Physiol Mol Biol Plants
https://doi.org/10.1007/s12298-022-01215-2

RESEARCH ARTICLE

Plant elicitation and TiO_2 nanoparticles application as an effective strategy for improving the growth, biochemical properties, and essential oil of peppermint

Akram Shenavaie Zare[1] · Ali Ganjeali[1] · Mohammad Reza Vaezi Kakhki[2] · Monireh Cheniany[1] · Mansour Mashreghi[1]

Received: 23 February 2022 / Revised: 22 July 2022 / Accepted: 26 July 2022

Abstract *Mentha piperita* L., which is an abundant source of essential oils (EO) and phenolic acids, is well known for its medicinal significance. The present research aimed to evaluate the impact of various concentrations of methyl jasmonate (MeJA; 0, 0.1, and 0.5 mM), titanium dioxide nanoparticles (TiO_2 NPs; 0 and 150 mg L^{-1}), and salicylic acid (SA; 0, 0.1, and 1 mM) on growth, EOs, and phenolic compounds of *M. piperita* L. The results demonstrated that the simultaneous application of SA (0.1 mM) and TiO_2 NPs (150 mg L^{-1}) enhanced shoot dry weight, the shoot length, and membrane stability index of peppermint by 56.17, 19.52, and 36%, respectively, compared to control. Moreover, phenolic content (76%), caffeic acid content (78%), rosmarinic acid content (87%), 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability (78%), and catalase (155%), ascorbate peroxidase activities (95%) were further improved by simultaneously applying MeJA (0.1 mM) and TiO_2 NPs (150 mg L^{-1}) compared to control. The highest menthol production (44.51%) was obtained with exogenous application of MeJA (0.1 mM) with 150 mg L^{-1} TiO_2 NPs. The findings of the current study presented an ideal combination of TiO_2 NPs with plant growth regulators for promoting antioxidant activities and increasing major components of EO in peppermint plants.

Keywords Antioxidant enzymes · DPPH · Medicinal plants · Menthol · Phenolic compounds

✉ Ali Ganjeali
ganjeali@um.ac.ir

[1] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
DAP	Days after planting
DDW	Double distilled water
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EO	Essential oil
GC-MS	Gas chromatography-mass spectrometry
MeJA	Methyl jasmonate
NPs	Nanoparticles
OD	Optical density
RA	Rosmarinic acid
ROS	Reactive oxygen species
SA	Salicylic acid
TiO_2 NPs	Titanium dioxide nanoparticles
TPC	Total phenol content
VOCs	Volatile organic compounds

Introduction

Peppermint (*Mentha piperita* L.), according to Spirling and Daniels (2001), is considered a natural hybrid of spearmint (*Mentha spicata* L.) and water mint (*Mentha aquatic* L.). It effectively improves irritable bowel syndrome, upper gastrointestinal disorders, respiratory problems, and muscle spasms. The antiviral and antimicrobial effects of peppermint essential oils (EO) have been confirmed in previous research (Pushpangadan and Tewari 2006). The shoots of peppermint include EOs, flavonoids, phenolic compounds, vitamins, fatty acids, minerals, and salicylic acid (SA) (Rita and Animesh 2011). Peppermint EO mainly consists of menthone (20–31%), menthol (29–48%), menthyl acetate (3–10%), and menthofuran (6.8%) (Singh et al. 2015). Menthol is considered the most essential component of the EO

Published online: 06 August 2022

which is biosynthesized and accumulated in the capillaries of the secretory glands on the surface of the epidermis. Additionally, peppermint EO contains compounds such as 1,8-Cineole, limonene, isopulegol, isomenthone, carvone, and pulegone (Loolaie et al. 2017). Zhao et al. (2005) indicated that the generation of secondary metabolites in plants is mostly associated with environmental stresses and their aggregation stimulated by elicitors. Among different elicitors, methyl jasmonate (MeJA) and SA are acknowledged as influential elicitors regarding the induction of secondary metabolite synthesis in plants (Yu et al. 2018).

SA is a growth regulator and phenolic composite that contributes to the growth of the plant and responses to environmental stresses. Zarinkamar et al. (2013) reported that SA effectively stimulates the production of different metabolites such as terpenoids, alkaloids, coumarin derivatives, and flavonoids. According to Kováčik et al. (2009), anthocyanins and flavonoids increase in chamomile (*Matricaria chamomilla*) treated with SA. As a plant growth regulator, SA influences the quantity and quality of EOs of medicinal plants (Nasiri et al. 2018). In their study, Pirbalouti et al. (2014) found that SA increases β-myrcene, carvacrol, thymol, and 1,8-Cineole in *Thymus vulgaris*.

MeJA functions as a signal molecule in different physiological processes of the plant, including root and plant growth, seed germination, the senescence process, and anther and pollen grain evolution. It is derived from linoleic acid in the octadecanoid pathway and exerts an essential role in activating plant defense responses such as the biosynthesis of specific secondary metabolites (Malekpoor et al. 2015). The findings of Malekpour et al. (2015) revealed that jasmonic acid increases EOs in basil. MeJA and SA typically change the EO chemical composition in various plants. Nanoparticles (NPs) are regarded as molecular or atomic assemblies with minimum dimensions of 1–100 nm (Marslin et al. 2017). Based on recent findings, they can help effectively increase the germination rate and plant growth as secondary metabolite production. For instance, the content of medicinal compounds (e.g., artemisinin and diosgenin) represented an increase in plants treated with NPs (Zheng et al. 2005).

Numerous studies indicated that treating photosynthetic plants and microorganisms with NPs increases the production of phenolic compounds. Gohari et al. (2020) have recently found that the application of NPs significantly alters physiological, morphological, and biochemical properties of *Dracocephalum moldavica*. Although growth regulators (MeJA, SA, and TiO_2 NPs) have a positive influence on plant growth and secondary metabolite production, no study has so far investigated the combined impact of TiO_2 NPs with MeJA and SA on peppermint.

Accordingly, the current study simultaneously applied plant growth regulators (SA and MeJA) and TiO_2 NPs, and then evaluated their effects on the growth, EO, and phenolic compounds of peppermint.

Materials and methods

Plant materials and growth conditions

The present experimental research was performed from May to September 2020 in the research field of the Botany Department, Hakim Sabzevari University (57° 43′ E longitude, 36° 12′ N latitude, and 977.6 m altitude), Sabzevar, Iran. Each experimental unit included a plastic pot (22 cm diameter × 20 cm height) that was filled with an autoclaved growing mixture (0.11 MPa, 120 °C, and 1 h) of vermicompost, cocopeat, perlite, and soil (1:1:1:1, v/v). Table 1 provides the chemical characteristics of the investigated soil. The pots were randomly arranged, and plants were grown at natural conditions with a relative humidity of 50–70% and average day and night temperatures of 32 and 17 °C, respectively. First, the rhizomes were planted in the pots, and then 30-day-old seedlings were transferred to the plastic pots. Each treatment was repeated in three pots, and each pot included three seedlings. The plants were watered daily.

TiO_2 NP treatment

TiO_2 NPs were provided from the US Research Nanomaterials (USA). The TiO_2 solution was prepared at the concentrations of 0 and 150 mg L^{-1} with filtered double-distilled water (DDW). In addition, 150 mg L^{-1} of TiO_2 NPs was applied to the 30-day-old seedlings as foliar spray. Further, DDW was employed as the control spray treatment. Moreover, 150 mg L^{-1} of TiO_2 NP treatment was applied with a 7-day interval (seven times) by a hand sprayer. The working solution was sonicated to prevent aggregation when necessary.

SA and MeJA treatments

According to some previous studies (Rahimi et al. 2013; Pérez et al. 2014) and evaluation of plant responses, one month after transplanting the seedlings, plants with different concentrations (e.g., 0, 0.1, and 1 mM) of water-soluble SA and 2%

Table 1 Chemical analysis of the studied soil

pH	EC (dS m^{-1})	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})
7.25	1.2	1.4	120	1250	842.8	36.6	62.8

ethanol-soluble MeJA (V/V; 0, 0.1, 0.5 mM) were separately sprayed with 150 mg L^{-1} of TiO_2 NPs. Various concentrations of SA and MeJA were considered at 17-day (three times) and 12-day (4 times) intervals using a hand sprayer, respectively. Additionally, control plants were sprayed with water.

Experimental design and treatment

The experiment was conducted with two elicitors (SA and MeJA) and two concentrations of TiO_2 NPs (0 and 150 mg L^{-1}). Thus, a factorial experiment was performed using a completely random design including a total of three replications.

Growth characteristics

Two months after treatments, plants were harvested, and morphological characteristics, including height (H) and aerial biomass of the plants, underwent measurement; three plants per pot were monitored to determine the above-mentioned parameters. The dry weight and aerial fresh weights were calculated (g), and the samples were dried in an oven at 70 °C for 48 h.

Leaf relative water content (RWC)

Leaf RWC was computed from the upper fully expanded young leaves according to Whetherley (1950). Leaf RWC was determined by the following equation:

$$\text{RWC}\ (\%) = ((\text{Fresh weight} - \text{Dry weight})/(\text{Saturated weight} - \text{Dry weight})) \times 100$$

Membrane stability index (MSI)

The MSI was assessed, according to Barrsu and Weatherley. (1992), using eight-leaf discs for each treatment. For this purpose, fully extended young leaf samples were washed thrice with deionized water in order to remove electrolytes adhered to the surface. Then, the leaf samples were put in closed vials consisting of 50 mL of deionized water and incubated for 24 h at 40 °C, followed by determining the first electrical conductivity of the solution (E1). Furthermore, the samples were autoclaved for 60 min at 100 °C, resulting in obtaining the second electrical conductivity (E2). The MSI was computed as follows:

$$\text{MSI}\% = \left[1 - (\text{E1} / \text{E2})\right] \times 100$$

Seed germination

Peppermint seeds were purchased from Pakan Bazr Institute in Isfahan, Iran. After separating healthy and uniform seeds, generally, 20 seeds were put in Petri dishes including filter papers, and three Petri dishes were considered as three repetitions for each treatment. For TiO_2 NP treatment, the TiO_2 NP solution (4 mL) with concentrations of 0 and 150 mg L^{-1} was added to Petri dishes containing the seeds. Moreover, for the combined treatments, seeds were soaked and transferred to Petri dishes with concentrations of 0, 1, and 0.5 mM MeJA or 0.1 and 1 mM SA (2 mL) solution with the TiO_2 NP (2 mL) solution. Next, the dishes were placed in a germinator at 16 °C. The germinated seeds in Petri dishes were daily counted up to eight days after planting. The percentage of germination was determined by the following formulas.

$$\text{Percentage of germination} = \text{Number of germinated seeds}/\text{Total number of seeds} \times 100$$

Sample preparation and extraction

To measure biochemical parameters, fresh leaves from each treatment were separately placed in aluminum foil and stored in a freezer at − 80 °C after putting them in liquid nitrogen.

Analyses of biochemical parameters

Estimation of total chlorophyll content

The content of chlorophyll was determined by the fresh leaves based on the method of Lichtenthaler and Buschmann (2001). Fresh tissues were grinded with 100% acetone by applying mortar-pestle. In addition, the optical density (OD) of the pigment solution was recorded at 662, 645 nm, and the contents of chlorophyll a and b were measured by a spectrophotometer (Analytik Jena, SPECORD 210, Germany). The total chlorophyll content was computed through summing up the chlorophyll a and b contents. Finally, the content of the total chlorophyll was represented as mg g^{-1} leaf fresh weight.

Calculation of total phenolic content (TPC)

The TPCs of the extracts were quantified by a spectrophotometer (Analytic Jena, SPECORD 210, Germany) according to the protocol explained by Sadasivam and Manickam. (2008). Leaf samples (500 mg) were ground, along with a 5-time volume of 80% ethanol by applying mortar and pestle. The homogenate was centrifuged for 10 min at 10,000 rpm at 4 °C, saving the supernatant, which was evaporated to dryness, followed by adding 5 mL of DDW. Subsequently, 2 mL of 20% Na_2CO_3 and 0.5 mL of the Folin–Ciocalteau reagent were added to each tube. The OD of the solution was

calculated at 650 nm against a reagent blank. Gallic acid was employed as a standard by representing the results with mg Gallic acid equivalents g^{-1} of fresh weight.

Determination of rosmarinic acid

Dry plant powder (0.1 g) was ground with 10 mL of 80% methanol and put in a shaker at 70 °C for 90 min. The resulting extracts were filtered using filter paper. The absorption of the methanolic extracts at 333 nm was read by a spectrophotometer (Analytik Jena, SPECORD 210, Germany). Then, the concentration of rosmarinic acid (RA) was determined using a standard curve (Arnaldos et al. 1995).

Determination of caffeic acid

The samples were ground on ice with 80% methanol at a ratio of 1.5 w/v until obtaining a homogeneous solution. The resulting homogenate was stirred at 40 °C for three hours and then centrifuged at 45 rpm for 45 min, and the obtained supernatant was employed to determine caffeic acid.

Next, 1 mL of 80% methanolic extracts was taken, followed by adding 1 mL of the ARNO reagent (containing 10% sodium molybdate and 10% sodium nitrate), 1 mL of sodium hydroxide 1 M, and 1 mL of hydrochloric acid 0.1 M. After vortex operation, the mixture absorbance was immediately measured at 490 nm by a spectrophotometer (Analytik Jena, SPECORD 210, Germany). In the control sample, 1 mL of 80% methanol was added instead of the extract (Sauvesty et al. 1992).

DPPH-radical scavenging activity

The protocol explained by Fernández-Agulló et al. (2013) was followed to determine the DPPH radical scavenging ability. The dried leaves of the plant (100 mg) were crushed with 2 mL methanol, and the mixture was centrifuged for 10 min at 3500 -rpm. Next, a 1.5 mL methanolic solution of DPPH was added to the solution, and the mixture was left to stand in the dark. After 30 min, the mixture absorbance was assessed at 517 nm by a spectrophotometer. Eventually, antioxidant activity was calculated according to the following formula (Analytic Jena, SPECORD 210, Germany).

$$\text{DPPH radical scavenging activity\%} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ = Absorbance in control, A_{sample} = Absorbance in treatments.

Measurement of antioxidant enzyme activities

Fresh leaves (100 mg) were ground in liquid nitrogen and then homogenized in 1 mL phosphate buffer (pH = 7) consisting of 1% (w/v) polyvinylpyrrolidone and 0.1 mM EDTA. Subsequently, the homogenate was centrifuged for 30 min at 4 °C at 13,000 rpm. Finally, the supernatant was applied for the enzyme assay.

Catalase (CAT) activity

This activity was determined using the method of Alici and Arabaci. (2016) and following the H_2O_2 consumption. The reaction mixture was composed of 100 µL of hydrogen peroxide (15 mM), 3 mL of 50 mM phosphate buffer, and 50 µL of the enzyme extract. After the extract addition, the absorption reduction at the wavelength of 240 nm for 1 minute was assessed by a spectrophotometer (Analytik Jena, SPECORD 210, Germany).

Ascorbate peroxidase (APX) activity

The APX activity was measured using the protocol of Nakano and Asada. (1981). The reaction mixture (1 mL) included 10 mM ascorbate, 50 mM potassium phosphate buffer (pH = 7), and 0.1 mM H_2O_2. The reaction was initiated by adding 100 µL of the crude enzyme. In addition, ascorbate oxidation was monitored for 1 minute through computing the decline in the absorbance at 290 nm at 10-s intervals by a spectrophotometer (Analytik Jena, SPECORD 210, Germany), and the activity was computed by the extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

EO extraction and analysis

Fresh leaves from different treatments were homogenized and mixed, and then the EO contents of the leaves were extracted by the distillation method for three hours by applying a Clevenger apparatus. Next, the EO was dried over anhydrous sodium sulfate and maintained in sealed glass vials at 4 °C for the gas chromatography-mass spectrometry (GC–MS) analysis.

The GC–MS analysis was performed by the gas chromatograph (Agilent 7890B) connected to a mass detector (Model 5977A, Agilent Technologies, USA). It should be noted that the chromatograph was equipped with an HP-5MS capillary column (Phenyl methyl siloxane [30 m × 0.25 mm ID 0.25 µm] and Agilent technologies). The temperature of the injector was set at 270 °C, and that of the oven was programmed from 60 °C (10 min) to 200 °C at the rate of 5 °C/minute. In addition, helium was chosen as the carrier gas while the flow rate was adjusted to 1 mL/minute and the injection volume was 1

µL. Additionally, the ionization energy in the mass was 70 eV, and the mass was in the range of 35–500 m/z. Further, the interface temperature was modified to 280 °C. The compounds were determined by comparing the retention times (RT) with those reported in the literature, as well as comparing their mass spectra with those stored in the Wiley library and the library of the National Institute of Standards and Technology.

Statistical analysis

The analysis of variance (Two-way ANOVA) was conducted by IBM SPSS (Version 23.0), and Duncan's multiple range test was employed to estimate significant differences at $P \leq 0.05$. All experiments were performed three times and represented as the mean ± standard error (SE).

Results

Growth characteristics

Based on the results of the two-way ANOVA, SA, MeJA, TiO_2 NPs, and their interaction could significantly affect all evaluated properties (Table 2). The results further demonstrated that using 0.1 mM SA and 150 mg L^{-1} TiO_2 NPs enhances plant growth, which was manifested as increased shoot length (21%), shoot dry weight (79.77%), and shoot fresh weight (14.3%) compared to control (Fig. 1a,b). The use of MeJA led to a significant reduction in shoot length and shoot dry weight in TiO_2 NP-sprayed plants and those without spraying. Our findings revealed that high concentrations of MeJA negatively affected plant growth. The lowest shoot dry weight (0.69) was observed with 0.5 mM MeJA without applying TiO_2 NPs. Overall, our results confirmed that the application of 0.1 mM SA exerted a positive impact on morphological properties, and this was also increased when using 150 mg L^{-1} TiO_2 NPs (Fig. 1a–c).

RWC

The RWC is an appropriate measurement for determining water status in plants (Lugojan and Ciulca 2011). The results showed that the RWC was significantly increased by employing SA, MeJA, and TiO_2 NPs. The highest RWC (70.6% ± 0.28) belonged to 0.1 mM SA with the application of 150 mg L^{-1} TiO_2 NPs, which increased by 30% compared to the control (Fig. 1d).

MSI

The highest MSI (70.4% ± 0.28) was detected in plants that were treated with 150 mg L^{-1} TiO_2 NPs and 0.1 mM MeJA, which increased by 36% compared to control plant (51.5% ± 1.75). Accordingly, the use of SA and MeJA separately and in combination with TiO_2 NPs could significantly increase MSI compared to control plants (Fig. 1e).

Percentage of germination

Based on the findings, the germination percentage was significantly enhanced by applying SA and TiO_2 NPs. The highest germination percentage (68.2% ± 0.15) was associated with 0.1 mM SA with the application of 150 mg L^{-1} TiO_2 NPs. The use of MeJA reduced the percentage of germination (Fig. 1f).

Total chlorophyll content

The results of the two-way ANOVA indicated that SA, MeJA, TiO_2 NPs, and their interaction had a significant effect on all measured biochemical characteristics (Table 3). Based on the results, the total chlorophyll content represented a significant enhancement by using SA and TiO_2 NPs. The highest total chlorophyll (1.87 ± 0.03 mg g^{-1} fresh weight) belonged to 0.1 mM SA with the application of 150 mg L^{-1} TiO_2 NPs, demonstrating an increase of 19.87% compared to control. The application of MeJA led to a significant decline in the total chlorophyll content (Fig. 2a).

Table 2 Analysis of variance for different growth properties of *Mentha piperita* L. plants

Source of variation	df	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	WRC (%)	MSI (%)
Elicitors (SA and MeJA)	4	70.5*	0.8*	0.37*	108.3*	123.07*
TiO_2 NPs	1	82.6*	1.2*	0.83*	203.8*	234.6*
Elicitors * TiO_2 NPs	4	5.4*	0.01^{ns}	0.02*	5.6*	4.5*
Error	20	0.1	0.01	0.001	0.2	0.3*

SA Salicylic acid, *MeJA* Methyl jasmonate, *TiO_2 NPs* Titanium dioxide nanoparticle, *WRC* Relative water content, *MSI* Membrance stability index

*Significant at $P \leq 0.05$

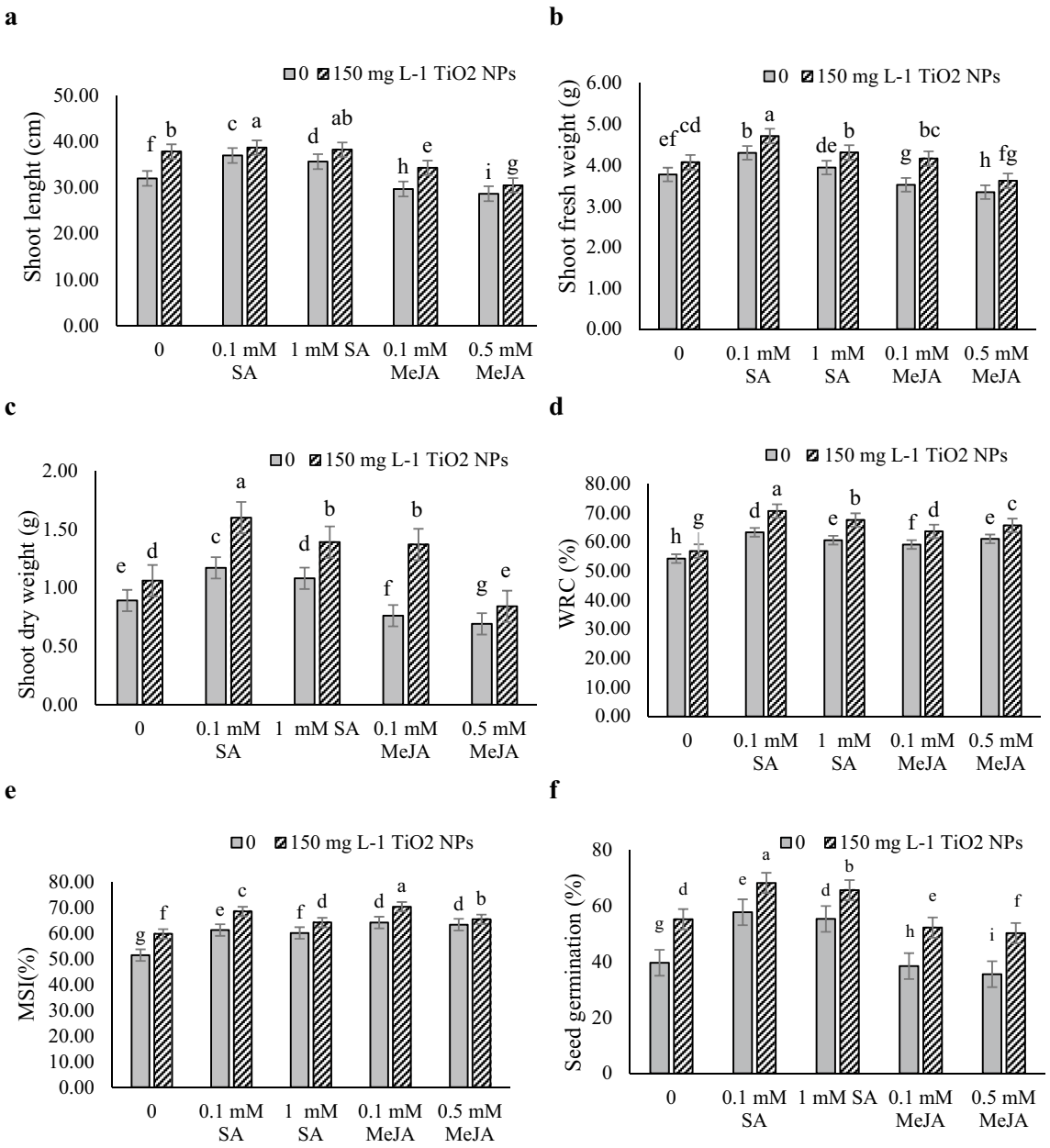

Fig. 1 Effect of SA, MeJA, and TiO_2 NPs on growth parameters. *Note*: **a** Shoot length, **b** shoot fresh weight, **c** shoot dry weight, **d** WRC, **e** MSI, and **f** seed germination. In each figure, means with the same letter(s) are not significantly different according to Duncan's test at $P \leq 0.05$. Means ± SE from the three replications. Error bars (⊤) show SE. *MSI* Membrance stability index, *WRC* Relative water content, *SA* Salicylic acid, *MeJA* Methyl jasmonate, *TiO_2 NP* Titanium dioxide nanoparticle, *SE* Standard error

TPC

Elicitors activate biosynthetic genes that are involved in secondary metabolites, including flavonoids, terpenoids, phenylpropanoids, and alkaloids through activating signal transduction cascade and play an important role in plant defense responses (Cappellari et al. 2019). Thus, it is essential to measure terpenoids and phenolic compounds in the plants under the influence of elicitation. In the current study, it has been observed that TiO_2 NPs significantly increased TPC in all levels of SA and MeJA application. The exogenous applications of SA and MeJA in all concentrations could significantly enhance TPC in plants sprayed with TiO_2 NPs and those not sprayed with TiO_2 NPs ($P \leq 0.05$). The highest TPC was obtained in plants that were treated with 150 mg L^{-1} TiO_2 NPs and 0.1 mM MeJA (76.15% higher

Table 3 Analysis of variance for the biochemical characteristics of *Mentha piperita* L. plants

Source of variation	df	Total phenolic contain (mg g^{-1})	Rosmarinic acid (mg g^{-1} dry weight)	Caffeic acid (mg g^{-1} dry weight)	DPPH (%)	Catalase activity (unit min^{-1} g^{-1}Fw)	Peroxidase activity (unit min^{-1} g^{-1} Fw)
Elicitors (SA and MeJA)	4	39.2*	693.6*	206.7*	622.9*	0.006*	0.008*
TiO_2 NPs	1	52.4*	1749.3*	893.7*	2818.4*	0.035*	0.028*
Elicitors * TiO_2 NPs	4	7.4*	9.02*	27.9*	21.2*	0.000*	0.001*
Error	20	20	0.07	0.5	0.7	0.000	0.000

SA Salicylic acid, *MeJA* Methyl jasmonate, *TiO_2 NP* Titanium dioxide nanoparticle, *DPPH* 2-diphenyl-1-picrylhydrazyl
*Significant at $P \leq 0.05$

than control). The results further revealed that the extracts obtained from SA- and MeJA-treated plants with lower concentrations generally possessed higher TPC in comparison to those treated with higher concentrations. Overall, the use of MeJA could have a more significant effect on the promotion of TPC compared to SA (Fig. 2b).

Rosmarinic acid and caffeic acid

Based on the results of Fig. 2c, d, the highest content of RA (98.65 ± 0.19 mg g^{-1} dry weight) and caffeic acid (68.97 ± 0.34 mg g^{-1} dry weight) belonged to the application of 0.1 mM MeJA and 150 mg L^{-1} TiO_2 NPs (87% and 78% higher than control (without elicitation), respectively). In general, the exogenous application of SA and MeJA significantly increased rosmarinic and caffeic acids in both TiO_2 NPs-treated plants and without TiO_2 NP plants.

CAT and APX activities

Elicitors such as SA, MeJA, and TiO_2 NPs generate a signal transduction pathway and *reactive oxygen species* (ROS). The plant increases the activity of antioxidant enzymes and antioxidant ability to scavenge ROS (da Silva et al. 2014). Hence, it is crucial to determine the antioxidant ability and activity of antioxidant enzymes, including CAT and APX.

In general, the CAT activity was significantly higher in TiO_2 NP-treated plants compared to control (without TiO_2 NPs). This trend continued for all elicitation levels. Exogenous application of MeJA and SA could significantly increase CAT activity in plants treated with and without TiO_2 NPs compared to control (without elicitation). CAT activity represented a considerable increase when applying 0.1 mM MeJA in TiO_2 NP-treated plants, which was 2.54 times higher than control (Fig. 2e). A similar trend was observed in peroxidase (POX) activity. The highest APX activity was found in plants sprayed with 0.1 mM MeJA and TiO_2 NPs (95% higher than control). Overall, MeJA was more influential on the activity of antioxidant enzymes compared to SA (Fig. 2e, f).

DPPH-radical scavenging activity

The antioxidant activities of the extracts were presented as the percentages of DPPH radical scavenging activities. TiO_2 NPs, MeJA, and SA led to a significant increase in the DPPH-scavenging activity compared to control (Fig. 2g). The highest DPPH radical scavenging activity ($65.17\% \pm 0.25$) was detected with the application of 0.1 mM MeJA in TiO_2 NP-treated plants (78% higher than control). In this regard, 1 mM SA was used in the next step. The obtained results confirmed a close relationship between TPC and DPPH-radical scavenging activity (Fig. 2g).

The EO content

Based on GC–MS analyses, 34 constituents were found in the EO of peppermint, mainly including menthol (24.21–44.51%) as the major component, isomenthone (4.01–15.11%), and 1,8-Cineole (5.65–29.3%). Other major constituents were α-pinene, β-pinene, sabinen, menthyl acetate, bornyl acetate, camphene, caryophyllene, germacrene D, humulene, pulegone, and menthofuran. The results (Table 4) revealed that the application of SA, MeJA, and TiO_2 NPs, could increase EO constituents compared to control in majority of studies. The percentage of menthol in plants treated with TiO_2 NPs at different concentrations of SA and MeJA exerted a significant increase compared to control. Compared to the control, the content of menthol was noticeably higher in TiO_2 NP-treated plants both, with and without SA elicitation by 62 and 58%, respectively. The highest menthol percentage was related to 0.1 mM MeJA in TiO_2 NP-treated plants (44.51%), which was 83% higher than control (24.21%). Moreover, other constituents in EO demonstrated significant fluctuations with elicitation compared to control. Elicitation with SA and TiO_2 NPs increased the 1,8-Cineole content (29.3%) compared with control (Table 4). The GC–MS analyses indicated that the use of

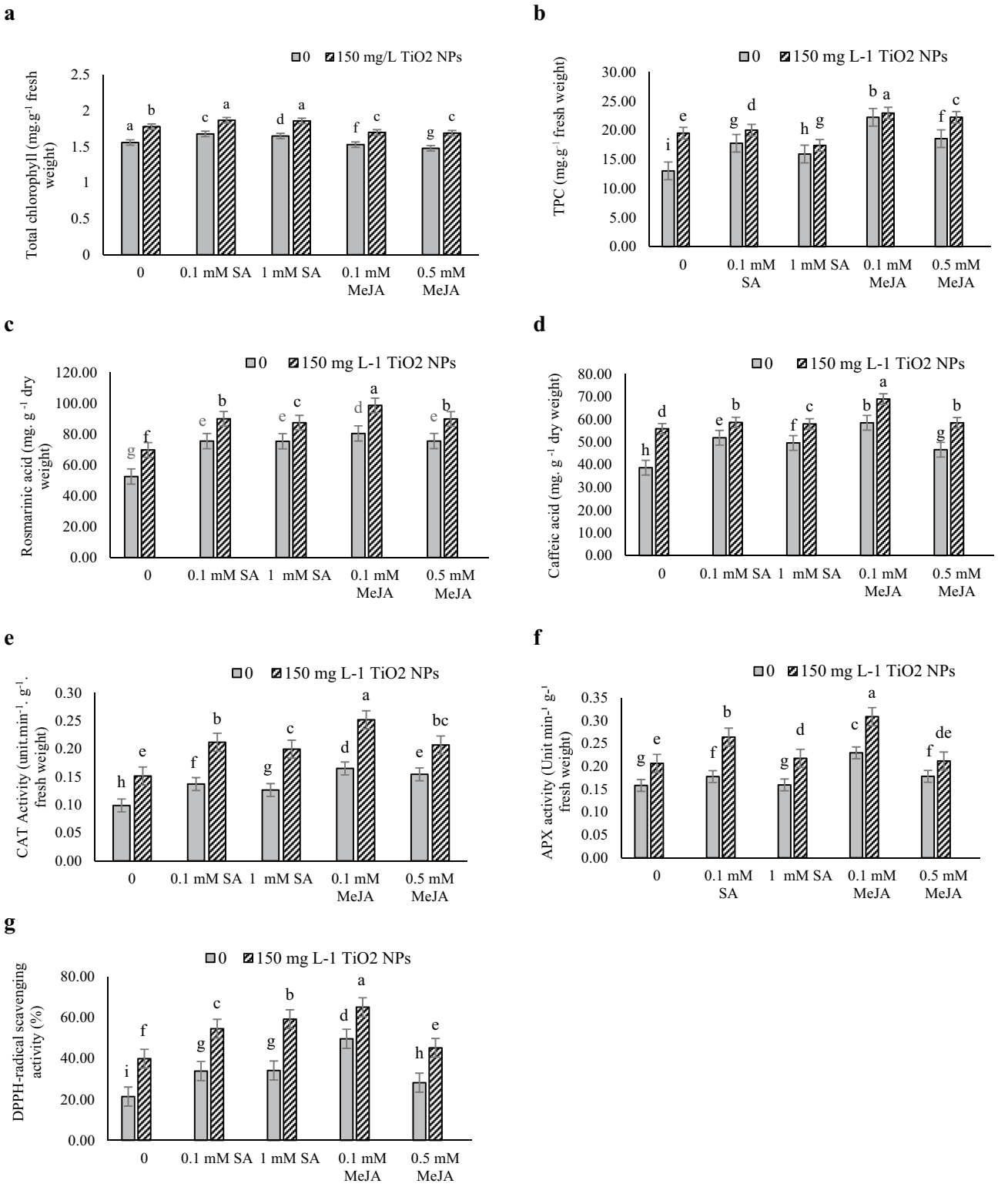

Fig. 2 Impact of SA, MeJA, and TiO_2 NPs on biochemical characteristics. *Note*: **a** Total chlorophyll content, **b** total phenolic content, **c** rosmarinic acid, **d** caffeic acid, **e** CAT activity, **f** APX activity, and **g** DPPH-radical scavenging activity (%). In each figure, means with the same letter(s) do not significantly differ according to Duncan's test at $P \leq 0.05$. Means ± SE from the three replications. Error bars (⊤) represent SE. *DPPH* 2-diphenyl-1-picrylhydrazyl, *CAT* Catalase, *APX* Ascorbate peroxidase, *SE* Standard error, *SA* Salicylic acid, *MeJA* Methyl jasmonate, *TiO2 NP* Titanium dioxide nanoparticle, *SE* Standard error

Table 4 Effect of SA, MeJA, and TiO_2 NP application on the percentage composition of *Mentha piperita* EO

No.	Compound	RT	Concentration (%)									
			0*					150*				
			0	0.1 mM SA	1 mM SA	0.1 mM MeJA	0.5 mM MeJA	0	0.1 mM SA	1 mM SA	0.1 mM MeJA	0.5 mM MeJA
1	α-Pinene	4.044	1.4	1.42	1.73	1.38	1.5	0.6	1.73	1.73	1.76	1.49
2	Comphene	4.35	ND	0.39	0.4	0.41	0.38	ND	0.53	0.54	0.51	0.47
3	Sabinen	4.757	0.88	1.3	1.49	1.3	1.49	0.51	1.66	1.6	1.48	1.46
4	Nopinen	4.866	2.91	2.56	2.94	2.97	2.69	ND	3.13	ND	ND	2.96
5	β-Pinene	4.873	ND	ND	ND	ND	ND	0.95	ND	3.32	3.33	ND
6	p-Menth-8-en-3-ol acetate	5.945	ND	ND	ND	ND	1.04	ND	ND	ND	ND	ND
7	1,8-Cineole	6.006	10.61	19.21	23.31	24.84	21.96	5.65	24.6	29.3	20.23	24.41
8	Limonene	6.14	ND	ND	ND	ND	ND	1.05	0.52	ND	ND	ND
9	4,7,dimethyl,undecane	6.23	ND	ND	ND	ND	ND	1.3	ND	ND	ND	ND
10	Isomenthone	9.027	4.01	15.11	12.03	0.34	12.24	4.24	12.11	13.89	8.01	4.54
11	Menthofuran	9.224	16.73	7.85	5.57	1.01	5.11	22.71	1.79	1.07	0.99	2.67
12	Isomenthol	9.394	ND	0.86	ND	ND	0.62	2.6	ND	ND	ND	ND
13	Linderol	9.448	7.09	0.76	0.81	ND	0.76	ND	1.69	ND	ND	1.26
14	(-)-Menthol	9.611	24.21	32.37	31.89	33.29	30.67	39.31	38.46	34.65	44.51	34.26
15	dl-Menthol	10.12	ND	ND	ND	ND	ND	0.78	ND	ND	ND	ND
16	Pulegone	11.172	8	7.6	9.51	9.86	10.16	1.19	2.01	0.55	1.48	10.5
17	Bornyl acetate	12.367	1.07	0.42	0.47	0.84	0.53	ND	0.58	0.84	0.75	0.95
18	Menthol, acetate	12.564	0.86	0.48	0.36	ND	0.4	10.48	ND	ND	ND	ND
19	Menthofurolactone	14.193	ND	ND	ND	ND	1.5	1.94	0.75	0.96	ND	1.12
20	Dihydrojasmone	14.56	1.18	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	4-(2-Methyl-3-oxocyclohexyl)butanal	15.565	1.91	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	Caryophyllene	15.843	7.22	6.16	5.55	15.49	4.96	0.91	6.74	7.35	11.01	8.95
23	Humulene	16.739	ND	0.38	0.33	0.98	0.34	ND	0.43	0.48	0.67	0.58
24	5Caranol,(1S,3R,5S,6R)-(-)-	16.746	3.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
25	Germacrene D	17.37	2.07	2.2	2.06	3.51	1.22	ND	1.77	1.42	3.26	2.03
26	Bicyclogermacrene	17.717	ND	0.34	0.34	ND	ND	ND	ND	ND	ND	ND
27	Elemene isomer	17.723	ND	ND	ND	ND	ND	ND	ND	ND	0.49	ND
28	Mint furanone	18.04	ND	ND	ND	ND	ND	1.29	ND	ND	ND	ND
29	Phenol, 3,5-bismethyl]-2,4,6-trimethyl	18.056	0.87	ND	ND	ND	ND	ND	ND	ND	ND	ND
30	7a-Hydroxymintlactone	19.72	ND	ND	ND	ND	ND	1.11	ND	ND	ND	ND
31	Caryophyllene oxide	19.76	2.34	ND	ND	1.31	0.8	ND	0.58	1.18	0.48	1.08
32	Cyclobarbital	20.928	0.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
33	(+)-T-Cadinol	21.179	1.31	0.58	0.66	1.4	0.69	ND	0.86	1.11	1.04	1.27
34	Di-n-decylsulfone	21.831	0.8	ND	ND	ND	ND	ND	ND	ND	ND	ND

RT Retention time, 0* and 150* = 0 and 150 mg L^{-1} TiO_2 NPs; *ND* Not detected, *TiO_2 NP* Titanium dioxide nanoparticle

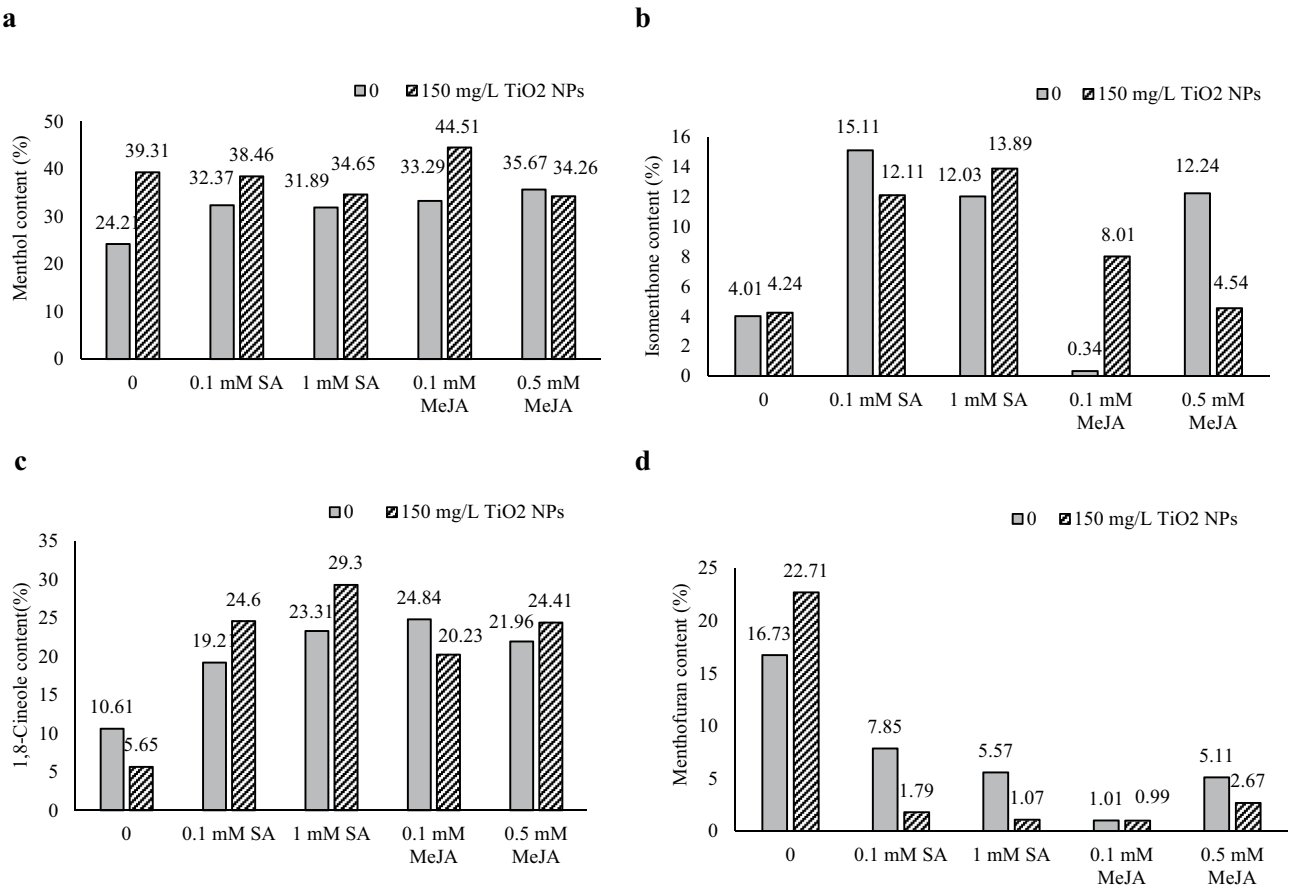

Fig. 3 Impact of SA, MeJA, and TiO_2 NPs on **a** menthol content, **b** isomenthone content, **c** 1,8-Cineole content, and **d** menthofuran content of peppermint (*Mentha piperita* L.). *Note*: *SA* Salicylic acid, *MeJA* Methyl jasmonate, *TiO_2 NP* Titanium dioxide nanoparticle

0.1 mM SA could increase the content of isomenthone by 276% compared to control. Based on the findings, the content of menthofuran represented a considerable decline with SA and MeJA elicitation, while it increased with the application of TiO_2 NP. Contrarily, the content of pulegone in 0.5 mM MeJA-elicited plants showed the highest rate both with and without TiO_2 NP application. Figure 3 illustrates the impact of SA, MeJA, and TiO_2 NPs on the menthol, isomenthone, 1,8-Cineole, and menthofuran contents of peppermint. Further, the EO chromatogram for some treatments is depicted in Fig. 4.

Discussion

Previous studies have thoroughly investigated the application of plant growth regulators and TiO_2 NPs, but no study has so far evaluated the combined effects of TiO_2 NPs with SA and MeJA on peppermint. In the current study, it has been observed that SA and MeJA in TiO_2 NP-treated plants had a positive effect on growth and secondary metabolite production. Bakry et al. (2012) reported that SA could improve nutrient uptake, chlorophyll content, photosynthesis, and plant growth, which is consistent with our results. This effect has been attributed to cell division induction (Bakry et al. 2012), more growth in the meristemic regions (Singh and Usha 2003), high auxin and gibberellin concentrations (Husain et al. 1990), and more enzyme activities (El-Tayeb 2005) in SA-treated plants. El-Tayeb (2005) indicated that increased plant growth using SA is related to its positive impact on net photosynthesis and increased activity of nitrate reductase and carbonic anhydrase. According to reports, SA is involved in the regulation of the H^+-ATPase activity in root tonoplast, leading to an increase in the cation content in roots and shoots, and thus a higher nutrient uptake. Thus, greater uptake of mineral nutrients in response to the application of SA enhances carbon dioxide (CO_2) uptake and photosynthesis rate, improving the growth and development of the plant (Gorni et al. 2020).

The results of our work are in agreement with Gharib (2006), confirming that SA enhanced fresh and dry weights and plant height in basil and marjoram. RWC is regarded as a measure of water status in plant tissues and is superior to cell water potential in this respect since the RWC of the

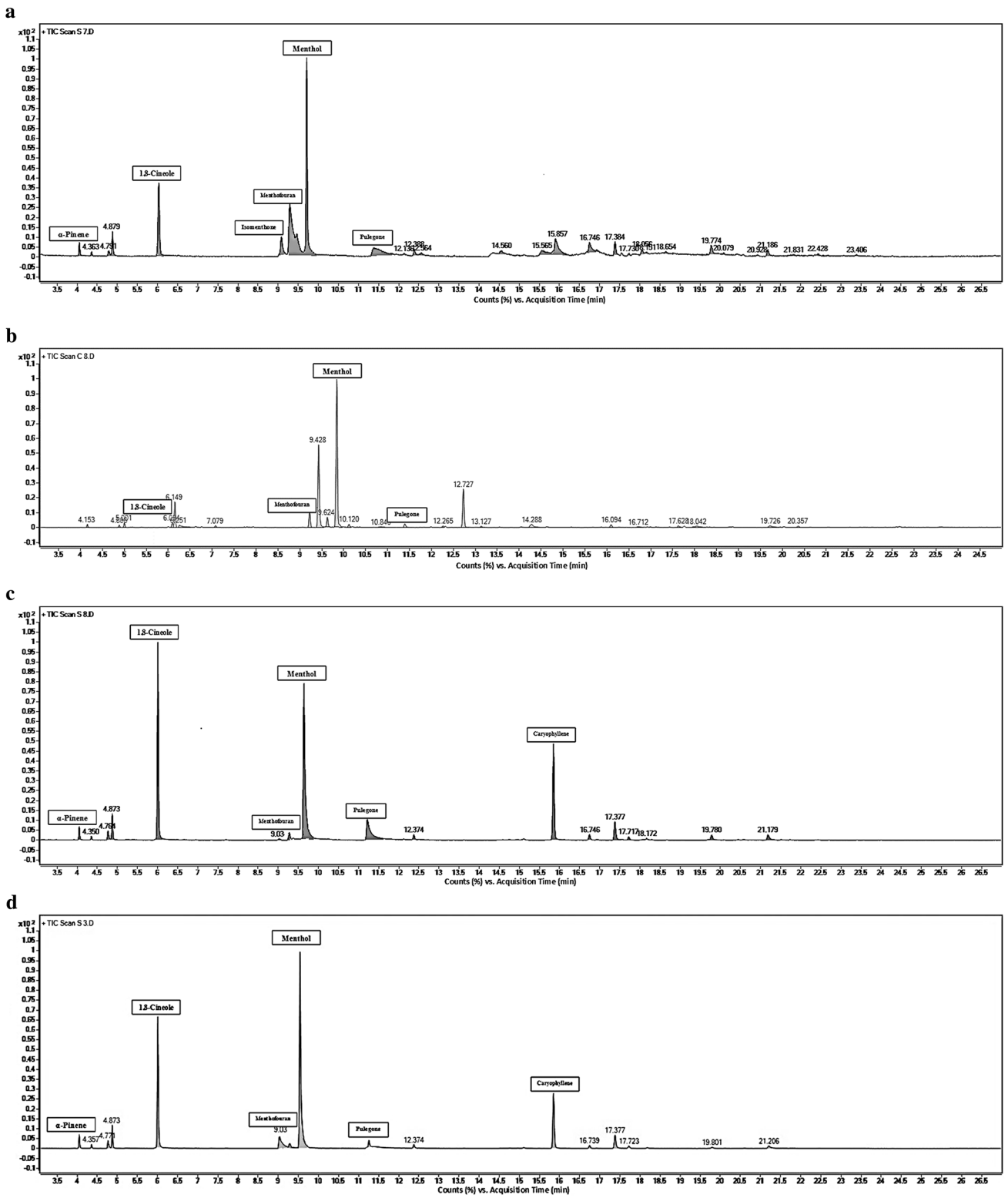

Fig. 4 *Mentha piperita* EO chromatograms in **a** control, **b** 150 mg L^{-1} TiO_2 NPs, **c** 0.1 mM MeJA, and **d** 0.1 mM MeJA × 150 mg L^{-1} TiO_2 NPs treatments. *Note*: *EO* Essential oil; *SA* Salicylic acid, *MeJA* Methyl jasmonate, *TiO_2 NP* Titanium dioxide nanopartic

leaves through direct correlation with the cell volume can balance plant water status and transpiration rate (Schonfeld et al. 1988). In their study, Agarwal et al. (2005) found that RWC in wheat increased with SA treatment. In other words, SA significantly decreased electrolyte leakage (Kang and Saltveit 2002).

Our results revealed that TiO_2 NP application enhanced the growth characteristics of the plant, which is probably associated with the amelioration of mineral absorption (Chen et al. 2018). Lei et al. (2007) concluded that TiO_2 NPs could significantly promote the uptake of minerals such as nitrogen and magnesium and accelerate nitrogen metabolism. Likewise, Ahmad et al. (2018) found that the content of nitrogen increased by 12.6% by applying 150 mg L^{-1} of TiO_2 NPs in *M. piperita* compared to control. The application of TiO_2 NPs could increase fresh and dry weight in spinach (Gao et al. 2008; Yang et al. 2006), as well as shoot length in wheat (Feizi et al. 2013). It is indicated that TiO_2 NPs can lead to an increase in the fresh and dry weights of plants through enhancing Rubisco enzyme activity and light absorption (Mingyu et al. 2007). The tendency of NPs to penetrate plant seed coats could enhance seed growth and germination (Singh et al. 2016). NPs can penetrate the leaves via stomatal apertures in the foliar application; next, they are translocated to a wide range of tissues through the apoplast and/or symplast pathways (Ramadan et al. 2022).

Based on the results of the current study, RWC demonstrated a significant improvement by applying TiO_2 NPs. Accordingly, the higher reactivity of TiO_2 NPs may extend root pores or result in higher water flow in the root (Larue et al. 2012). Subramanian et al. (2006) reported that TiO_2 NPs could enhance water absorption from the root toward the shoot and increase the RWC in the plant. Higher membrane stability would be relative to an increase in the cell water content, phosphorus uptake, or activity of enzyme and non-enzyme antioxidants (Feng et al. 2002), which corroborates with the findings of the present study. In our study, TiO_2 NP application improved membrane stability.

In the current study, MeJA treatment reduced shoot dry weight and plant height. Decreased plant growth may be associated with the induction of plant senescence by MeJA (Kim and Hwang 2014). Moreover, MeJA has a restricting impact on the synthesis and accumulation of gibberellins, leading to postponed plant growth. Additionally, MeJA may negatively interact with other hormones involved in growth regulation, including auxin, cytokinins, and brassinosteroids (Cappellari et al. 2019). It has been recommended that MeJA can inhibit the cell division cycle, especially in mitosis (Świątek et al. 2002). According to a number of studies, the use of plant hormones such as MeJA and jasmonic acid in the absence of environmental stresses exerts an inhibitory impact on the growth of the plant (Pessarakli 1994; Ueda and Saniewski 2006; Zhao et al. 2007). A considerable decline was observed in the shoot dry weight of *Hypericum hirsutum* and *Hypericum maculatum* (Coste et al. 2011) and *Hypericum* (Wang et al. 2015) when treated with jasmonic acid. It was reported that the reduction in the biomass might be owing to membrane lipoxidation induced by elicitor treatments. In their experiment, Ma et al. (2014) found that MeJA improves water use efficiency through decreasing stomatal conductance. Furthermore, MeJA could enhance the stability of the cell membrane, while reducing oxidative stress through increasing antioxidant capacity. MeJA increased the RWC by maintaining moisture within the plant tissue (Miranshahi and Sayyari 2016). Previous research represented an inverse relationship between electrolyte leakage and CAT activity in plants that were treated with MeJA (Khalvandi et al. 2019).

In the present research, TiO_2 NPs and SA could increase total chlorophyll content. Similarly, Morteza et al. (2013) reported the positive impact of TiO_2 NPs on the photosynthetic pigments of maize (chlorophyll a, chlorophyll b, total chlorophyll content, and carotenoids contents). In the current study, treatment of plants with MeJA led to a decrease in the total chlorophyll content, and MeJA elicitation increased the TPC. This is a possibility that SA increases flavonoid and phenolic compounds by increasing the activities of phenylalanine ammonia-lyase (PAL) and chalcone synthase. Our results revealed that the phenolic content increased in plants treated with TiO_2 NPs. In line with our findings, the use of nano-sized ZnO and CuO particles in *Glycyrrhiza glabra* seedlings positively affected secondary metabolite production (Oloumi et al. 2015). It is extensively accepted that elicitors increase secondary metabolite biosynthesis through activating the signal transduction pathway and specific genes (Ali et al. 2019). In addition, it was reported that SA induce the genes involved in the biosynthetic pathway of phenolic compounds (Thulke and Conrath 1998, Li et al. 2016), increases the activity of the PAL enzyme, eliminates free radicals, and prevents cell membrane damage (Bagal et al. 2012). Khanam and Mohammad (2018) associated the beneficial impact of SA on the synthesis of secondary metabolites with an improvement in the peltate gland number and growth characteristics. MeJA application could increase secondary metabolites, including phenolics, alkaloids, and terpenes in medicinal and aromatic plants (Cappellari et al. 2019). According to Saeed et al. (2017), MeJA can interact with the surface receptors of plant cells, triggering a cascade of plant defense reactions due to the transcription of various vital secondary metabolism genes, including the *PAL* gene.

They further indicated that MeJA had a stimulating impact on the production of phenolic compounds.

MeJA could improve lithospermic acid B and RA in the hairy root cultures of *Salvia miltiorrhiza* (Xiao et al. 2009; Zhang et al. 2014). Additionally, MeJA application in the hairy root cultures of *Coleus forskohlii* and *Coleus blumei*, as well as the cell cultures of *Agastache rugosa* Kuntze, could increase the accumulation of RA (Xiao et al. 2009; Kim et al. 2013). An enhancement in the RA content was observed with the application of the MeJA-treated cells of *Lithospermum erythrorhizon* (Mizukami et al. 1993). Further, MeJA increased RA, chlorogenic acid, caffeic acid, and cinnamic acid contents in *Mentha spicata* (Yousefian et al. 2020). The increase of flavonoids and phenolic compounds by using SA and MeJA highlighted the inductive impact of elicitors on the metabolic pathways of phenylpropanoids (Mendoza et al. 2018).

In the current study, the treatment of plants with MeJA, SA, and TiO_2 NPs could significantly improve the DPPH-scavenging activity. The activities of antioxidant enzymes could be altered relying on the type and concentration of NPs. In plants treated with TiO_2 NPs, ROS overproduction may result in inducing antioxidant machinery to scavenge ROS (Castiglione et al. 2014). However, in our experiment, the application of TiO_2 NP led to an increase in the content of proline as a component of the antioxidant defense system, reducing ROS production. Furthermore, the production of ROS under stress conditions can be controlled by the activities of antioxidant enzymes, including POX, superoxide dismutase (SOD), and CAT (Ghabel and Karamian. 2020). Ramadan et al. (2022) have recently reported the positive impact of TiO_2 NPs on CAT activity in *Helianthus annuus*.

Based on some reports, SA could increase the activity of POX, SOD, and CAT in *Oryza sativa* and *Zea mays* (Guo et al. 2007; Krantev et al. 2008). High levels of antioxidant enzymes exert an essential role in inhibiting free radicals and decreasing oxidative damage. In an experiment, the total phenolic compound was significantly higher in MeJA-treated plants and led to higher DPPH free radical scavenging activities (Kim et al. 2006). MeJA has a role in the signal transduction pathway in oxidative stress by increasing CAT, POX, and SOD activities (Abdelgawad et al. 2014). Similarly, MeJA could increase SOD, POX, and CAT activities in peanut, arabidopsis, and rapeseed seedlings (Jung 2004; Kumari et al. 2006).

In the present research, MeJA, SA, and TiO_2 NPs could significantly influence the content of terpenoids. Previous reviews demonstrated that TiO_2 NPs increase the photosynthetic capacity of plants by directly affecting chlorophyll and indirectly by the content of other factors, resulting in progress in glucose synthesis (Ahmad et al. 2018). Glucose is a suitable precursor for the synthesis of EOs, especially monoterpenes (Croteau et al. 1972). The application of TiO_2 NPs exerted a positive impact on EO production through enhancing the expression of enzymes that were involved in the biosynthesis of monoterpenes (Ahmad et al. 2019). In their study, Ahmad et al. (2018) reported that an increase in the concentration of menthol could be because of an increase in the expression of the menthone reductase enzyme, decreasing menthone to menthol. Generally, better plant performance (Chen et al. 2014), improved photosynthesis (Zhang et al. 2015), higher expression involved in terpenoids biosynthesis (Ahmad et al. 2018), and increased density of oil glands were found to be associated with the use of TiO_2 NPs.

The positive effect of TiO_2 NPs on EO production has been reported in *Salvia officinalis* (Ghorbanpour 2015), *Rosmarinus Officinalis* (Golami et al. 2018), and *Vetiveria zizanioides* (Shabbir et al. 2019). In a study on *Mentha piperita*, Ahmed et al. (2018) found an increase in the amount of menthol, menthyl acetate, and menthone within the foliar-applied TiO_2 NPs compared to control. The foliar application of SA and MeJA could increase the thymol amount and the other components of EO in *Salvia officinalis* (Yadegari 2018). Moreover, SA increased the amount of EO in *Salvia macrosiphon* (Rowshan et al. 2010).

The increase in EO by SA is probably due to greater uptake of nutrients by the roots, increased vegetative growth, and increased photosynthetic activity of the plant, along with alterations in the population of glands producing EOs in flowers and leaves (Gharib 2006). Likewise, higher oil yields in MeJA-treated basil plants could be attributed to an increase in plant growth, improvement in nutrient uptake, and changes in the biosynthesis of monoterpenes and leaf oil gland population (Talebi et al. 2018).

Conclusion

Overall, plant elicitation, due to its promising role in crop-growing systems, can be considered an essential strategy. Based on our observations, SA is more effective in enhancing physiological characteristics in TiO_2 NP-treated plants, while phenolic compound, antioxidant activity, and important constitutents of EO were noticeably improved with the application of MeJA. The results of the current study confirmed the positive impacts of TiO_2 NPs on improving the biochemical and morphological properties of the plant.

Acknowledgements This study received financial support from Ferdowsi University of Mashhad, Ministry of Science, Research, and Technology, Iran (grant number 350426).

Declarations

Conflict of interest The authors of this study declare that they have no conflict of interest.

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