.00 | 0.00 | 3.46 | 7.89 |
| 50 mg $L^{-1}$ GO+50 mM NaCI | 0.89 | 0.00 | 0.00 | 4.38 | 0.00 | 0.00 | 12.05 | 6.41 | 112.86 | 94.50 | 7.13 | 0.00 | 0.89 | 1.74 |
| 50 mg $L^{-1}$ GO+100 mM NaCI | 0.59 | 2.33 | 5.30 | 8.91 | 1.72 | 0.00 | 13.28 | 14.81 | 336.28 | 70.10 | 1.27 | 0.00 | 5.74 | 2.05 |
| 25 mg $L^{-1}$ NP+50 mM NaCI | 0.00 | 0.00 | 1.34 | 0.00 | 5.16 | 0.00 | 30.24 | 3.88 | 152.98 | 10.70 | 0.00 | 0.00 | 5.20 | 0.59 |
| 25 mg $L^{-1}$ NP+100 mM NaCI | 4.8 | 0.00 | 0.00 | 6.73 | 0.00 | 0.00 | 8.50 | 13.50 | 56.46 | 36.93 | 0.00 | 0.00 | 0.90 | 5.71 |
| 50 mg $L^{-1}$ NP+50 mM NaCI | 31.25 | 6.19 | 0.00 | 8.32 | 0.99 | 0.00 | 6.15 | 8.88 | 174.44 | 98.22 | 1.70 | 0.00 | 4.82 | 3.95 |
| 50 mg $L^{-1}$ NP+100 mM NaCI | 6.39 | 6.55 | 0.71 | 5.13 | 2.91 | 0.00 | 31.05 | 5.07 | 382.09 | 30.44 | 1.33 | 0.00 | 4.16 | 2.58 |

M.S.: M. *spicata*. M.P.: M. *piperita*

Phenolic averages of *Mentha* species under salt stress (g $kg^{-1}$)

| Treatments | Quercetin | | Cinnamic acid | | Naringenin | | Chrysin | | Flavone | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M.S | M:P | M.S | M:P | M.S | M:P | M.S | M:P | M.S | M:P |
| Control | 2.63 | 0.53 | 0.00 | 0.00 | 0.00 | 2.89 | 130.23 | 0.00 | 2.06 | 0.00 |
| 50 mM NaCI | 9.46 | 2.01 | 0.00 | 0.00 | 1.88 | 3.04 | 150.67 | 0.00 | 0.00 | 0.00 |
| 100 mM NaCI | 1.44 | 4.30 | 0.00 | 0.00 | 0.00 | 1.61 | 0.00 | 0.00 | 0.00 | 0.00 |
| 25 mg $L^{-1}$ QC | 4.67 | 1.79 | 3.70 | 2.06 | 1.81 | 1.53 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50 mg $L^{-1}$ QC | 9.44 | 8.42 | 0.00 | 3.47 | 1.80 | 1.59 | 0.00 | 0.00 | 0.00 | 0.00 |
| 25 mg $L^{-1}$ GO | 4.09 | 2.69 | 0.00 | 0.76 | 2.86 | 1.37 | 0.00 | 0.00 | 0.00 | 0.99 |
| 50 mg $L^{-1}$ GO | 4.05 | 3.61 | 0.00 | 2.75 | 2.08 | 1.43 | 0.00 | 0.00 | 0.00 | 0.00 |
| 25 mg $L^{-1}$ NP | 3.83 | 3.53 | 0.00 | 0.00 | 0.00 | 1.53 | 29.13 | 0.00 | 1.07 | 2.64 |
| 50 mg $L^{-1}$ NP | 5.18 | 1.14 | 0.00 | 0.00 | 0.00 | 3.95 | 0.00 | 45.91 | 2.20 | 2.98 |
| 25 mg $L^{-1}$ QC+50 mM NaCI | 7.86 | 1.71 | 0.00 | 0.00 | 2.20 | 2.31 | 0.00 | 25.12 | 0.00 | 1.57 |
| 25 mg $L^{-1}$ QC+100 mM NaCI | 3.31 | 1.53 | 3.85 | 0.00 | 1.50 | 1.63 | 13.03 | 16.93 | 1.90 | 1.57 |
| 50 mg $L^{-1}$ QC+50 mM NaCI | 5.13 | 1.19 | 3.46 | 3.78 | 1.69 | 1.67 | 18.86 | 8.70 | 2.81 | 0.57 |
| 50 mg $L^{-1}$ QC+100 mM NaCI | 4.68 | 3.33 | 0.00 | 1.72 | 0.00 | 1.46 | 37.36 | 0.00 | 2.32 | 0.00 |
| 25 mg $L^{-1}$ GO+50 mM NaCI | 5.42 | 1.06 | 0.76 | 0.56 | 0.00 | 1.82 | 14.78 | 1.24 | 2.48 | 2.22 |
| 25 mg $L^{-1}$ GO+100 mM NaCI | 3.00 | 4.14 | 0.00 | 4.73 | 0.00 | 1.75 | 40.71 | 0.00 | 2.41 | 3.13 |
| 50 mg $L^{-1}$ GO+50 mM NaCI | 8.7 | 2.08 | 3.60 | 4.49 | 10.02 | 3.26 | 73.3 | 0.00 | 1.98 | 0.00 |
| 50 mg $L^{-1}$ GO+100 mM NaCI | 6.01 | 3.53 | 0.64 | 5.98 | 6.27 | 2.83 | 9.27 | 0.00 | 2.50 | 0.00 |
| 25 mg $L^{-1}$ NP+50 mM NaCI | 2.21 | 1.30 | 4.39 | 7.02 | 1.98 | 3.03 | 24.10 | 11.57 | 0.89 | 0.00 |
| 25 mg $L^{-1}$ NP+100 mM NaCI | 3.21 | 2.07 | 0.97 | 0.00 | 3.61 | 3.21 | 18.35 | 0.00 | 0.90 | 0.00 |
| 50 mg $L^{-1}$ NP+50 mM NaCI | 2.53 | 3.04 | 0.71 | 0.00 | 7.62 | 3.27 | 38.55 | 0.00 | 1.88 | 0.00 |
| 50 mg $L^{-1}$ NP+100 mM NaCI | 4.94 | 1.63 | 3.42 | 3.91 | 1.19 | 3.39 | 64.20 | 0.00 | 4.17 | 0.00 |

M.S. – M. *spicata*, M.P. – M. *piperita*

Table 3

Eigenvalues and variance explained by principal components of phenolic compounds in *Mentha spicata*

| Component | Eigenvalue | Variance (%) | Cumulative (%) |
|---|---|---|---|
| 1 | 4.32144 | 24.008 | 24.01 |
| 2 | 2.87895 | 15.994 | 40.1 |
| 3 | 2.3647 | 13.137 | 53.15 |
| 4 | 2.11342 | 11.741 | 64.9 |
| 5 | 1.35395 | 7.522 | 72.43 |
| 6 | 1.25029 | 6.9461 | 79.38 |
| 7 | 0.838698 | 4.6594 | 84.04 |
| 8 | 0.769886 | 4.2771 | 88.09 |
| 9 | 0.630279 | 3.5015 | 88.32 |
| 10 | 0.585322 | 3.2518 | 91.58 |
| 11 | 0.287807 | 1.5989 | 93.18 |
| 12 | 0.2503 | 1.3906 | 94.58 |
| 13 | 0.130316 | 0.72398 | 95.31 |
| 14 | 0.0934062 | 0.51892 | 95.83 |
| 15 | 0.0586834 | 0.32602 | 96.16 |
| 16 | 0.0411656 | 0.2287 | 96.39 |
| 17 | 0.0212802 | 0.11822 | 100.0 |

correlation was observed between resveratrol and several compounds in the second cluster, including caffeic acid, rosmarinic acid, vanillic acid, chlorogenic acid, and catechin hydrate. Conversely, resveratrol showed a negative correlation with salicylic acid.

These clustering patterns suggest that treatments involving nanoparticles (NPs) and graphene oxide (GO) were more closely associated with the accumulation of compounds in the second cluster, which are typically linked to antioxidant defense and phenylpropanoid metabolism. In contrast, the third cluster – comprising salicylic acid, rutin, and quercetin – appears to reflect responses associated with salinity-induced stress signaling. This distribution indicates that the GO and NP treatments modulated phenolic metabolism differently compared with quercetin and control treatments, leading to a distinct separation of phenolic profiles among the experimental groups.

Figure 2 illustrates the changes in phenolic acid components in *Mentha spicata* under various treatments. The highest flavon content was observed in the 50 mg $L^{-1}$ QC + 100 mM NaCl treatment, while the lowest was found in the 50 mg $L^{-1}$ GO group. Similarly, caffeic acid peaked in the 25 mg $L^{-1}$ GO treatment compared to the control. Notably, rutin, vanillic acid, catechin hydrate, and chlorogenic acid levels were elevated in treatments containing

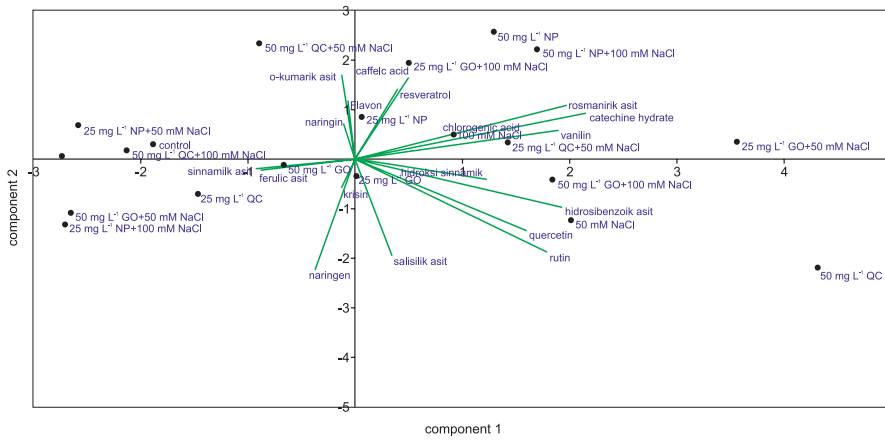


Fig. 1. Principal component analysis (PCA) of the phenolic compound properties in *Mentha spicata*

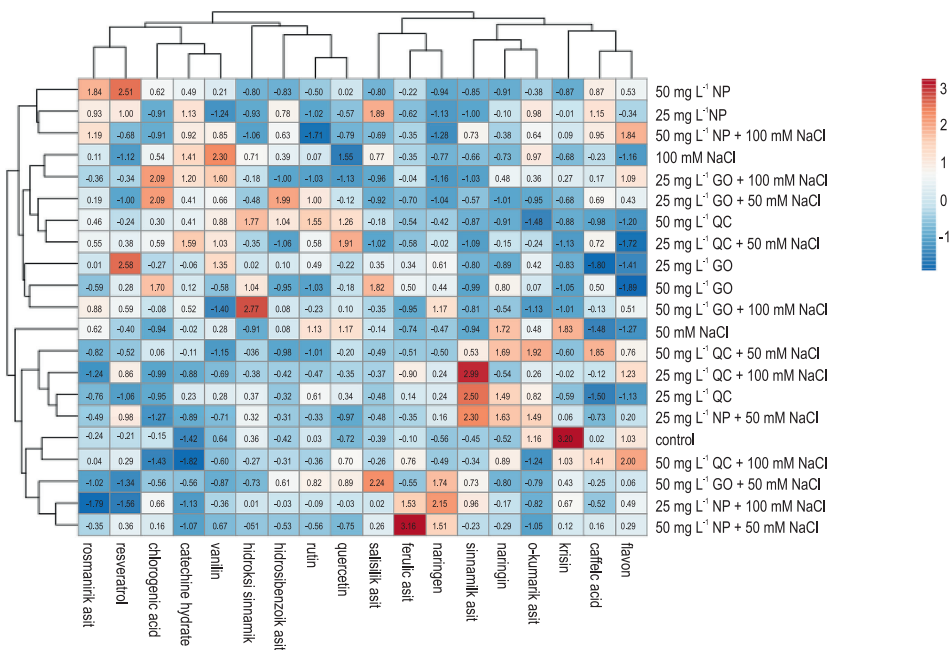


Fig. 2. Heat map of phenolic acid components in *Mentha spicata*

graphene oxide, whereas quercetin and salt stress generally reduced their concentrations. Resveratrol and rosmarinic acid increased with both nanoparticle and graphene oxide applications, indicating potential roles in stress mitigation. Overall, these data suggest that QC and GO treatments modulate phenolic acid profiles, potentially enhancing the plant's adaptive responses under salt stress conditions.

According to the principal component analysis (PCA) of phenolic compound parameters in *Mentha piperita*, seven components with eigenvalues greater than 1 were extracted from 18 variables (PC1: 4.55; PC2: 2.80; PC3: 2.19; PC4: 1.53; PC5: 1.49; PC6: 1.25; PC7: 1.15) – Table 4. The first principal component (PC1) accounted for approximately 26% of the total variance, and the second (PC2) explained 16%, together totaling about 42% of the variance, which was considered insufficient for robust statistical interpretation. The applied treatments did not significantly affect flavonoid levels; however, the highest concentrations of chrysin and caffeic acid were observed under the combined quercetin + salt treatment.

Table 4

The distribution of the first and second principal components of the phenolic compound properties of *Mentha piperita*

| Component | Eigenvalue | Variance (%) | Cumulative (%) |
|---|---|---|---|
| 1 | 4.55246 | 25.91 | 25.91 |
| 2 | 2.80734 | 15.596 | 41.51 |
| 3 | 2.198 | 12.211 | 53.72 |
| 4 | 1.53983 | 8.5546 | 62.28 |
| 5 | 1.4967 | 8.315 | 70.60 |
| 6 | 1.25409 | 6.9672 | 77.57 |
| 7 | 1.15506 | 6.417 | 83.99 |
| 8 | 0.872404 | 4.8467 | 88.74 |
| 9 | 0.563141 | 3.1286 | 91.87 |
| 10 | 0.532094 | 2.9561 | 94.73 |
| 11 | 0.341834 | 1.8991 | 96.13 |
| 12 | 0.252685 | 1.4038 | 97.54 |
| 13 | 0.152099 | 0.84499 | 98.39 |
| 14 | 0.132208 | 0.73449 | 99.03 |
| 15 | 0.0874832 | 0.48602 | 99.51 |
| 16 | 0.050139 | 0.27855 | 99.79 |
| 17 | 0.00761901 | 0.042328 | 99.83 |
| 18 | 0.00481689 | 0.02676 | 100.0 |

The PCA biplot (Figure 3) demonstrates clear separations in phenolic and flavonoid profiles among the different treatments. The first two components capture the majority of the variance, distinctly separating the QC- and GO-containing treatments from the control and NaCl-only treatments. Rutin, caffeic acid, and chlorogenic acid showed strong associations with the QC and GO treatments, while naringenin and resveratrol were more closely clustered with the NaCl treatments. This distribution suggests that QC and GO may enhance plant tolerance to salt stress by modulating the accumulation

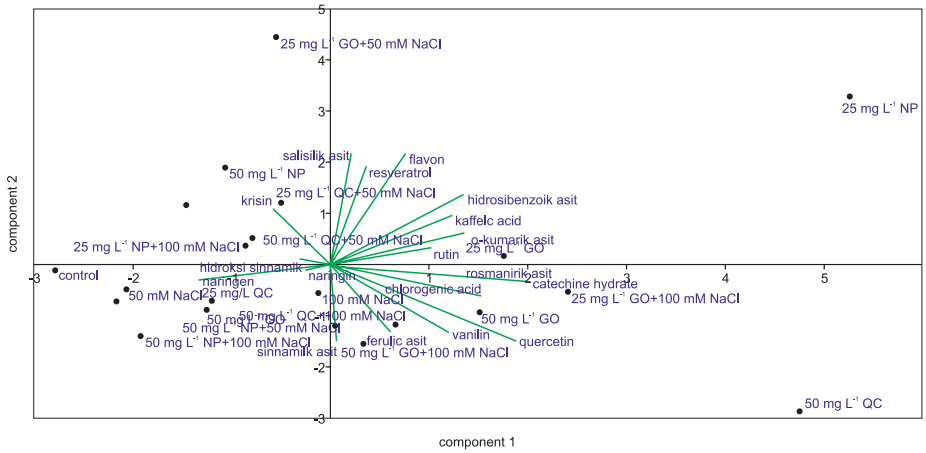


Fig. 3. Principal component analysis of phenolic acid components in *Mentha piperita*

of secondary metabolites. Overall, the combined application of QC and GO appears to guide phenolic and flavonoid profiles toward a more balanced metabolic state under NaCl stress.

The heatmap (Figure 4) clearly shows how different treatments affected phenolic and flavonoid levels. Salt stress (NaCl) generally reduced the accu-

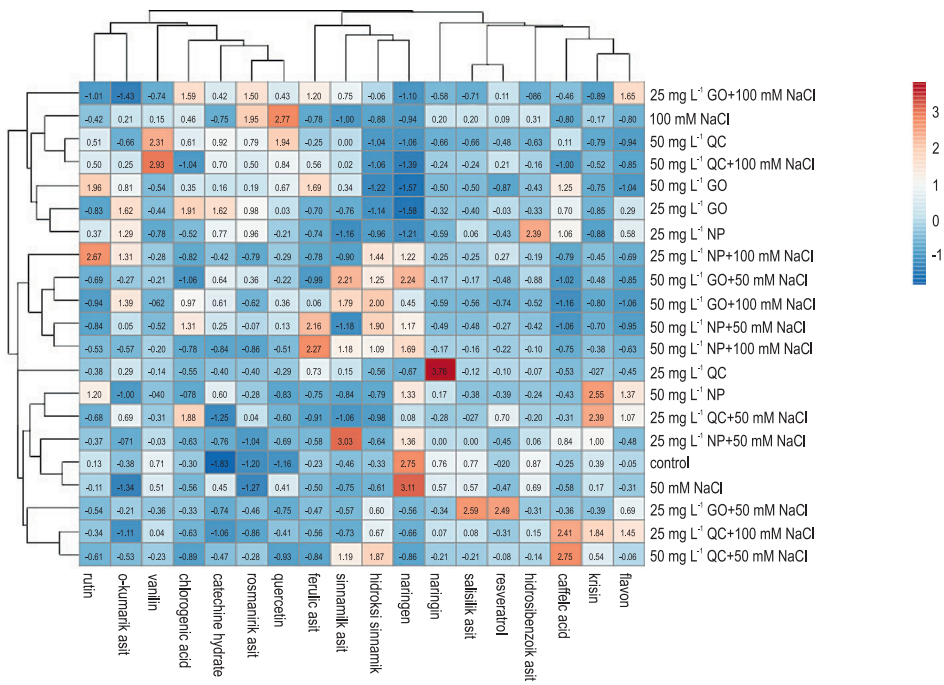


Fig. 4. Heat map of phenolic acid components in *Mentha piperita*

mulation of most metabolites, suggesting that ionic stress limits secondary metabolism. In contrast, applying quercetin (QC), either alone or together with NaCl, increased certain antioxidant compounds, including naringenin, resveratrol, and caffeic acid. These changes suggest that QC may help the plant cope with oxidative stress.

Interestingly, treatments combining graphene oxide (GO) and QC formed a separate cluster. In particular, GO + QC + NaCl treatments displayed a more balanced profile than NaCl alone, implying that GO might support QC's protective effects. Hydroxybenzoic acid, ferulic acid, and rutin were the most responsive metabolites, highlighting their importance in stress adaptation. Overall, the data suggest that co-applying GO and QC under salt stress can modulate phenolic metabolism and potentially enhance plant resilience.

## CONCLUSIONS

This study investigated the effects of salt stress and the applications of nanoparticles (NP), graphene oxide (GO), and quercetin (QC) on the phenolic compound profiles of *Mentha piperita* and *Mentha spicata*. The results demonstrated that NP and GO treatments positively modulated phenolic metabolism by increasing levels of rosmarinic acid, resveratrol, and certain flavonoids. QC application enhanced specific compounds, including caffeic acid, rutin, and quercetin, supporting plant responses under salinity stress. Principal Component Analysis (PCA) and clustering revealed that NP and GO treatments produced distinct phenolic profiles compared to QC and control groups. Overall, NP and GO applications effectively mitigated the adverse effects of salt stress and enhanced phenolic compound accumulation, suggesting their potential utility for improving stress tolerance and phenolic content in Mentha species under saline conditions.

### Author contributions

All authors have contributed to the completion of this research. All authors have read and agreed to the published version of the manuscript.

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