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Enhancing salt tolerance in *Mentha*×*gracilis* through foliar applications of titanium and nano-titanium

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Abstract

Salinity is an abiotic stress that negatively affects plant growth and the synthesis of secondary metabolites. This study aimed to evaluate the effects of foliar applications of titanium (Ti) and nano-titanium (nano-Ti) at concentrations of 0, 50, and 100 mg/L under salinity levels of 0, 50, and 100 mM NaCl in controlled greenhouse conditions. A factorial experiment based on a completely randomized design with four replications was conducted. A comprehensive set of morphological (plant height, fresh and dry biomass), physiological (photosynthetic pigments, soluble carbohydrates, proline, and protein content), and biochemical parameters (antioxidant enzyme activities including superoxide dismutase, guaiacol peroxidase, and ascorbate peroxidase), as well as essential oil (EO) content and composition, were assessed. Salinity stress markedly reduced plant growth, chlorophyll content, and EO yield, while increasing oxidative stress markers such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). The application of 100 mg/L nano-titanium under non-stress conditions significantly increased plant height (47.01 cm), fresh weight (87.33 g), and essential oil yield (0.639 g/pot). Moreover, essential oil content reached a maximum of 1.84% under 50 mM salinity with 100 mg/L nano-titanium, representing a 212% increase compared to the control. Nano-titanium application under salinity stress increased APX and SOD activities by 176% and 237%, respectively, compared to the control. GC–MS analysis revealed linalool, trans-caryophyllene, 1,8-cineole, and germacrene D as the major EO constituents, whose concentrations were notably influenced by both salinity level and Ti treatments. These findings suggest that nano-Ti has the potential to be used as a sustainable biostimulant to enhance growth and secondary metabolite production in *M.*×*gracilis* under saline environments.

Keywords Antioxidants, Chlorophyll, Mentha, Nanoparticles, Salinity

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Introduction

Medicinal plants have long been valued for their therapeutic properties, playing a vital role in both traditional and modern healthcare systems. Their bioactive constituents, including essential oils, alkaloids, and flavonoids, contribute to the treatment and prevention of a wide range of human diseases [1], in addition to their therapeutic applications in healthcare systems, they have also gained attention in agriculture as natural and effective alternatives to chemical materials [2, 3]. The increasing prevalence of chronic illnesses and a growing demand for natural and sustainable remedies have further emphasized the importance of identifying and cultivating high-quality medicinal plants with enhanced therapeutic value [4–6]. The genus *Mentha* from the Lamiaceae family contains compounds with interesting pharmacological and therapeutic properties [7]. Among these, *Mentha* × *gracilis*, commonly known as ginger mint, has attracted attention due to its aromatic essential oil and pharmacological applications in the food, pharmaceutical, and cosmetic industries [7–10]. However, medicinal plants, like other crops, are vulnerable to environmental stressors, particularly salinity, which is one of the most critical abiotic stresses affecting plant growth, metabolism, and yield worldwide [11–13]. According to the Food and Agriculture Organization (FAO), about 20% of cultivated land and 33% of irrigated land worldwide are affected by salinity, threatening global food security [14]. Salinity leads to osmotic imbalance and oxidative stress, negatively impacting photosynthesis, nutrient uptake, and secondary metabolite production [15–17]. While moderate stress may stimulate the accumulation of valuable phytochemicals, excessive salt levels can impair plant performance and reduce the quantity and quality of essential oils [18, 19]. This dual effect necessitates the development of strategies to enhance plant tolerance to salinity while preserving or improving their phytochemical composition. *Mentha* × *gracilis* is primarily found in temperate and humid regions, particularly in parts of Europe and Asia, where it grows naturally; however, due to its unique phytochemical properties, it has been widely cultivated across the globe, including in semi-subtropical regions of Iran such as East Azerbaijan, Kermanshah, and Fars [20, 21].

Similar to salinity, drought stress has been shown to reduce plant biomass while enhancing essential oil content and the accumulation of phenolic and flavonoid compounds in medicinal plants such as Salvia officinalis, highlighting the general impact of abiotic stresses on secondary metabolism [22, 23]. Previous studies have shown that improving soil or foliar inputs under salinity stress—whether through organic amendments or molecular priming—can significantly enhance plant physiological and secondary metabolite responses, including the accumulation of phenolics and improved antioxidant capacity, especially in aromatic and medicinal species [24, 25]. Foliar application of bioactive compounds has been demonstrated to alleviate the negative effects of drought stress by enhancing antioxidant enzyme activities and essential oil biosynthesis, suggesting a potential parallel with the effects of nano-titanium under salinity conditions [22, 23, 25–30].

Recent studies have demonstrated that eco-friendly nanomaterials, particularly the application of metal oxide nanoparticles can significantly improve plant stress tolerance [31–34]. These responses vary depending on the plant species, the form of titanium, the properties of the nanoparticle at the nanoscale, its concentration, and the method of application [15, 35–37]. Titanium dioxide nanoparticles (nano-TiO_2) are among the most widely used nanomaterials in modern industries, including agriculture, due to their high chemical stability, strong photocatalytic activity, and low cost. TiO_2 exists in three primary crystalline forms—anatase, rutile, and brookite—with anatase being the most active and commonly used form in plant-related applications [38]. As a semiconductor nanomaterial, nano-TiO_2 has gained attention for its ability to interact with light and biological systems, making it a valuable input in the formulation of antimicrobial agents, fungicides, and agricultural bio-stimulants [39]. Although titanium dioxide nanoparticles (TiO_2 NPs) can enhance plant growth traits at low concentrations, they may exhibit phytotoxic effects at higher doses; therefore, selecting an appropriate dosage is essential to avoid plant damage [40].

Recent studies have demonstrated the beneficial effects of nano-TiO_2 on plant growth, photosynthesis, chlorophyll synthesis, enzymatic activity, and essential oil production [15, 41, 42]. Several studies have reported that nano-TiO_2 application improves germination rate, biomass accumulation, and enzymatic antioxidant activity in crops such as fennel, rosemary, basil, and spinach under both normal and stress [15, 43]. Recent studies have confirmed the positive effects of nanomaterials such as titanium dioxide, cerium oxide, and iron oxide nanoparticles on improving salt stress tolerance in mint species. These treatments enhanced plant growth, photosynthetic pigments, antioxidant activities, and essential oil yield and composition under salinity conditions [26, 44, 45]. Although some investigations have explored the role of nano-titanium in enhancing plant growth and essential oil yield under stress conditions, there is a lack of specific research focusing on *Mentha* × *gracilis*. Therefore, the present study aimed to examine the impact of various forms and concentrations of titanium, including nano-titanium, on the morphological, physiological, and phytochemical traits of *Mentha* × *gracilis* under salinity

stress, with a particular focus on essential oil composition and production.

Materials and methods

Experimental site and design

This experiment was conducted in a glass greenhouse located in the Faculty of Agriculture, University of Maragheh, East Azerbaijan Province, Iran. Environmental conditions in the greenhouse were maintained at 20–27 °C, 30–60% relative humidity, and a photosynthetic photon flux density (PPFD) of approximately 100 µmol m^{-2} s^{-1} to ensure uniform seedling growth and controlled treatment conditions. To investigate the effect of salinity stress (0, 50, and 100 mM NaCl), and foliar application of titanium (in four forms: control (distilled water), 50 mg/L nano-titanium, 100 mg/L nano-titanium, and 50 mg/L titanium) a factorial experiment in a completely randomized design (CRD) with four replications was laid out. The charachteristics of used nano titanium has been presented in our previous work [46].

Growth conditions and treatments

Mentha × *gracilis* rhizomes were obtained from the research filed of university of Maragheh Iran. The plants were obtained under national and international guidelines and all authors comply with all the local and national guidelines. The identification of plant was done by Dr. Valiollah Mozaffariana a botanist from the Research Institute of Forests and Rangelands, Iran. Voucher specimen was deposited in the herbarium of Department of Horticulture, University of Maragheh, Iran under voucher number of 4366. The rhizomes were grown in 5-L plastic pots filled with a mixture of cocopeat and perlite (2:1). Four rhizomes (5 cm each) of *Mentha* × *gracilis* were planted in each pot to ensure uniform establishment. Hoagland nutrient solution application started at 25% strength during early growth, increasing to full strength as plants matured.

Salinity treatments were applied from the six-leaf stage to full flowering by irrigating with NaCl solutions. To avoid salt accumulation, root zones were leached with distilled water every six days. Titanium treatments were sprayed once, three days before salinity stress, and subsequently three times during stress at 10-day intervals. Depending on the plant growth stage and canopy around 10–20 mL of foliar spray was applied per application.

Sampling and measurements

Sampling was performed at full flowering stage after all treatments. For chlorophyll, carotenoid, proline, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and antioxidant enzyme activity (SOD, GPX, APX). The fresh leaves (3g) were frozen in liquid nitrogen and stored at −20°C until analysis. For essential oil extraction and phytochemical analysis, aerial parts were harvested and dried at room temperature for one week [47].

Measured traits and analytical methods

Plant height and biomass

Plant height was measured from the base of the stem at the soil surface to the tip of the apical meristem using a ruler (in cm) at the time of harvest. For fresh weight, whole shoots were harvested and immediately weighed using a digital scale. For dry weight, samples were placed in a paper bag and dried in a hot-air oven at 70 °C for 48 h until a constant weight was achieved, and then weighed.

Photosynthetic pigments

Chlorophyll a, b, and total carotenoids were extracted based on Arnon method [48]. For this reason, 0.1 g of fresh tissue were extracted in 10 mL acetone (80%), centrifuged at 6000 rpm for 10 min, and absorbance read at 663, 647, and 470 nm using a spectrophotometer (UV-1800 Shimadzu, Japan). Photosynthetic Pigments were determined as follows:

$$Chlorophyll\ a = 12.7 \times A663 - 2.69 \times A645$$

$$Chlorophyll\ b = 22.9 \times A645 - 4.68 \times A663$$

$$Total\ carotenoids\ 100\ \times\ A470 -\ (1.82\ Chl\ a)\ -\ (85.02\ Chl\ b)$$

Hydrogen peroxide (H_2O_2)

Fresh leaf tissue (0.5 g) was homogenized in 10 mL of 1% (w/v) trichloroacetic acid (TCA) in an ice-cold mortar. The extract was centrifuged at 12,000 rpm for 15 min at 4 °C. Then, 0.5 mL of the supernatant was mixed with 0.5 mL of 50 mM phosphate buffer (pH 7.0) and 1.0 mL of 1 M potassium iodide (KI). The mixture was incubated at room temperature for 10 min, and the absorbance was recorded at 390 nm using a spectrophotometer (UV-1800 Shimadzu, Japan). H_2O_2 concentration was calculated using a standard curve and expressed as µmol g^{-1} fresh weigh [49].

Malondialdehyde (MDA)

The MDA content was determined using the thiobarbituric acid (TBA) method (Heath and Packer, 1968). Briefly, 0.5 g of fresh leaf tissue was homogenized in 1.5 mL of 0.1% TCA, and the extract was centrifuged at 1000 rpm for 10 min at 4 °C. Then, 0.5 mL of the supernatant was mixed with 1 mL of 0.1% TBA in 20% TCA. The mixture was incubated in a water bath at 95 °C for 30 min, cooled in ice for 30 min, and absorbance was measured at 532 and 600 nm using a spectrophotometer (UV-1800 Shimadzu, Japan). MDA concentration was calculated as µmol g^{-1} FW after subtracting non-specific absorbance at 600 nm [50].

GPX (Guaiacol Peroxidase) activity

GPX activity was assayed following MacAdam et al. (1992), using a reaction mixture containing 1000 µL of 100 mM phosphate buffer (pH 7.0), 250 µL of 0.1 mM EDTA, 1000 µL of 15 mM H_2O_2, 1000 µL of 5 mM guaiacol, and 50 µL of enzyme extract. The change in absorbance at 470 nm was recorded for 180 s [32].

Ascorbate peroxidase (APX) activity

APX activity was determined according to Nakano and Asada (1981). The assay mixture contained 1500 µL of 50 mM phosphate buffer (pH 7.0), 600 µL of 0.1 mM EDTA, 400 µL of 0.5 mM ascorbic acid, 400 µL of 3% H_2O_2, and 100 µL of enzyme extract. Absorbance was measured at 290 nm [33].

SOD (Superoxide dismutase) activity

SOD activity was measured according to the method of Dhindsa et al. (1981). A control sample without enzyme extract was kept in the dark throughout the assay and used as a blank. Absorbance was recorded at 560 nm using a spectrophotometer [31].

Proline content

Proline content was determined based on the method of Bates et al. (1973), with modifications. Fresh leaf tissue (0.5 g) was homogenized in 10 mL of 3% sulfosalicylic acid and centrifuged at 10,000 rpm for 20 min at 4°C. Two milliliters of the supernatant were mixed with 2 mL of acid ninhydrin solution (prepared by dissolving 1.25 g of ninhydrin in 30 mL glacial acetic acid and adding 20 mL of 6 M phosphoric acid), and 2 mL of glacial acetic acid. The mixture was incubated in a hot water bath at 95 °C for 1 h, then transferred to an ice bath. Subsequently, 4 mL of toluene was added, and the mixture was vortexed for 20 s. The chromophore-containing toluene phase was separated and absorbance was read at 520 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan). A standard curve with known proline concentrations (0–20 mg/L) was used to quantify proline content [51].

Total soluble protein

Protein content was determined using Bradford reagent. Extracts were mixed with the dye solution, incubated, and absorbance measured at 595 nm using BSA (Bovin serum albumin) as the standard [52].

Total phenolics

Extraction, was carried out according to the method described by [53]. 100 µL of extract was reacted with Folin–Ciocalteu reagent and $NaHCO_3$. After incubation at 45 °C, absorbance was measured at 765 nm [53]. The results were expressed as mg of gallic acid equivalent per g dry weight (mg of GAE/g FW).

Total flavonoids

The aluminum chloride method was employed using a 2% w/v $AlCl_3$ solution in 80% methanol. In brief, 1 mL of methanolic plant extract was mixed with 1 mL of 2% $AlCl_3$ and incubated for 1 h at room temperature. Absorbance was read at 415 nm [53]. The total flavonoid content was expressed as mg of rutin equivalents per g of dry weight (mg of RE/g FW).

Essential Oil (EO) extraction

A 20 g of dried plant material was hydrodistilled for 3 h using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and stored in sealed amber vials at 4 °C for up to one week before GC–MS and GC-FID analysis [54].

GC–MS and GC-FID Analysis

EO constituents were identified by GC–MS (Agilent 5977 A) using an HP-5MS column. Quantification was performed by GC-FID (Agilent 7990B). Compound identification was based on retention indices and comparison with NIST database (Adams, 2007; NIST 08, 2008). The percentage of essential oil constituents was determined using a gas chromatograph (Agilent 7990B, USA) equipped with a flame ionization detector (FID) and a VF-5MS capillary column. The injector and detector temperatures were set at 230 °C and 240 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min with a split ratio of 24:1. Essential oil samples were diluted 1:100 in hexane, and 1 µL of each sample was injected. Quantification of the oil components was carried out based on peak area normalization without the use of correction factors [54].

Statistical analysis

All collected data were analyzed using analysis of variance (ANOVA) via SAS software (version 9). Mean comparisons were made using LSD at 5% probability level. Graphs and charts were prepared using Microsoft Excel 2013.

Results

Plant height

The analysis of variance revealed that salinity stress, fertilizer treatments, and their interaction had a significant effect on plant height (Table 1). The highest plant height (47.01 cm) was recorded under non-stress conditions with the application of 100 mg of nano-titanium, while the lowest height (18.41 cm) was observed under 100 mM salinity stress without fertilizer application (Fig. 1). Furthermore, plant height under non-stress conditions was 25% and 72% greater than under 50 mM and 100 mM salinity stress, respectively. The application of 100 mg nano-titanium under non-stress conditions increased plant height by 155% compared to 100 mM

Table 1 Analysis of variance (ANOVA) for growth traits of *Mentha* × *gracilis* under salinity stress and fertilizer treatments

Source of Variation	df	Plant Height (cm)	Fresh Weight (gr per plant)	Plant Height (gr per plant)
Salinity Stress	2	837.05**	2039.06**	6671.13**
Fertilizer Treatments	3	505.09**	1469.81**	276.79**
Salinity × Fertilizer	6	8.17**	97.12**	28.22**
Error	36	1.42	2.16	2.65
Coefficient of Variation (%)		3.64	2.27	5.31

ns non-significant; ** = significant at 1% level; * = significant at 5% leve

salinity without fertilizer. Additionally, the use of 50 mg nano-titanium under 50 mM salinity increased height by 30%, while 100 mg under 100 mM salinity led to a 68% increase in height compared to no fertilizer within the same stress level (Fig. 1).

Fresh weight

Salinity stress, fertilizer treatments, and their interaction significantly affected fresh plant weight (Table 1). The maximum fresh weight (87.33 g) was observed under non-stress conditions with the application of 100 mg nano-titanium, while the minimum (39.75 g) occurred under 100 mM salinity without fertilizer (Fig. 2). Compared to the control, salinity stress at 50 mM and 100 mM reduced fresh weight by 14% and 31%, respectively. Conversely, fertilizer treatments improved fresh weight under both stressed and non-stressed conditions. The application of 100 mg nano-titanium under non-stress conditions led to a 130% increase in fresh weight compared to 100 mM salinity without fertilizer. Under 50 mM stress, the use of 100 mg nano-titanium increased fresh weight by 79%, and under 100 mM stress, 50 mg nano-titanium resulted in a 70% increase (Fig. 2).

Dry weight

Dry weight was significantly influenced by salinity stress, fertilizer treatments, and their interaction (Table 1). The highest dry weight (42.62 g) was recorded under non-stress conditions with 100 mg nano-titanium, and the lowest (18.28 g) under 100 mM salinity without fertilizer (Fig. 3). Dry weight decreased with increasing salinity, showing a 27% and 51% increase under non-stress conditions compared to 50 mM and 100 mM stress,

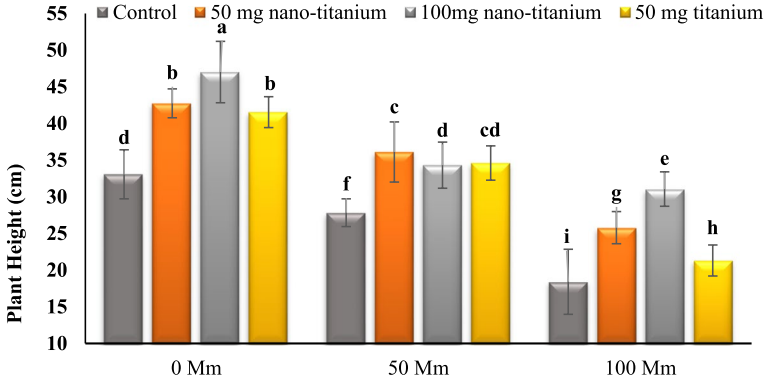

Fig. 1 Interaction effect of salinity and fertilizer treatments on height of *Mentha* × *gracilis*. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences ($P < 0.05$) among the treatments (LSD test at 5% level)

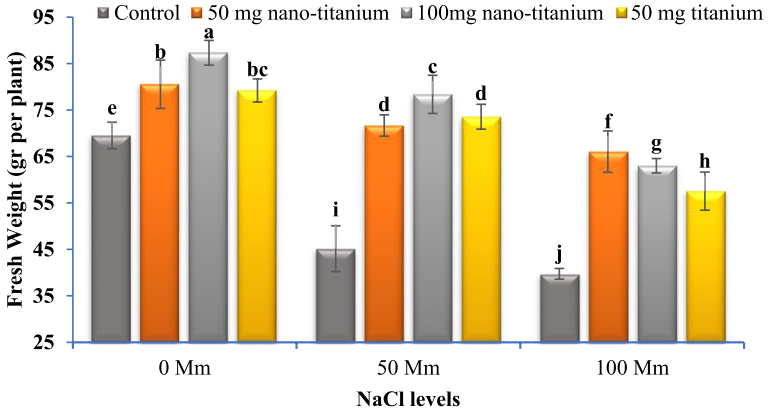

Fig. 2 Interaction effect of salinity and fertilizer treatments on fresh weight of *Mentha* × *gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

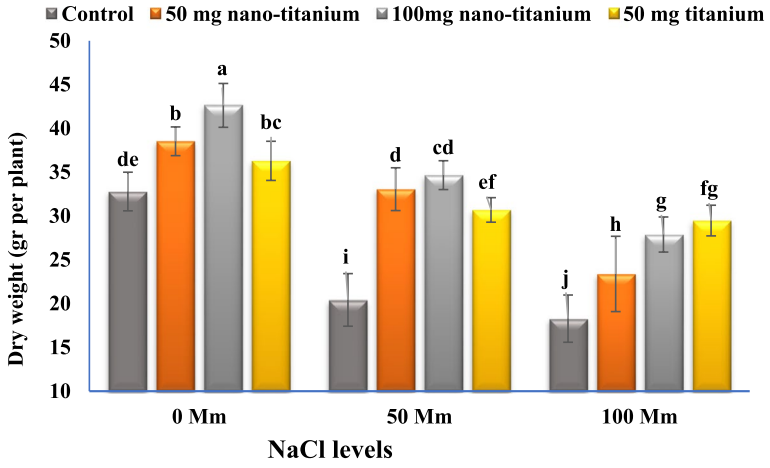

Fig. 3 Interaction effect of salinity and fertilizer treatments on dry weight of *Mentha*×*gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

Table 2 Analysis of variance (ANOVA) for photosynthetic pigments of *Mentha*×*gracilis* under salinity stress and fertilizer treatments

Source of Variation	df	Chlorophyll a (mg/gFW)	Chlorophyll b (mg/gFW)	Carotenoids (mg/gFW)
Salinity Stress	2	42.55**	23.03**	24.98**
Fertilizer Treatments	3	13.01**	0.719**	5.71**
Salinity × Fertilizer	6	1.23**	0.081**	1.22**
Error	36	3.89	0.029	0.005
CV (%)		0.08	3.15	1.23

**Significant at $p \leq 0.01$, *Significant at $p \leq 0.05$, ns = not significant

respectively. Nevertheless, fertilizer treatments improved dry weight. On average, applying 100 mg nano-titanium increased dry weight by 65% compared to no fertilizer. Furthermore, under non-stress conditions, applying 100 mg nano-titanium resulted in a 155% increase in dry weight compared to the 100 mM salinity without fertilizer (Fig. 3).

Chlorophyll a content

Chlorophyll a content was significantly affected by salinity stress, fertilizer treatments, and their interaction (Table 2). The highest chlorophyll a content (10.41 mg/g FW) was recorded under non-stress conditions with 100 mg nano-titanium, while the lowest value (5.18 mg/g FW) was obtained under 100 mM salinity stress without fertilizer (Fig. 4). Salinity stress led to a reduction in chlorophyll content: chlorophyll a decreased by 40% and 52% under 50 mM and 100 mM salinity, respectively, compared to the non-stress condition. Conversely, fertilizer treatments mitigated the adverse effects of salinity. Chlorophyll a content under non-stress conditions with 100 mg nano-titanium was 101% higher than that under 100 mM salinity without fertilizer (Fig. 4).

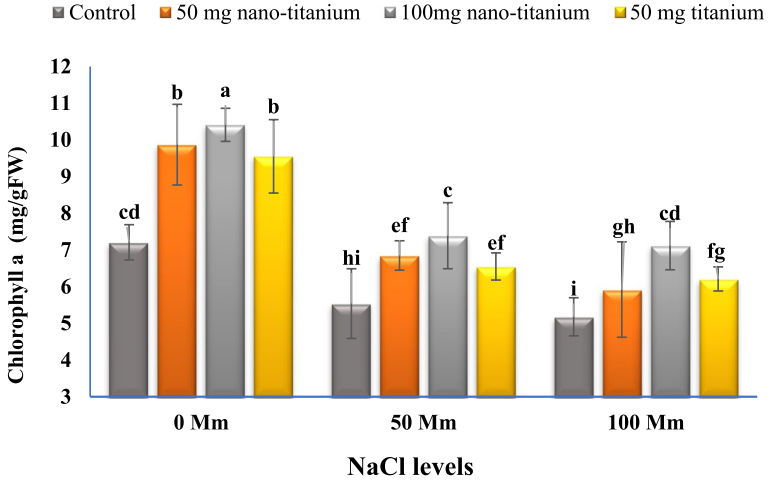

Fig. 4 Interaction effect of salinity and fertilizer treatments on Chlorophyll a content of *Mentha*×*gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

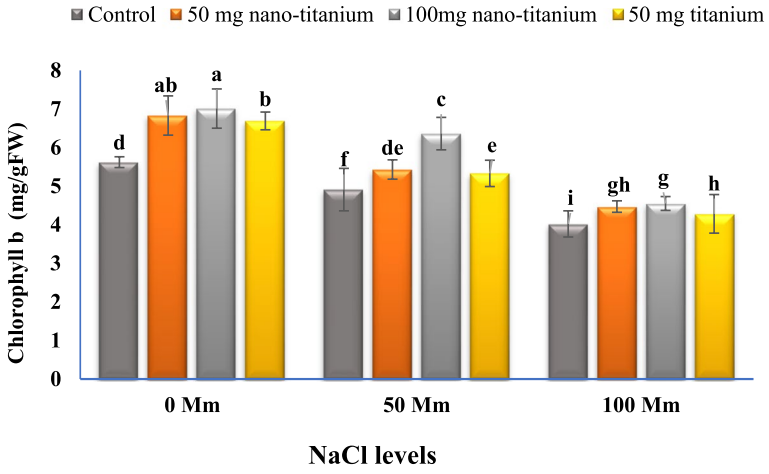

Fig. 5 Interaction effect of salinity and fertilizer treatments on Chlorophyll b content of *Mentha* × *gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

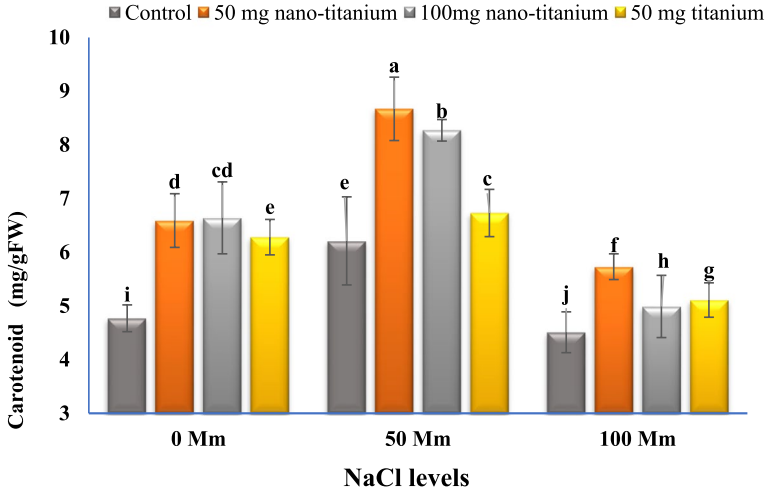

Fig. 6 Interaction effects of salinity stress levels and fertilizer treatments on carotenoid content of *Mentha* × *gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

Chlorophyll b content

The results indicated that salinity stress, fertilizer treatments, and their interaction significantly affected chlorophyll b content (Table 2). The maximum chlorophyll b content (7.01 mg/g FW) was observed under non-stress conditions with 100 mg nano-titanium, which did not significantly differ from 50 mg nano-titanium under the same condition. The minimum value (4.02 mg/g FW) was recorded under 100 mM salinity without fertilizer (Fig. 5). Salinity stress caused a decline in chlorophyll b content, decreasing it by 27% and 57% under 50 mM and 100 mM stress, respectively, compared to the control. However, fertilizer treatments significantly improved chlorophyll b levels. Application of 100 mg nano-titanium under non-stress conditions increased chlorophyll b content by 74% compared to 100 mM salinity without fertilizer (Fig. 5).

Carotenoid content

The results revealed that carotenoid content was significantly influenced by salinity stress, fertilizer treatments, and their interaction (Table 2). The highest carotenoid content (8.67 mg/g fresh weight) was recorded under 50 mM salinity stress with the application of 50 mg of nano-titanium, while the lowest value (4.51 mg/g fresh weight) was observed under 100 mM salinity stress without fertilizer (Fig. 6). Carotenoid content under 50 mM salinity increased by 47% compared to 100 mM stress and by 23% compared to non-stress conditions. Furthermore, the carotenoid content under 50 mM salinity with 50 mg nano-titanium was 93% higher than under 100 mM salinity without fertilizer and 81% higher than under non-stress conditions without fertilizer (Fig. 6).

Table 3 Analysis of variance (ANOVA) for antioxidant enzymes, hydrogen peroxide, and malondialdehyde content of *Mentha*×*gracilis* under salinity stress and fertilizer treatments

Source of Variation	df	H_2o_2 (μmol g^{-1} FW)	MDA (μmol g^{-1} FW)	GPX (U mg^{-1} protein)	APX (U mg^{-1} protein)	SOD (U mg^{-1} protein)
Salinity Stress	2	19.46**	30.51**	4.27**	4.96**	4.96**
Fertilizer Treatments	3	1.61**	3.78**	1.87**	0.99**	0.99**
Salinity × Fertilizer	6	0.826**	0.741**	0.07**	0.199**	0.199**
Error	36	0.029	0.113	0.009	0.003	0.003
CV (%)		3.34	7.47	4.49	3.82	3.82

Significant at $p \leq 0.01$, *Significant at* $p \leq 0.05$, *ns* not significant

Table 4 Interaction effects of different salinity stress levels and fertilizer treatments on antioxidant enzyme activities, MDA, and hydrogen peroxide content in *Mentha*×*gracilis*

	Treatments	H_2o_2 (μmol g^{-1} FW)	MDA (μmol g^{-1} FW)	GPX (U mg^{-1} protein)	APX (U mg^{-1} protein)	SOD (U mg^{-1} protein)
	Control	5.23 g	3.12 h	1.19 i	4.38 i	0.721 i
Control	50 mg Nano-Ti	4.64 i	2.45 i	1.76 g	5.68 h	0.99 h
	100 mg Nano-Ti	4.96 h	3.08 h	2.09 ef	6.74 g	1.28 g
	50 mg Ti	5.15 gh	3.42 gh	1.41 h	5.18 h	1.02 h
	Control	7.04 c	5.66 bc	2.18 de	9.13 de	1.33 fg
50 mM NaCl	50 mg Nano-Ti	6.01 e	4.48 f	3.01 b	11.28 ab	2.43 a
	100 mg Nano-Ti	5.95 f	3.71 g	3.15 a	12.06 a	2.36 ab
	50 mg Ti	6.11 de	4.85 ef	2.25 d	10.55 bc	2.34 b
	Control	7.68 a	6.61 a	1.87 g	8.14 f	1.42 f
100 mM NaCl	50 mg Nano-Ti	7.37 b	5.38 cd	2.51 c	8.48 e	1.53 e
	100 mg Nano-Ti	6.29 d	5.02 de	2.42 c	9.82 cd	1.76 c
	50 mg Ti	7.51 ab	6.07 b	2.01 f	9.80 cd	1.67 d
	LSD (0.05)	0.247	0.482	0.139	0.781	0.086

Values with the same letter within each column are not significantly different according to the LSD test at the 5% probability level

Hydrogen Peroxide (H_2o_2) Content

The results of the analysis of variance showed that salinity stress, fertilizer treatments, and their interaction significantly affected hydrogen peroxide content (Table 3). The highest hydrogen peroxide content (7.68 μmol g^{-1} fresh weight) was recorded under severe salinity stress (100 mM) without fertilizer application. In contrast, the lowest content (4.64 μmol g^{-1} fresh weight) was observed under non-stress conditions with the application of 50 mg of nano-titanium (Table 1). Compared to the non-stress condition, H_2o_2 content increased by 33% under 50 mM salinity and by 56% under 100 mM salinity stress (Table 4). However, fertilizer treatments reduced hydrogen peroxide levels. Under non-stress conditions, the application of 50 mg of nano-titanium decreased hydrogen peroxide content by 40% compared to the 100 mM stress treatment without fertilizer. Furthermore, the application of 100 mg nano-titanium reduced hydrogen peroxide content by 16% under 50 mM salinity and by 19% under 100 mM salinity compared to the corresponding no-fertilizer treatments (Table 4).

Malondialdehyde (MDA) content

MDA content was significantly affected by salinity stress, fertilizer treatments, and their interaction (Table 3). The maximum MDA content (6.61 μmol g^{-1} fresh weight) was recorded in the 100 mM salinity stress treatment without fertilizer, whereas the minimum (2.45 μmol g^{-1} fresh weight) was observed under non-stress conditions with 50 mg nano-titanium application (Table 4). In comparison with non-stress conditions, MDA content increased by 55% under 50 mM salinity and by 92% under 100 mM salinity. Furthermore, under 100 mM salinity without fertilizer, MDA content increased by 112% compared to the control. On the other hand, nano-titanium foliar application reduced MDA levels. Specifically, under non-stress conditions, the use of 100 mg nano-titanium reduced MDA content by 63% compared to the severe salinity stress without fertilizer (Table 1). In addition, under 50 mM salinity, the application of 100 mg nano-titanium decreased MDA by 35% relative to the no-fertilizer treatment, and under 100 mM salinity, it led to a 24% reduction in MDA compared to the unfertilized condition (Table 4).

Guaiacol peroxidase activity

The results of the analysis of variance indicated that guaiacol peroxidase activity was significantly affected by salinity stress, fertilizer treatments, and their interaction (Table 3). The findings revealed that the highest guaiacol peroxidase activity (3.15 U mg^{-1} protein) was recorded under 50 mM salinity stress combined with 100 mg of nano-titanium treatment (Table 4). In contrast, the lowest activity (1.19 U mg^{-1} protein) was observed under

non-stress conditions without any fertilizer application (Table 4). Furthermore, at 50 mM salinity, guaiacol peroxidase activity increased by 65% compared to non-stress conditions and by 21% compared to 100 mM salinity stress. Application of 100 mg nano-titanium under 50 mM salinity increased guaiacol peroxidase activity by 165% compared to the control and by 69% compared to 100 mM salinity stress without fertilizer (Table 4).

Ascorbate Peroxidase (APX) activity

According to the analysis of variance, APX activity was significantly influenced by salinity stress, fertilizer treatments, and their interaction (Table 3). The highest APX activity (12.06 U mg^{-1} protein) was recorded under 50 mM salinity stress with 100 mg nano-titanium, while the lowest (4.38 U mg^{-1} protein) was observed in the control (Table 4). Under 50 mM salinity, APX activity increased by 92% compared to the non-stress condition and by 16% compared to 100 mM salinity. Additionally, application of 100 mg nano-titanium under 100 mM salinity enhanced APX activity by 176% relative to the control and by 48% compared to 100 mM salinity without fertilizer. Moreover, under 100 mM salinity, 100 mg nano-titanium increased APX activity by 21% compared to the unfertilized treatment under the same stress condition (Table 4).

Superoxide Dismutase (SOD) activity

As shown in the analysis of variance, salinity stress, fertilizer treatments, and their interaction significantly affected SOD activity (Table 3). The maximum SOD activity (2.43 U mg^{-1} protein) was observed with the application of 50 mg nano-titanium under 50 mM salinity, while the minimum (0.721 U mg^{-1} protein) was recorded in the untreated control (Table 3). Under 50 mM salinity, SOD activity increased by 110% compared to the non-stressed condition and by 33% relative to 100 mM salinity. Moreover, application of 50 mg nano-titanium under 50 mM salinity resulted in a 237% increase in SOD activity compared to the control and 71% compared to 100 mM salinity without fertilizer. On average, the use of 50 mg nano-titanium led to a 39% increase in SOD activity compared to the unfertilized condition (Table 4).

Soluble carbohydrate content

The results indicated that soluble carbohydrate content was significantly affected by the individual effects of salinity stress and fertilizer treatments (Table 5). Among the fertilizer treatments, the highest soluble carbohydrate content (21.98 mg/g fresh weight) was observed with 50 mg nano-titanium, while the lowest value (15.58 mg/g fresh weight) was recorded in the control treatment (Fig. 7). Application of 50 mg nano-titanium increased soluble carbohydrates by 41%, 16%, and 29% compared to the control, 100 mg nano-titanium, and 50 mg titanium, respectively (Fig. 7).

Additionally, under salinity stress conditions, the highest soluble carbohydrate content (22.02 mg/g fresh weight) was observed at 100 mM salinity, while the lowest (15.03 mg/g fresh weight) occurred under non-stress conditions (Fig. 7). Notably, soluble carbohydrate content increased by 47% under 100 mM salinity and by 20% under 50 mM salinity compared to non-stress conditions (Fig. 7).

Proline content

Proline content was significantly affected by salinity stress, fertilizer treatments, and their interaction (Table 5). The highest proline content (4.23 µmol g^{-1} fresh weight) was observed under 100 mM salinity stress combined with the application of 50 mg of nano-titanium. Conversely, the lowest content (1.68 µmol g^{-1} fresh weight) was recorded in the control group under non-stress conditions without fertilizer application. Moreover, under 100 mM salinity, proline content increased by 71% compared to the non-stress condition and by 20% compared to the 50 mM salinity treatment. Notably, the application of 50 mg of nano-titanium under 100 mM salinity resulted in a 151% increase in proline content compared to the untreated control. Furthermore, this treatment led to a 28% increase in proline compared to the no-fertilizer treatment under 50 mM salinity (Fig. 8).

Total soluble protein

According to results, soluble protein content was significantly influenced by salinity stress and fertilizer treatments. Among fertilizer treatments, the highest protein

Table 5 Analysis of variance (ANOVA) for physiological traits of *Mentha* × *gracilis* under salinity stress and fertilizer treatments

Source of Variation	df	Soluble Carbohydrates	Proline(µmol g^{-1} FW)	Soluble Protein (mg/gFW)	Total Phenolics (mg GAE/gFW)	Total Flavonoids (mg RE/g FW)
Salinity Stress	2	196.35**	9.84**	2.56**	330.16**	402.60**
Fertilizer Treatments	3	93.21**	1.61**	0.401**	175.47**	151.79**
Salinity × Fertilizer	6	1.06 ns	0.088**	0.228 ns	9.74 ns	17.18**
Error	36	0.610	0.018	0.007	0.432	0.124
CV (%)		4.24	4.45	5.44	1.34	1.39

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$, *ns* not significant

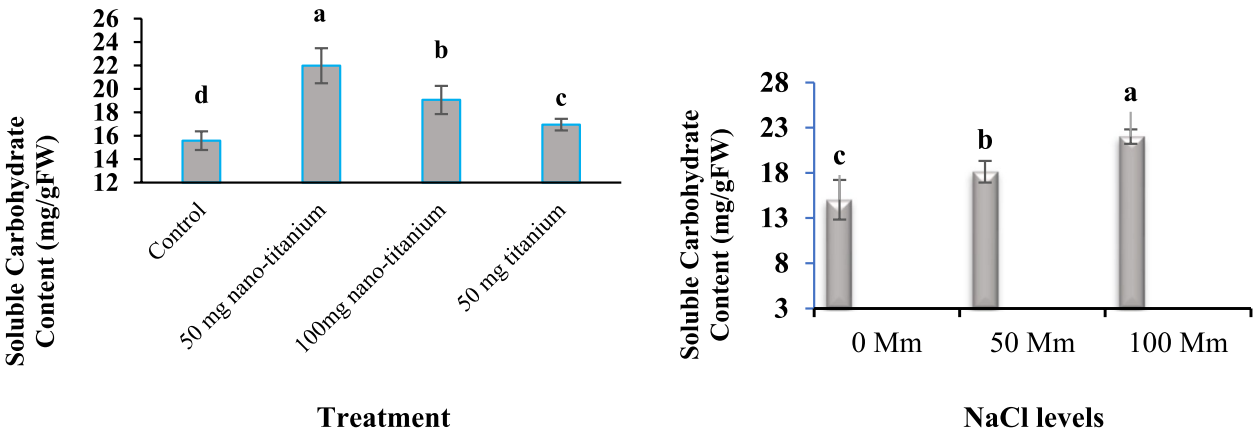

Fig. 7 Comparison of the effects of fertilizer treatments and salinity stress on soluble carbohydrate content of *Mentha*×*gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

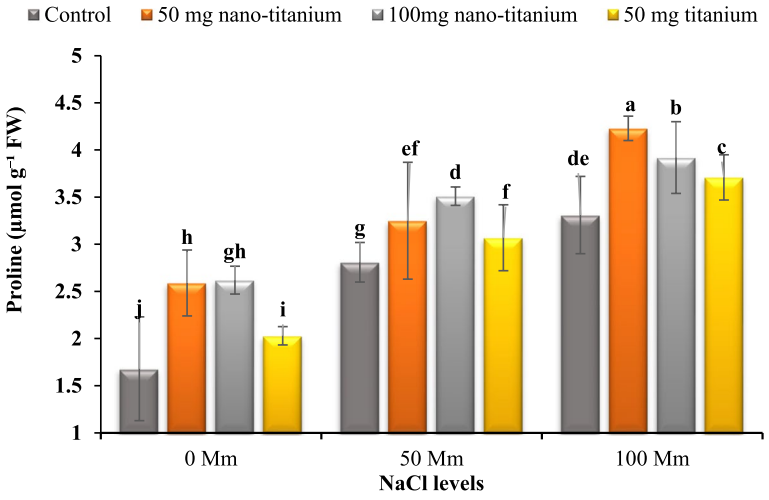

Fig. 8 Interaction effects of different salinity levels and fertilizer treatments on proline content in *Mentha*×*gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

content (1.79 mg g^{-1} fresh weight) was obtained with the application of 100 mg nano-titanium, while the lowest (1.35 mg g^{-1} fresh weight) was observed in the no-fertilizer control (Fig. 9). Protein content in the 100 mg nano-titanium treatment increased by 33% compared to the control. Additionally, salinity stress enhanced soluble protein content, with the highest value (1.93 mg g^{-1} fresh weight) observed under 100 mM salinity and the lowest (1.12 mg g^{-1} fresh weight) in the non-stressed control. Under 100 mM salinity, soluble protein content increased by 74% and 23% compared to the control and 50 mM salinity treatments, respectively (Fig. 9).

Total phenolic content

Analysis of variance revealed that salinity stress and fertilizer treatments significantly affected total phenolic content (Table 5). Among fertilizer treatments, the highest phenolic content (49.44 mg GAE g^{-1} fresh weight) was observed in the 100 mg nano-titanium treatment, while the lowest (39.33 mg GAE g^{-1} fresh weight) was found in the untreated control (Fig. 10). The application of 100 mg nano-titanium resulted in a 26% increase in phenolic content compared to the no-fertilizer control. In addition, the highest phenolic content (53.18 mg GAE g^{-1} fresh weight) was recorded under 50 mM salinity, while the lowest (44.92 mg GAE g^{-1} fresh weight) was observed in the non-stressed control. Salinity stress at 50 mM increased total phenolics by 18% relative to the non-stressed condition (Fig. 10).

Total flavonoid content

Results showed that salinity stress, fertilizer treatments, and their interaction significantly affected total flavonoid content (Table 5). The highest flavonoid content (37.44 mg

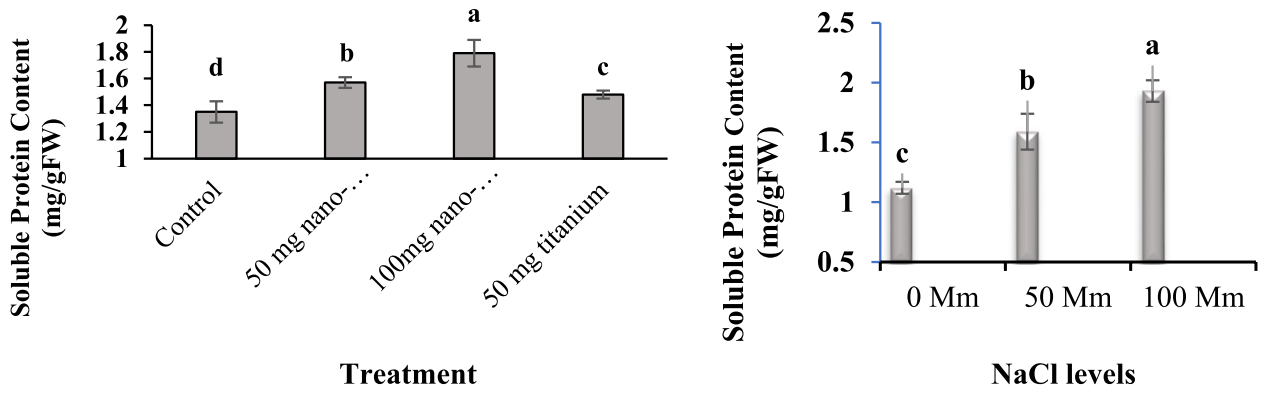

Fig. 9 Mean comparison of the effects of salinity stress and fertilizer treatments on soluble protein content in *Mentha*×*gracilis*.. Data shown are mean values of $n=3$ and the error bars represent standard errors of the means. Different letters indicate significant differences ($P<0.05$) among the treatments (LSD test at 5% level)

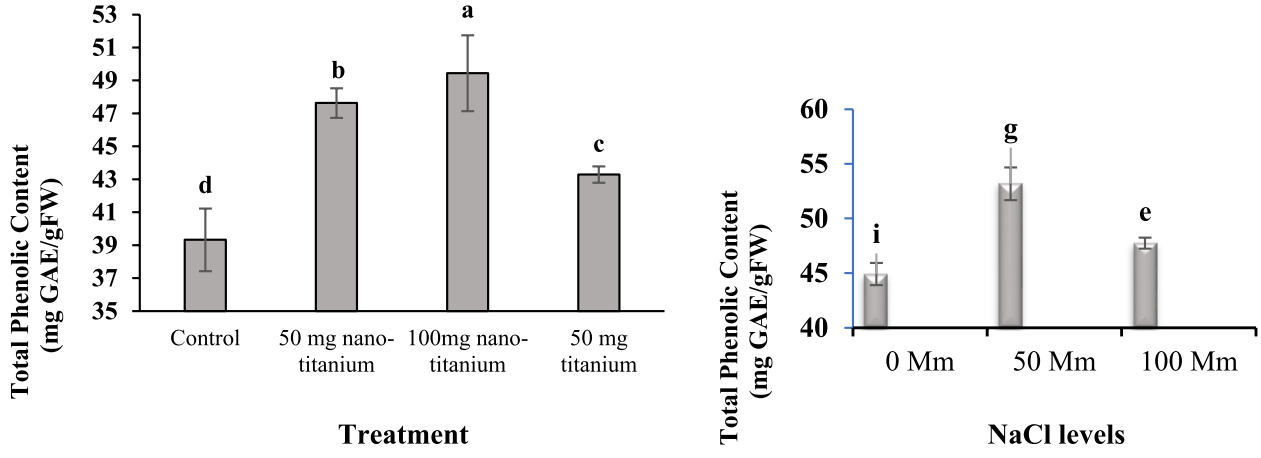

Fig. 10 Mean comparison of the effects of salinity stress and fertilizer treatments on total phenolic content in *Mentha*×*gracilis*.. Data shown are mean values of $n=3$ and the error bars represent standard errors of the means. Different letters indicate significant differences ($P<0.05$) among the treatments (LSD test at 5% level)

RE g^{-1} fresh weight) was recorded under 50 mM salinity combined with 100 mg nano-titanium, while the lowest (17.14 mg RE g^{-1} fresh weight) was found in the unfertilized control under non-stress conditions (Fig. 11). Flavonoid content increased by 43% and 32% under 50 and 100 mM salinity, respectively, compared to non-stress conditions. Application of 100 mg nano-titanium under 50 mM salinity enhanced flavonoid content by 59% compared to the unfertilized treatment under the same stress level. Similarly, under 100 mM salinity, the same treatment increased flavonoid content by 25% relative to the unfertilized treatment. Furthermore, in 50 mM salinity conditions, the use of 100 mg nano-titanium led to a 118% increase in flavonoid content compared to the control (Fig. 11).

Essential oil content

The effects of salinity stress, fertilizer treatments, and the interaction between salinity stress and fertilizer treatments were significant for essential oil content (Table 6). Additionally, the highest essential oil content (1.84%) was observed under 50 mM salinity stress with the application of 100 mg of nano-titanium, while the lowest essential oil content (0.59%) was recorded for the control treatment (Fig. 12). It is noteworthy that essential oil content under 50 mM salinity stress increased by 65% compared to 100 mM salinity stress, and by 62% compared to the non-stressed condition (Fig. 4). However, the use of fertilizers led to an improvement in essential oil content. For instance, the application of 100 mg of nano-titanium increased the essential oil content by 137%, 16%, and 60% under non-stress, 50 mM, and 100 mM salinity stress, respectively, compared to the no-fertilizer treatment. Moreover, the application of 100 mg of nano-titanium under 50 mM salinity stress resulted in a 212% increase in essential oil content compared to the control (non-stressed, no fertilizer) (Fig. 12).

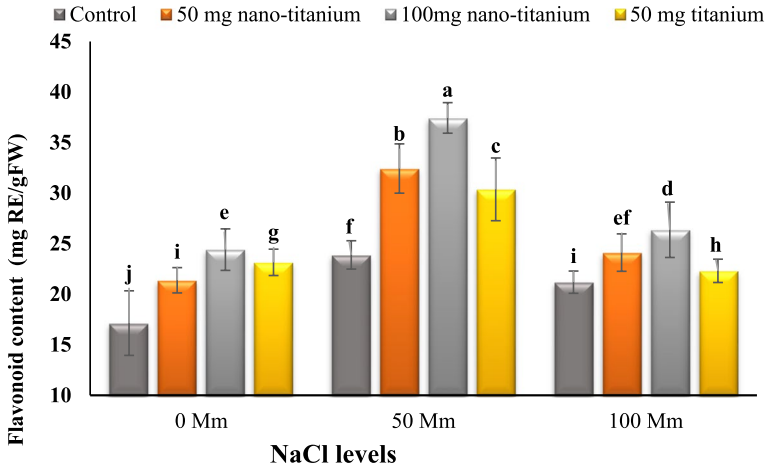

Fig. 11 Interaction effects of different salinity levels and fertilizer treatments on total flavonoid content in *Mentha* × *gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

Table 6 Analysis of variance (ANOVA) for essential oil content and yield of *Mentha* × *gracilis* under salinity stress and fertilizer treatments

Source of Variation	df	Essential Oil Content (%)	Essential Oil Yield
Salinity Stress	2	2.28**	0.245**
Fertilizer Treatments	3	0.521**	0.193**
Salinity × Fertilizer	6	0.06**	0.009**
Error	36	0.009	0.001
CV (%)		7.91	10.86

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$, *ns* = not significant

Essential oil yield

Results showed that essential oil yield was significantly affected by salinity stress, fertilizer treatments, and their interaction. According to the results, the highest essential oil yield (0.639 g per pot) was observed under non-stressed conditions with the application of 100 mg of nano-titanium, while the lowest essential oil yield (0.143 g per pot) was recorded under 100 mM salinity stress without fertilizer (Fig. 13). Additionally, essential oil yield increased by 27% under 50 mM salinity stress compared to non-stressed conditions and by 95% compared to 100 mM salinity stress. It is also worth mentioning that under non-stressed conditions, essential oil yield improved by 55% compared to severe salinity stress

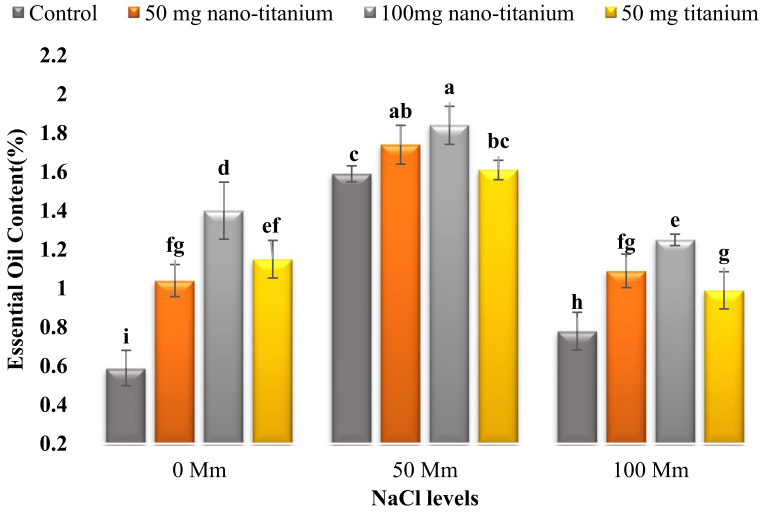

Fig. 12 Interaction effect of different salinity stress levels and fertilizer treatments on the essential oil content of *Mentha* × *gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

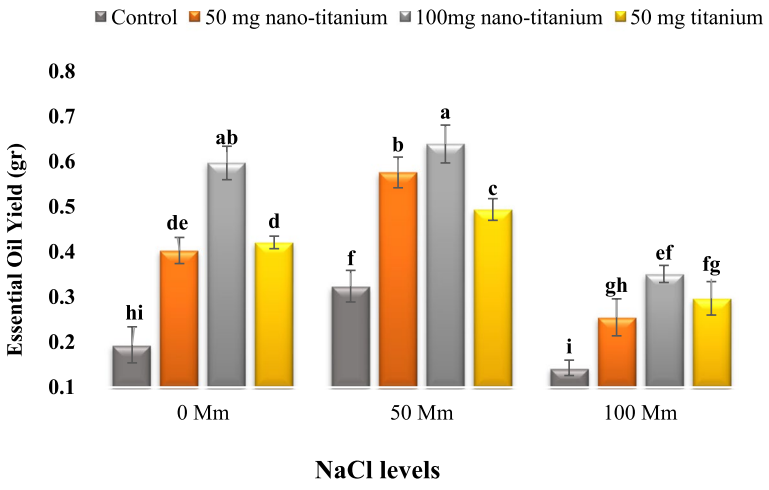

Fig. 13 Interaction Effect of Different Salinity Stress Levels and Fertilizer Treatments on the Essential Oil Yield of *Mentha* × *gracilis*.. Data shown are mean values of n = 3 and the error bars represent standard errors of the means. Different letters indicate significant differences (P < 0.05) among the treatments (LSD test at 5% level)

(Fig. 13). Furthermore, the foliar application of different forms of titanium had a positive effect on essential oil yield, with the best result achieved using 100 mg ofTiO_2 nanoparticles. This treatment increased the essential oil yield by an average of 140% compared to the no-fertilizer treatment. Also, under non-stressed conditions, the application of 100 mg of nano-titanium resulted in a 211% increase in essential oil yield compared to 100 mM salinity stress without fertilizer (Fig. 13).

Essential oil composition

Analysis of ginger mint essential oil identified 22 compounds, which accounted for 90.2% to 98.24% of the total composition (Table 7). Among the essential oil compounds, linalool (21.6% to 46.49%), trans-caryophyllene (6.82% to 17.99%), 1,8-cineole (5.75% to 12.47%), germacrene D (4.28% to 10.42%), gamma-terpinene (5.01% to 9.98%), β-pinene (2.97% to 9.39%), thymol (4.77% to 6.52%), and n-decane (1.31% to 6.16%) were the major components. Furthermore, the highest amount of linalool was obtained using 50 mg of nano-titanium under non-stressed conditions, while the highest levels of trans-caryophyllene, 1,8-cineole, and germacrene D were found under severe salinity stress with the application of 100 mg of nano-titanium. The highest amounts of gamma-terpinene and β-pinene were observed under severe salinity stress with 50 mg of titanium, while the maximum levels of thymol and n-decane were recorded under mild salinity stress with 100 mg of nano-titanium.

Discussion

Morphological traits

The present study revealed that salinity stress significantly affects the growth and height of *Mentha spicata*. High salinity levels alter soil structure and reduce nutrient availability, leading to ion toxicity that impairs water uptake and reduces osmotic potential. This leads to reduced growth of both root and shoot systems [55]. Additionally, salinity stress suppresses cell division and induces programmed cell death, further reducing plant height [56, 57]. Our findings, showed that the application of TiO_2 nanoparticles significantly improved plant height under both saline and non-saline conditions. Ti nanoparticles were found to enhance root development, regulate transpiration, and boost chlorophyll biosynthesis, which in turn promoted vegetative growth [42, 55]. TiO_2 nanoparticles enhance plant growth through multiple mechanisms. They improve nutrient uptake by interacting with root cells and activating transport and signaling pathways [58–60]. TiO_2 nanoparticles also stimulate chlorophyll biosynthesis and photosynthesis efficiency [61, 62], which enhanced photosynthesis is proposed to result from titanium's ability to stimulate the electron transport rate within chloroplasts, potentially by enhancing the Hill reaction (Hong et al., 2005), while reducing oxidative stress by enhancing antioxidant enzyme activities such as SOD and APX (Machanu, et al. 2024). These combined effects support root development, regulate transpiration, and promote vegetative growth under both normal and stress conditions. Previous studies support these results, with Waani et al. [57] demonstrating that TiO_2 nanoparticles (500 mg kg^{-1}) increased plant height in rice[57], and Sheikhalipour et al. [35] showing that 20 mg L^{-1} of TiO_2 nanoparticles improved shoot and root growth in *Stevia rebaudiana* under saline conditions.

In addition to growth enhancement, salinity stress also reduced both fresh and dry biomass in *Mentha x gracilis*, as elevated salinity increased osmotic pressure and induced ionic toxicity, which disrupted plant metabolism [63, 64]. TiO_2 nanoparticles mitigated these effects

Table 7 Essential Oil Composition of Ginger Mint under Salinity Stress and Fertilizer Treatments

Composition	Inhibition Index	100 mM NaCl				50 mM NaCl				0 mM NaCl			
		50 mg Ti	100 mg nano-Ti	50 mg nano-Ti	Control	50 mg Ti	100 mg nano-Ti	50 mg nano-Ti	Control	50 mg Ti	100 mg nano-Ti	50 mg nano-Ti	Control
n-Nonane	900	0.58	0.40	0.46	0.52	0.43	0.44	0.42	0.44	0.1	0.21	0.24	0.20
α-Thujene	924	1.36	1.41	1.14	1.87	0.98	0.85	1.11	1.35	0.43	0.90	0.67	0.7
α-PINENE	932	1.09	0.92	0.89	0.79	0.88	0.62	0.84	0.76	0.49	0.57	0.62	0.54
β-Pinene	**974**	**9.39**	**4.78**	**7.64**	**4.24**	**6.58**	**3.97**	**6.61**	**6.97**	**2.97**	**4.085**	**4.51**	**4.06**
β -Myrcene	988	2.37	1.57	1.94	1.96	1.92	1.42	1.86	1.96	1.46	1.28	1.43	1.26
n-Decane	**1000**	**2.26**	**3.26**	**3.56**	**5.65**	**1.82**	**6.16**	**1.75**	**4.00**	**5.74**	**1.99**	**1.73**	**1.31**
α-Phellandrene	1002	0.00	0.17	0.00	0.41	0.00	0.32	0.00	0.00	0.1	0	0	0.1
alpha.-Terpinene	1014	0.75	0.52	0.62	0.74	0.68	0.64	0.65	0.49	0.375	0.365	0.54	0.41
p-cymene	1024	2.85	1.75	2.49	2.04	2.50	1.77	2.43	1.82	1.59	1.875	1.68	1.29
1,8-Cineole	**1026**	**12.02**	**12.47**	**9.78**	**6.18**	**10.80**	**5.75**	**9.88**	**8.12**	**7.11**	**6.62**	**8.69**	**7.12**
(Z)-β-Ocimene	1032	0.85	0.70	0.87	1.07	1.08	1.01	1.05	0.96	1.03	0.91	0.71	1.08
(E)-β-ocimene	1044	1.72	1.23	1.35	1.24	1.41	1.13	1.44	1.27	1.1	0.95	0.84	1.06
γ-Terpinene	**1054**	**9.98**	**6.74**	**7.70**	**6.45**	**7.79**	**4.01**	**7.59**	**6.44**	**6.91**	**5.31**	**6.58**	**5.44**
Linalool oxide	1084	0.44	0.42	0.72	0.59	0.23	0.97	0.75	0.95	0.1	0.465	0.44	0.69
Linalool	**1096**	**22.17**	**21.60**	**30.23**	**24.9**	**40.09**	**37.39**	**39.51**	**29.3**	**33.28**	**41.31**	**46.49**	**44.7**
Borneol	1165	0.48	1.23	0.75	4.07	0.37	1.12	0.34	0.84	1.255	0.425	0.33	0.28
α-Terpineol	1186	0.48	0.56	0.38	0.83	0.44	0.78	0.37	0.67	0.765	0.365	0.46	0.46
Thymol	**1289**	**4.77**	**6.52**	**5.38**	**6.53**	**5.21**	**7.03**	**5.60**	**6.20**	**4.88**	**5.88**	**5.48**	**5.24**
Carvacrol	1298	0.00	0.40	0.00	0.41	0.34	0.67	0.28	0.00	0.245	1.44	0.335	0.34
Piperitenone oxide	1366	0.00	0.29	0.63	0.50	0.59	0.45	0.35	0.15	0.405	0.455	0.66	0.63
Trans-Caryophyllene	**1417**	**11.98**	**17.99**	**11.32**	**13.5**	**6.82**	**14.62**	**8.22**	**13.4**	**13.41**	**9.9**	**6.83**	**8.90**
Germacrene D	**1481**	**6.56**	**10.41**	**6.52**	**6.59**	**3.69**	**9.17**	**4.94**	**7.95**	**8.54**	**6.165**	**4.085**	**4.28**
SUM		93.41	95.3	92.94	91.1	94.61	98.24	97.92	93.96	92.3	91.49	93.37	90.2

by improving root metabolic efficiency, enhancing water uptake, and promoting biomass accumulation [65]. This improvement can be attributed to the ability of TiO_2 nanoparticles to stimulate photosynthetic activity and activate antioxidant enzymes, helping to reduce oxidative stress [46]. These findings are consistent with the results of the present study, where foliar application of TiO_2 nanoparticles significantly improved plant height, biomass accumulation, and photosynthetic pigment content in *Mentha* × *gracilis* under salinity stress. The enhancement of physiological traits and antioxidative responses in treated plants indicates that TiO_2 nanoparticles effectively mitigate salt-induced damage. Therefore, our results confirm the potential of TiO_2 nanoparticles as a promising nanotechnological approach to enhance salt tolerance and overall growth performance in medicinal and aromatic plants.

Pigment content

Salinity stress significantly reduced chlorophyll content in *Mentha* × *gracilis*, which is likely attributed to chloroplast structural damage and impaired photosynthetic enzyme activity under ionic and osmotic stress conditions[11, 66]. The observed decline in chlorophyll a and b in our study supports the notion that salt stress disrupts thylakoid membranes and inhibits chlorophyll biosynthesis, ultimately reducing photosynthetic efficiency [35, 67]. The reduction in chlorophyll a and b content under salt stress may also be attributed to the disruption of chloroplast ultrastructure caused by high salt concentrations [68], as well as the inhibition of pigment biosynthesis pathways [69]. Based on the findings of previous studies, TiO_2 nanoparticles have been shown to protect the chlorophyll structure under abiotic stress such as drought and salinity by improving light absorption and accelerating the transport and conversion of light energy, thereby preventing chloroplast senescence [70]. These mechanisms collectively support chlorophyll biosynthesis and stability during stress. In the present study, a significant decrease in chlorophyll a and b content was observed under salinity stress, with a reduction of up to 52% and 57%, respectively, under 100 mM NaCl. However, foliar application of TiO_2 NPs, particularly at 100 mg/L, significantly mitigated this reduction, resulting in chlorophyll a and b levels comparable to control treatments. These observations align well with earlier reports showing that TiO_2 NPs can alleviate salt-induced declines in chlorophyll by maintaining chloroplast ultrastructure [70–72].

Oxidative damage caused by stress is often mitigated by enzymatic and non-enzymatic antioxidant systems, including carotenoids, phenolics, flavonoids, ascorbates, α-tocopherols, and antioxidant enzymes, which protect cell components and prolong cell lifespan [47]. The increase in carotenoid content under mild salinity stress can be attributed to its antioxidant role in reducing reactive oxygen species (ROS) and enhancing plant resistance to stress [11]. Supporting studies have shown that mild stress leads to higher carotenoid levels in medicinal plants like *Salvia officinalis* [15]. Furthermore, titanium dioxide (TiO_2) nanoparticles improved carotenoid content under salinity stress, likely due to reduced oxidative degradation and enhanced biosynthetic enzyme activity, along with better photosynthetic performance and increased light absorption by leaves [73].

Biochemical traits

Regarding proline and soluble carbohydrates, salinity stress significantly affected their levels. Proline, an osmotic regulator, plays a vital role in osmotic adjustment and protecting cellular structures under stress [47]. Under salinity stress, proline levels significantly increased, aiding in oxidative protection and enhancing plant tolerance [74]. Similarly, soluble carbohydrates, serving as energy sources and osmolytes, help regulate osmotic pressure and improve stress resistance [75]. Under salinity conditions, soluble carbohydrate levels typically increase, providing energy and mitigating the negative effects of stress [73]. TiO_2 nanoparticles also enhanced proline and soluble carbohydrate levels in *Mentha x gracilis* under saline stress. This improvement is attributed to the regulation of proline biosynthesis genes, enhancing plant resilience to stress [76, 77]. This is consistent with previous studies demonstrating that TiO_2 nanoparticles significantly increased proline and carbohydrate levels under salinity stress [78]. Also, this aligns with previous findings, where TiO_2 nanoparticles significantly increased leaf soluble sugar content, likely due to enhanced stress-related metabolic adjustments and starch hydrolysis under abiotic stress [79]. As drought and salinity both disrupt water potential and metabolic stability, the accumulation of soluble sugars acts as an adaptive osmoprotective strategy that maintains turgor pressure, protects macromolecules, and provides energy reserves during stress. Therefore, the findings of the present study confirm that TiO_2 nanoparticles can enhance proline and soluble carbohydrate accumulation in *Mentha* × *gracilis*, potentially improving osmotic adjustment and salinity tolerance by modulating carbon metabolism and reducing stress-induced damage.

Also, salinity stress significantly influenced the soluble protein content, which plays a key role in stress responses and metabolic regulation [47]. Under saline conditions, plants may exhibit an increase or decrease in soluble proteins, depending on stress type and duration. Salinity often leads to the production of stress-resistant proteins and antioxidant enzymes, which aid in osmotic regulation and reduce oxidative damage [80]. Conversely, some studies report a reduction in soluble proteins due to

protein degradation and decreased synthesis under stress [81]. Our findings showed that TiO_2 nanoparticles positively impacted soluble protein levels in *Mentha x gracilis* under salinity stress by enhancing enzymatic activity, and reducing oxidative damage [82]. These results are supported by other research showing significant increases in soluble protein levels in plants under salinity stress with TiO_2 nanoparticle application [35]. The observed increase in protein content may also be attributed to the role of proteins in maintaining membrane stability and supporting enzymatic functions, which requires protection against protein denaturation under stress conditions. In this context, the accumulation of compatible solutes such as proline plays a key role by stabilizing protein conformation and preserving enzymatic activity through direct interaction with proteins and cellular structures [83]. This protective mechanism helps maintain soluble protein content under salinity stress, as observed in the present study where both salinity and TiO_2 nanoparticle treatments significantly enhanced soluble protein levels in *Mentha* × *gracili*.

The results indicated that salt stress significantly affected the content of phenols and flavonoids in plants. Phenols, known for their high antioxidant properties, increased significantly under salt stress. This increase is attributed to their role in combating oxidative damage and reducing cellular stress [84]. Phenols, acting as defensive molecules, reduce ROS production and help protect cellular structures from salt-induced stress. Similarly, flavonoids, another group of antioxidants, are also reported to increase significantly under stress conditions [47]. Flavonoids help mitigate oxidative damage by scavenging reactive oxygen species (ROS), thus playing a critical role in enhancing plant tolerance to salt stress [17]. In line with our findings, researchers have shown that salt stress increases phenol and flavonoid levels in plant [75], suggesting a defense response to salt stress. Based on the findings of this study, salinity stress significantly enhanced the accumulation of phenolic and flavonoid compounds in *Mentha* × *gracilis* leaves. The highest levels of total phenolics (53.18 mg GAE/g FW) and flavonoids (37.44 mg RE/g FW) were recorded under moderate salinity (50 mM NaCl). These results are consistent with the observations in artichoke (*Cynara scolymus*), [85] and *Tagetes erecta* [86], where moderate salinity combined with stress-alleviating treatments enhanced phenolic and flavonoid contents. This suggests that moderate salinity may activate defense-related metabolic pathways, promoting the biosynthesis of polyphenols as part of the plant's stress adaptation mechanism [85]. Moreover, the synergistic effect of TiO_2 NPs likely enhances these responses by boosting ROS-related signaling or stimulating key enzymes in the phenylpropanoid pathway [87]. Also, it is hypothesized that TiO_2 nanoparticles, by enhancing Rubisco protein levels and activity, boost the Calvin cycle reactions, leading to enhanced photosynthesis and increased plant resistance to salt stress [67, 88]. Therefore, the combined use of nano-titanium and controlled salinity could be a promising strategy to enrich bioactive compounds with pharmacological value in medicinal plants.

Antioxidant activity

Salt stress significantly increased the levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), both of which are indicators of oxidative stress and cellular damage [89]. Elevated MDA levels reflect lipid peroxidation due to ROS activity, while increased H_2O_2 levels signify the accumulation of reactive oxygen species, leading to oxidative damage. The presence of these compounds under stress conditions suggests a detrimental effect on plant health, as they contribute to the degradation of cellular components [90].TiO_2 nanoparticles (TiO_2) were found to help reduce the increase in both MDA and H_2O_2 by enhancing the plant's antioxidant defense system, thus mitigating oxidative damage caused by salt stress [67]. Based on the findings of the present study, the application of TiO_2 nanoparticles at 100 mg L^{-1}, especially under moderate salinity stress (50 mM NaCl), significantly enhanced the activity of key antioxidant enzymes including SOD, APX, and GPX. These results are consistent with Weisany et al. [56], who reported that increased enzyme activities under salinity stress are mainly due to the accumulation of H_2O_2 and the activation of plant defense systems.

Salt stress notably affected the activity of antioxidant enzymes. Under salt stress, the activity of GPX (glutathione peroxidase) generally increased to counteract high hydrogen peroxide and ROS levels. This increase in GPX activity helps reduce oxidative damage and enhances plant resistance to salinity stress [18]. Additionally, superoxide dismutase (SOD), a key antioxidant enzyme, also showed increased activity under salt stress, as plants respond to higher superoxide and ROS levels [91, 92]. On the other hand, ascorbate peroxidase (APX) acts as a crucial enzyme in reducing hydrogen peroxide, preventing oxidative damage. However, the enzyme activity may vary with the intensity and duration of the stress, showing an initial increase followed by a potential decrease due to enzyme system damage [47].TiO_2 nanoparticles were found to effectively enhance antioxidant enzyme activity under salt stress by improving cellular structure and function [26, 65].

The present study revealed that foliar application of nano-titanium significantly enhanced antioxidant enzyme activities (SOD, APX, GPX) in *Mentha* × *gracilis* under salinity stress. Specifically, guaiacol peroxidase activity increased by 165%, and ascorbate peroxidase

activity rose by 176% under 50 and 100 mM salinity stress, respectively, when treated with 100 mg of TiO_2 nanoparticles. These findings are in line with previous studies indicating that TiO_2 NPs enhance antioxidant defense systems in various plant species. For instance, Jacob et al. [93], reported increased activity of antioxidant enzymes at 10–30 ppm TiO_2 NPs in beans, while similar enhancements were observed in *Spinacia oleracea* [94], *Vitex agnus* [95], Grapevine Saplings [96], *Trigonella foenum-graecum* [97]. Such activation likely contributes to improved ROS detoxification, membrane protection, and overall stress tolerance in *M.* × *gracilis*. Furthermore, elevated intracellular levels of H_2O_2 under salt stress may act as a signaling molecule that induces APX activity, as also observed in *Dracocephalum moldavica* [15]. In our study, the lowest H_2O_2 content was observed in plants treated with 100 mg L^{-1} TiO_2, likely due to the significant upregulation of APX, GPX, and SOD activities in these treatments [15]. The detoxification of ROS following TiO_2 nanoparticle application may be attributed to the stabilization of cellular structures and the enhancement of membrane physical properties. Supporting this, Lei et al. [11] demonstrated that TiO_2 nanoparticles, when applied under drought conditions, boosted antioxidant enzyme activities by reducing lipid peroxidation and strengthening membrane integrity.

Essential oil yield and content

The results indicated that salinity stress significantly impacts the percentage and yield of essential oils. Salinity stress is one of the most crucial environmental factors affecting the metabolism of medicinal plants. Under mild stress conditions, essential oil production increases in plants such as ginger mint. This increase is typically due to the activation of defensive metabolic pathways that naturally occur in response to environmental stress [91]. Researchers have stated that, in response to stress, the activity of enzymes involved in the biosynthesis of essential oils and other secondary metabolites increases [17]. Additionally, the accumulation of precursors (such as isoprenoids) and the reduction of their use in growth and biomass production pathways may also contribute to the increased essential oil production under mild stress conditions [47]. However, under severe salinity stress, with increased osmotic pressure, water and nutrient absorption by roots decreases, and this resource shortage leads to a reduction in the production of secondary metabolites involved in essential oil production [15, 98]. Salinity stress also results in increased production of reactive oxygen species, which can damage cells and reduce essential oil yield [64].

In contrast, the application of TiO_2 nanoparticles to reduce the negative effects of salinity stress and improve essential oil yield showed significant results. TiO_2 nanoparticles improve soil structure and enhance the availability of water and nutrients, thereby increasing their absorption by the roots, which leads to increased secondary metabolite and essential oil production [46]. Moreover,TiO_2 nanoparticles can enhance the activity of enzymes related to essential oil synthesis, thus increasing the production of these compounds [99]. Additionally,TiO_2 nanoparticles help reduce the production of reactive oxygen species and increase the activity of antioxidant enzymes, which helps maintain cell health and improve both the quality and quantity of essential oils under salinity stress [100]. In support of our findings, various studies have shown that TiO_2 nanoparticles can effectively improve essential oil yield and content in plants under salinity stress conditions [15, 26, 77]. It should be noted that since essential oil yield is determined by multiplying the dry matter yield by the essential oil content, and these two factors are directly related, any factor that increases these two indices will lead to increased essential oil yield. Therefore, it is expected that with the improvement of dry matter yield and essential oil content through titanium nanoparticle application, essential oil yield will ultimately increase [47].

Essential oil components

Salinity stress can significantly impact the composition of essential oils in plants like mint. Essential oils, which contain volatile and aromatic compounds, play a critical role in the quality and properties of medicinal plants. Salinity stress can alter the composition and concentration of these compounds. The increase in essential oil compounds under stress conditions is due to the higher allocation of carbon fixed during photosynthesis to the production of secondary metabolites, which enhances the plant's resistance to environmental stress by reducing reactive oxygen species damage. In line with our findings, Bahreininejad et al. [101], reported that the thymol content in the essential oil of *Thymus daenensis* increased under both mild and severe stress conditions compared to the control.

Our results indicated that TiO_2 nanoparticles can effectively improve essential oil composition under salinity stress. TiO_2 nanoparticles enhance the activity of enzymes involved in essential oil biosynthesis and reduce oxidative damage, which can help preserve or increase the quality and diversity of essential oil compounds [26]. These nanoparticles improve growth conditions and reduce the negative effects of salinity stress, leading to increased concentration and diversity of essential oil compounds [73]. Furthermore, since elements such as nitrogen and phosphorus are essential for the formation of these compounds, the application of TiO_2 nanoparticles may supply the necessary nutrients, thereby increasing chlorophyll content, Rubisco enzyme activity, and

consequently photosynthetic activity, essential oil yield, and composition [46, 102]. In another study, foliar application of nanoliposome containing Fe^{2+} element in sweet basil, enhanced the Fe content and essential oil composition [36].

Supporting our results, a study on coriander showed that applying 4 gr/L of titanium dioxide nanoparticles increased the percentage of linalool compared to the absence of fertilizers [103]. Additionally, Shenavaie Zare et al. [77] demonstrated that spraying 150 mg per liter of titanium dioxide nanoparticles increased stem dry weight, total chlorophyll, and main compounds of peppermint essential oil, such as menthol and menthyl acetate. Another study on sage showed that the percentage of essential oil, oil yield, and main compounds such as cis-thujone, camphor, and 1,8-cineole increased by 75%, 174.5%, 87.5%, 30.8%, and 123.1%, respectively, when 200 mg per liter of titanium dioxide nanoparticles was applied compared to the control.

Conclusion

This study clearly demonstrated that salinity stress significantly reduced both fresh and dry biomass of *Mentha* × *gracilis,* while inducing marked increases in proline, soluble protein, and carbohydrate levels, suggesting an adaptive physiological response to osmotic stress. Application of TiO_2 nanoparticles, particularly at a concentration of 100 mg L^{-1}, effectively alleviated the adverse effects of salinity, resulting in improved plant growth parameters and enhanced essential oil yield. The activity levels of key antioxidant enzymes—guaiacol peroxidase (GPX), superoxide dismutase (SOD), and ascorbate peroxidase (APX)—were notably elevated under salinity, especially in treatments involving Nano-Ti, indicating strengthened oxidative defense and reduced cellular damage. Furthermore, GC–MS and GC-FID analyses revealed that Nano-Ti treatment increased the concentrations of major essential oil constituents, including linalool, trans-caryophyllene, 1,8-cineole, germacrene D, and γ-terpinene. Collectively, these findings highlight the potential of Nano-Ti application as a promising approach to enhance both physiological resilience and phytochemical quality of *Mentha* × *gracilis* under saline conditions, supporting its use in sustainable crop production systems facing salinity stress.

Abbreviations

APX	Ascorbate peroxidase
Chl a	Chlorophyll a
Chl b	Chlorophyll b
DW	Dry weight
EO	Essential oil
FW	Fresh weight
GPX	Guaiacol peroxidase
H_2O_2	Hydrogen peroxide
LAI	Leaf area index
MDA	Malondialdehyde
Nano-Ti	Nano titanium
NaCl	Sodium chloride
SOD	Superoxide dismutase
Ti	Titanium
GC–MS	gas chromatography–mass spectrometry

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Authors' contributions

Mohammad Reza Morshedloo, Nastaran Jabbari: Performing experimental works, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing—original draft. Majid Azizi, Mansoureh Nazari, Seyed Morteza Zahedi and Agnieszka Viapiana: Conceptualization, Investigation, Project administration, Validation, Visualization, Writing—review & editing. All authors approved the final version.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interest.

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