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Changes in growth, essential oil composition and biochemical traits of peppermint in response to coapplication of zinc and methyl jasmonate in soilless culture

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ABSTRACT

Zinc (Zn), a micronutrient, is a source of energy for essential oil (EO) biosynthesis. Methyl jasmonate (MeJA) is a plant growth regulator that has favorable effects on EO quality and quantity. The main objective of this study was to evaluate the interaction effect of Zn and MeJA on the phytochemical traits and EO compositions of peppermint cultivated under soilless conditions. Zn omission and the highest Zn concentration (0.1 mgL^{-1}), which are stress conditions for plants, increased phytochemical traits as well as EO content, and 1 mM MeJA was more suitable for increasing these parameters. The major classes of peppermint EO components were oxygenated monoterpenes, sesquiterpene hydrocarbons, and monoterpene hydrocarbons. The major constituents included menthol, menthone, menthofuran, and 1,8-cineole. Elicitation of peppermint with MeJA at different Zn levels modified the EO composition. The highest menthol content (50.1%) was observed in the 1 mM MeJA and 0.025 mgL^{-1} Zn treatment groups. In conclusion, this study indicated that Zn and MeJA could be appropriate compounds for changing phytochemical compounds, antioxidant activity, and EO composition.

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KEYWORDS
Essential oil; menthol; peppermint; phenolic compounds; plant growth regulator

Introduction

A soilless agricultural system is one of the main parts of a sustainable protected culture suitable for urban areas with rare agricultural land and limited water sources (Barrameda-Medina et al. 2017). Under greenhouse conditions, agronomic practices such as crop management through good control of plant growth and nutrient management are needed to improve plant quality (Alvarenga et al. 2015). In soilless culture, the required nutrients only come from formulated nutrient solutions. This technology can be applied to study nutrient uptake, which can increase plant growth and productivity (Alvarenga et al. 2015; Chrysargyris et al. 2017). One of the most important factors that increase plant production and mineral accumulation is plant fertilization. Micronutrients play a significant role in plant growth and development *via* different biochemical processes (Derakhshani et al. 2011). Zinc (Zn), which is generally absorbed as a bivalent cation (Zn^{2+}), is a necessary micronutrient for plant growth and yield improvement (Marschner 2012;

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Wang et al. 2018). Zn, as a micronutrient, plays different roles in plant physiology. It acts in plants as a metal component of enzymes or as a structural, functional, and regulatory co-factor of different enzymes (Barrameda-Medina et al. 2017). In addition, it regulates the function of cofactors in carbohydrate metabolism and cellular photosynthesis (Marschner 2012; Wang et al. 2018). The total phenol and flavonoid contents of medicinal plants play several roles in the cellular defense system against reactive oxygen species (ROS) (Zaid and Wani 2019). Heavy metals and nutrient deficiency increase heavy metal levels (Tavallali, Gholami, and Espargham 2020). ROS are key signaling molecules that play important roles in the response of plants to abiotic and biotic stresses. ROS play an important role in numerous signaling pathways that mediate defense responses in plants (Zaid and Wani 2019). Zn spraying on medicinal plants of the Lamiaceae family enhanced plant dry matter (DM) and oil content according to a previous report (Hegazy et al. 2016). In addition, the foliar application of Zn increased plant yield (Baghaie and Aghilizefree 2020) and the antioxidant activities of peppermint (Mohammadi, Hosseini, and Dashtaki 2016).

On the other hand, plant growth regulators (PGRs) have favorable effects on EO yield and quality (Wasternack and Song 2016). PGRs improve EO yield and the physiological activities and photosynthetic efficiency of plants (Sharafzadeh and Zare 2011). They play a significant role in the growth and development of plants through processes such as photomodulation (Loake, Ayyar, and Howat 2017; Bhatla 2018), trichome formation, reproduction, senescence, and elongation (Wasternack and Strnad 2017). Biostimulants have the potential to increase medicinal and aromatic plant productivity (Ahmed, El-Kady, and Khalid 2018). The results from a previous study revealed that L-tryptophan treatment produced the greatest changes in plant growth characteristics, total soluble sugars, EO yield, and the main components of *Petroselinum crispum* (Ahmed, El-Kady, and Khalid 2018). Jasmonates, a family of phytohormones, are an important class of signaling molecules and fatty acid (linolenic acid or linoleic acid) derivatives (Loake, Ayyar, and Howat 2017). Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), are widely distributed in different plant species to improve and regulate plant growth (Wasternack and Strnad 2017). The role of MeJA under metal stress conditions has been reported in previous studies (Hanaka et al. 2022: Kamali et al. 2024; Kaushik et al. 2024). Exogenous JA and MeJA have been shown to improve antioxidant defense activities under heavy metal toxicity (Dai et al. 2020; Raza et al. 2020). MeJA application reduced the effects of metal stress on different plant species, such as wheat (Kaya et al. 2021), rice (Li et al. 2022), and pea (Manzoor et al. 2022). Jasmonates also play an important role in the elicitation of secondary metabolites (Loake, Ayyar, and Howat 2017; Bhatla 2018). The application of phytohormones could improve the quality of important medicinal and aromatic plants under heavy metal stress (Verma et al. 2020). For instance, JA controls the number of secondary metabolites produced, such as terpenoids, and upregulates the enzymes and genes of secondary metabolite biosynthetic pathways (Wasternack and Strnad 2017). Furthermore, the individual effects of PGRs on the EO content and composition of peppermint were previously examined (Ali 2021). One key factor in improving the qualitative and quantitative characteristics of the EO as well as biomass production in medicinal and aromatic plants is nutrient availability (Amani Machiani et al. 2018).

Mentha piperita L. (peppermint) is one of the most important aromatic and medicinal herbs in the Lamiaceae family and is rich in secondary metabolites such as essential oils (EOs), which are mainly composed of monoterpenes (Gupta et al. 2017). EO is extracted from different parts of aromatic plants, such as leaves, stems, flowers, and bark, through the hydrodistillation method and then used in different industries (Keshavarz, Modarres-Sanavy, and Mahdipour Afra 2018). Peppermint contains ~3% EO, which is > 50% different. The major EO components, including menthol, menthyl acetate, menthone, 1,8-cineole, β-caryophyllene, linalool, germacrene D, menthofuran, and pulegone, make up ~60% of the total oil (Amani Machiani et al. 2018).

Zn deficiency is a worldwide problem in different agroclimatic regions (Tewari, Kumar, and Sharma 2019), but there is not enough information on the role of Zn in biochemical characteristics and secondary metabolite production. In addition, MeJA is a chemical signal that affects growth and plant metabolism, causing biochemical and phytochemical reactions (Yan, Borrego, and Kolomiets 2013; Wasternack and Song 2016). Therefore, due to the important effects of Zn, as a source of energy for terpenoid biosynthesis, on EO content and composition and MeJA concentrations on biochemical variables, as well as *via* gene expression linked to the biosynthesis of EO and the chemical composition of medicinal plants, the present study was designed to study the interaction effects of exogenously applied MeJA and Zn levels on the biochemical characteristics, EO content, and components of peppermint as well as its growth traits.

Materials and methods

Experimental design

The soilless greenhouse factorial experiment was a randomized complete block design (RCBD) with four replications in a greenhouse at Ferdowsi University of Mashhad, Iran, which is situated at latitude 36° 18′ N and longitude 59° 31′ E and an altitude of 985 meters above sea level, in 2021. The plastic pots (40 × 30 cm) were filled with finely washed sand (0.05–0.1 mm) as growth media (Safari et al. 2019), and five similar peppermint rhizomes (5–6 cm in length) were cultivated in each pot. First, to adjust and establish the plants completely, they were irrigated with deionized water for two weeks. Then, to perform the treatments, Hoagland solution was prepared (Hoagland and Arnon 1950). The plants received a solution containing different Zn concentrations three times a week. Zn was applied at four levels, 0 (Zn omission from the Hoagland solution), 0.025, 0.05, and 0.10 mgL^{-1}, while all other nutrient concentrations were kept steady. The source of Zn used in this experiment was $ZnSO_4.7H_2O$. During the experiment, the pH (5.8–6.5) and EC (1–1.3 dSm^{-1}) of the nutrient solution were kept stable. Every week, the solution was changed to resupply nutrients (Maggini et al. 2012; Abbasi Khammar et al. 2021). On the other hand, the other foliar spraying treatments included I) distilled water (control), II) distilled water + 1% ethanol (as solvent), III) MeJA at 1 mM, and IV) MeJA at 2 mM. The flow chart of RCBD including one replicate for the treatment eas shown in Figure 1. Every 10 days, foliar application of MeJA with a hand sprayer was performed at the dew point until one week before harvesting. Finally, the plants were harvested at the flowering stage, and their characteristics were measured.

Determination of biomass and DM

Three plants from each pot were selected at the flowering stage, and the aboveground parts were harvested, dried in an oven at 72 °C for 48 h and weighed. Then, the DM percentage was calculated by using the following formula according to the method of Salem et al. (2014):

$$\text{DM } (\%): \ \text{DMW/FMW} \times 100$$

where DMW: dry matter weight, FMW: fresh matter weight

Determination of leaf area (LA)

Peppermint leaves were used for measuring LA. Image software and an LA meter (Model LI-3100c, LICOR, Lincoln, NE, USA) device were used for reporting LA.

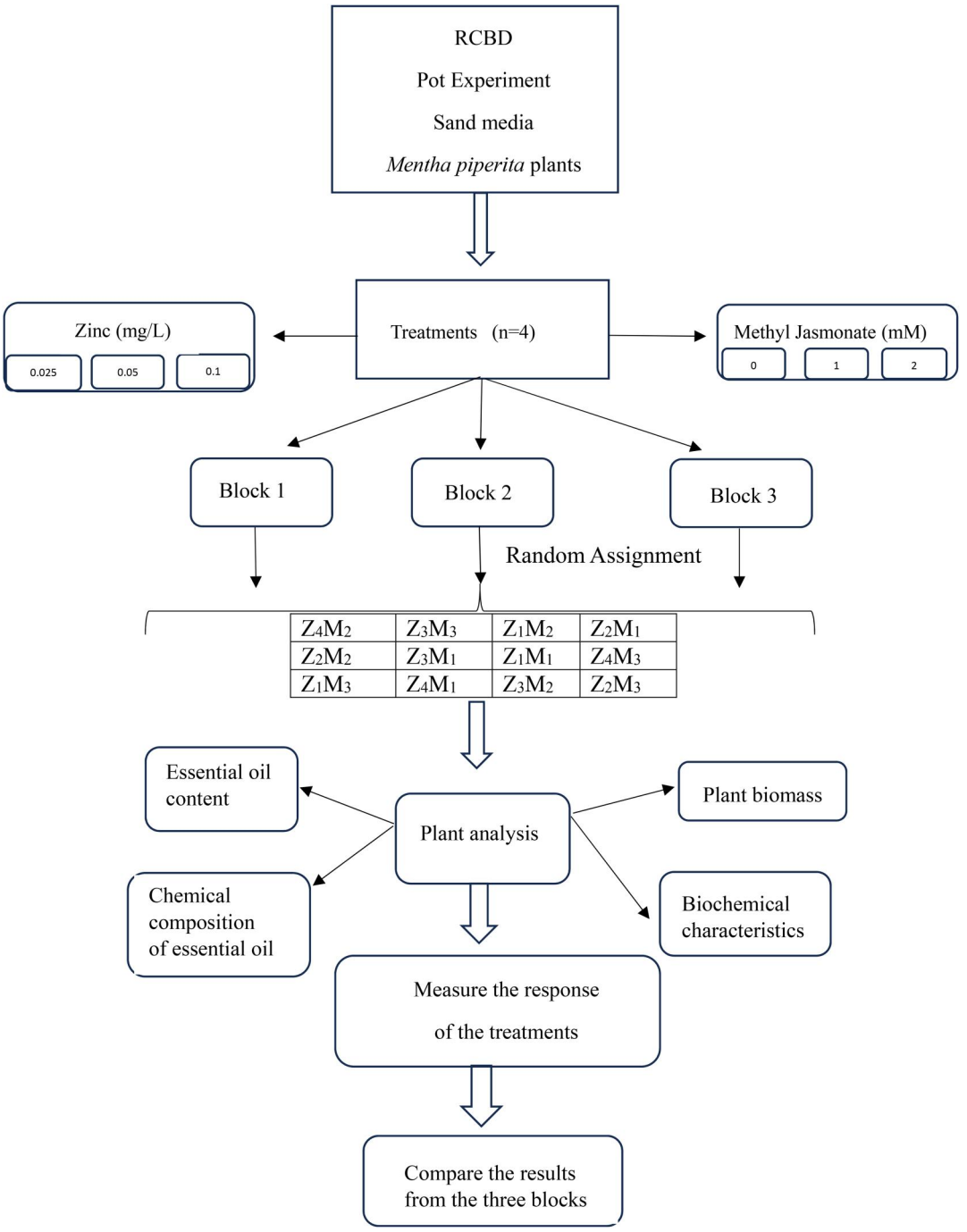

Figure 1. The flow chart of randomized complete block design (RCBD) including one replicate for the treatment. *Z_1: 0; Z_2:0.025; Z_3: 0.05; Z_4: 0.1 mg L^{-1} Zinc. *M1:0; M_2:1, M_3: 2 mM methyl jasmonate.

Determination of chlorophyll (chl) meter reading (SPAD)

SPAD was utilized for the relative leaf greenness (level of Chl) measurement in peppermint leaves (SPAD 502, Minolta Ltd., Osaka, Japan). The SPAD values of peppermint were measured on the middle part of the leaf blade.

Determination of total anthocyanin content

To assess this parameter, 0.25 g leaf samples were extracted in 3 mL of acidified methanol (methanol: HCl, 99: 1, vv^{-1}) and put in dark conditions in a refrigerator for 24 h. After centrifugation for 10 min at 12000 rpm at room temperature, the extracts were filtered, and the total anthocyanin content was measured spectrophotometrically (Bio Quest C2502, UK) at 550 nm (Krizek, Britz, and Mirecki 1998). To calculate the anthocyanin concentration, the light extinction coefficient (ε) of 33 $mol^{-1}\,cm^{-1}$ was considered in the following formula:

$$A = \varepsilon bc$$

A = absorption, b = cuvette width, c = the concentration of the intended solution

Determination of the preparation of the methanolic extracts

To prepare the methanolic extract, 0.5 g of dried leaves was ground, and then 5 mL of methanol (99%) was added and placed on a shaker for 48 h. After that, the prepared extracts were centrifuged at room temperature at 4500 rpm for 20 min. Then, the insoluble portions were separated by filtration. Finally, the prepared extracts were stored at 4 °C in a refrigerator until final analysis.

Determination of total phenolic content

The total phenolic content of the leaves was evaluated by the Folin-Ciocalteu method (Singleton and Rossi 1965). The mixture included the methanolic extract (100 μL), Folin-Ciocalteu reagent (Sigma–Aldrich Chemical Company, Germany) (200 μL), and distilled water (2 mL). Three minutes later, 20% Na_2CO_3 (1 mL) was added to the solution. After 60 min of incubation at room temperature, the absorbance was measured at 765 nm by a spectrophotometer (Bio Quest C2502, UK). A standard curve of gallic acid (GA) solution (25–200 μg mL^{-1}) was prepared, and the total phenolic content was reported as mg GA equivalent g^{-1} dry weight (DW) extract sample (mg GAE g^{-1} DW).

Determination of the total flavonoid content

The flavonoid content was determined by using a spectrophotometer based on the formation of a flavonoid–aluminum complex. The mixture included 500 μL of the extract, 2 mL of distilled water, and 150 μL of 5% (w/v) sodium nitrite. Five minutes later, 300 μL of 10% (w/v) $AlCl_3$ was added. Finally, after 6 min, 1.0 mL of 1.0 M NaOH and 1050 μL of distilled water were added. Then, the mixture was shaken, and the absorbance was read immediately at 510 nm (Menichini et al. 2009). Quercetin (QE) solution was applied to prepare the standard curve flavonoid content determined as mg QE equivalent per gram of DW.

Determination of total flavone and flavonol contents

The method suggested by Popova et al. (2004) was applied to analyze the flavone content of the leaves. Aliquots (2 mL) of the test solution, 20 mL of methanol, and 1.0 mL of 5% aluminum chloride in methanol (wv^{-1}) were mixed in a volumetric flask, and the volume was adjusted to 50 mL with methanol. The mixture was left for 30 min, after which the absorbance at 425 nm was measured. The flavonol content was determined according to the methods of Yermakov, Arasimov, and Yarosh (1987). The reaction mixture included 0.5 mL of methanolic extract with 2 mL of $AlCl_3$ (20 mg mL^{-1}) and 6 mL of sodium acetate (50 mg mL^{-1}). The absorbance was

measured after 2.5 h at 440 nm. A QE solution was used to prepare the standard curve, and the total flavone and flavonol contents were determined as mg QE equivalent per gram of DW.

Determination of the total soluble sugars content

The total soluble sugars were measured according to Mc Cready et al. (1950) method. To evaluate the total soluble sugars, the reaction mixture included 3 mL of anthrone (0.15 g in 100 mL of 72% H_2SO_4) and 100 μL of methanolic extract. After mixing for 10 min, they were placed in a 100 °C bath and cooled to room temperature. Finally, the absorbance was recorded at 630 nm. The comparable content of total soluble sugars was calculated against the standard curve produced by different concentrations of glucose. The data were estimated in mg g^{-1} DW for total soluble sugars.

Determination of free radical-scavenging activity

The free radical-scavenging activity of the extracts was estimated with the modified method of Wang et al. (2008), which is based on the measurement of the reducing ability of antioxidants toward the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. For each sample, different concentrations ranging from 500 to 8000 µg mL^{-1} were prepared with methanol (99%). The reaction mixtures (in 96-well plates) consisted of 100 μL of sample and 100 μL of 0.2 mM DPPH radical dissolved in methanol. The mixture was shaken vigorously and then kept in the dark for 15 min at room temperature. Then, the absorbance of the resulting solutions was measured by using a Bio Quest C2502, UK, spectrophotometer at 517 nm against a blank. All determinations were performed in triplicate. The percent inhibition of the free radical DPPH was calculated as follows:

$$\%\text{DPPH radical scavenging} = [1 - (A_{1\text{-}}A_2)/A_0] \times 100$$

A_0: The control reaction absorbance (with no sample); A_1: Plant sample absorbance; A_2: The sample with no DPPH radical absorbance. The scavenging ability of the samples was reported as IC_{50} values, i.e. the useful concentration at which 50% of DPPH radicals were scavenged. The 50% inhibition (IC_{50}) of the extract was determined by plotting the inhibition percentage against the concentration of the extract solution.

Determination of EO extraction

At the flowering stage, three other peppermint plants from each pot were selected, and the aerial parts were harvested and dried at room temperature to extract their EO. Dried aerial parts of peppermint were subjected to hydrodistillation for 3 h by a Clevenger-type apparatus. The samples were collected over water, separated, dried over anhydrous sodium sulfate and stored in a dark glass bottle at 4 °C until chemical analysis. The EO content was recorded as %v/w (British Pharmacopeia Commission 1980).

Determination of gas chromatographic (GC) flame ionization detector (FID) analysis

To analyze the chemical composition of the EO, a Shimadzu GC-17A, Agilent (HP), Japan, GC coupled with an FID was used. The chemical components were separated on an HP5-MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The oven temperature was programmed for 1 min at 60 °C, subsequently increased to 250 °C at 5 °C/min, and then held isothermally for 2 min at 250 °C. The injector and FID detector temperatures were adjusted to 250 °C and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 1.1 mL/min, the initial pressure was 35.3 kPa, and the split ratio was 1:100.

Determination of gas chromatography–mass spectrometry (GC–MS) analysis

An Agilent 7990B GC coupled to a 5977 A MS and an HP5-MS column (30 $m \times 0.25$ mm, film thickness 0.25 μm) was used to analyze the EO. The temperature program and conditions were similar to those previously described in section 2.13. Scanning (0.6 scan/s, cycle time: 0.2 s) was carried out in the range of 40 to 460 m/z *by* applying electron impact ionization at 70 eV at an inert ion source, a high-efficiency electron ionization (HES EI) temperature of 350 °C, an MS interface temperature of 250 °C, and a quadrupole temperature of 150 °C in a mass spectrometer.

Determination of chemical compound identification

The quantity of each compound was measured based on the GC peak area and organized in order of GC elution. The identification of EO compounds was performed by comparison of the mass spectra with the database equipment (Wiley 7n. L and NIST) and compared with the calculated linear retention indices (RIs) (based on retention times of homologous linear hydrocarbons (C_5-C_{28}) under the same experimental conditions, with values reported in the literature (Adams 2007).

Statistical analysis

All the analytical data are reported as the means ± standard errors (SE). Statistical analysis was performed with Minitab 17 software. The significance of differences between the treatments was examined by two-way analysis of variance (ANOVA) was used for analyzing the data and the mean values were compared using Tukey's post hoc test at $p < 0.05$ (McLaughlin and Wakefield 2005).

Results

The findings of this study showed that the interaction effect of Zn concentration and MeJA application had a significant effect on all the studied traits.

Biomass and DM

The aboveground fresh biomass and DM of peppermint were influenced by the Zn concentration and MeJA application (Figure 2a,b). The lowest fresh biomass (18.63 g plant^{-1}) was observed in the Zn omission and 2 mM MeJA treatments. The lowest DM (19.69%) was obtained at 0.1 mgL^{-1} with distilled water application (Figure 2a,b). The highest aboveground fresh biomass (45.23 g plant^{-1}) and DM (38.97%) were obtained in the 0.025 and 0.05 mgL^{-1} Zn with 1 mM MeJA treatments, respectively (Figure 2a,b). Therefore, the application of 1 mM MeJA at the abovementioned concentrations of Zn increased the aboveground fresh biomass and DM by approximately 109.7% and 61.16%, respectively, compared with those of the control (Figure 2a,b).

LA

The highest LA (2710.44 cm^2 plant^{-1}) was reported for 0.1 mgL^{-1} Zn and distilled water (Figure 2c), which was approximately 50.88% greater than that of the control. The lowest LA (808.2 cm^2 plant$^{-1)}$ was observed in the 0.1 mgL^{-1} Zn and 1 mM MeJA treatments. This treatment decreased LA by approximately 55.01% in comparison with the control (Figure 2c).

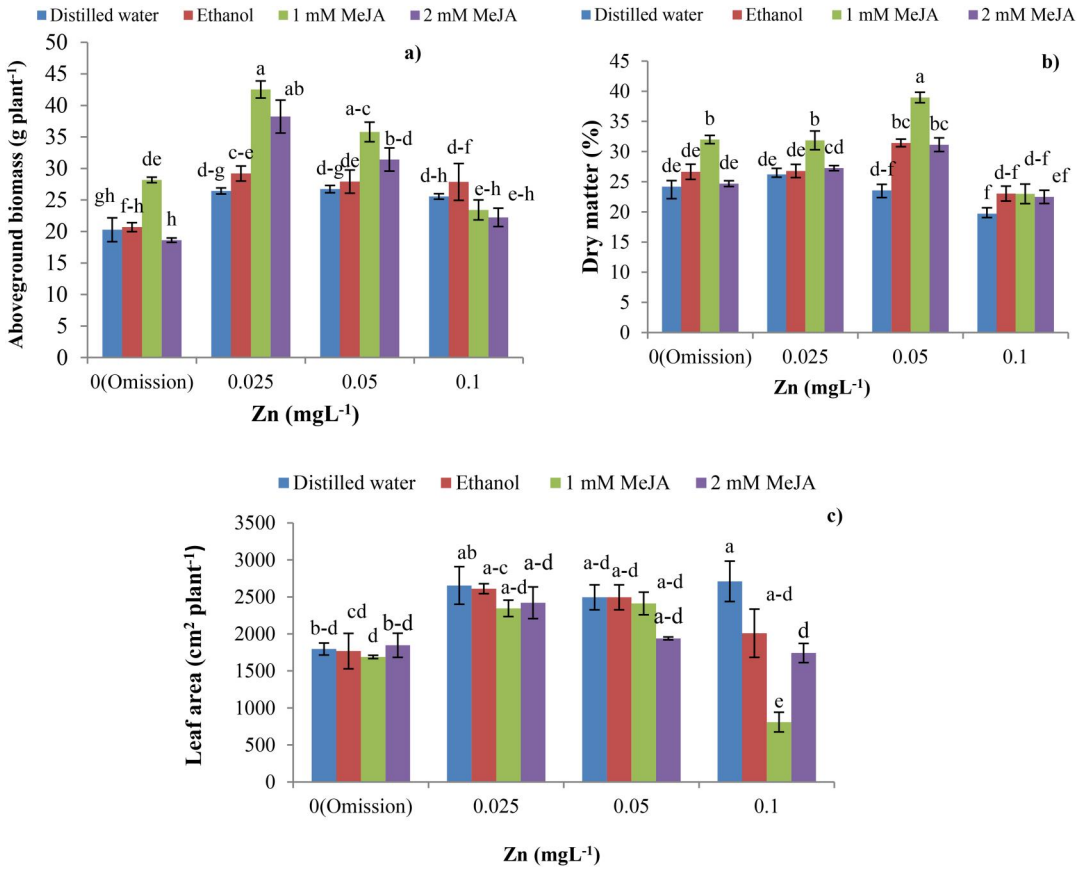

Figure 2. (a) Aboveground biomass, (b) dry matter, and (c) leaf area of peppermint at flowering stage exposed to different levels of Zn and methyl jasmonate (MeJA) application. Values are mean ± SE ($n = 4$). Different lowercase letters indicate a significant difference among the treatments at $p < 0.05$.

SPAD

Determination of the photosynthetic pigments of peppermint leaveses, SPAD index, was evaluated. With increasing Zn concentration and MeJA application, the SPAD index increased (Table 1). The highest SPAD value (60.61) was obtained for the 0.05 mgL^{-1} Zn with 2 mM MeJA application (Table 1). The simultaneous use of MeJA (2 mM) and 0.025 mgL^{-1} Zn increased the SPAD index by 43.97% in comparison with that of the control (Table 1).

Total anthocyanin content

Exogenous MeJA application had a stimulatory effect on the anthocyanin content of peppermint leaves (Table 1). The highest anthocyanin content (0.126 mg g FW^{-1}) was reported in the Zn omission and 1 mM MeJA treatments because Zn omission from the Hoagland solution was similar to the stress conditions and changed the color of the leaves. The foliar application of 1 mM MeJA in this treatment significantly increased the anthocyanin content by 215% compared with that in the control (Table 1).

Total soluble sugars

The application of different concentrations of Zn and MeJA had significant effects on the total soluble sugar accumulation in the peppermint leaves. The highest total soluble sugars

Table 1. Phenolic content and antioxidant activity of M. piperita affected by different levels of Zn and methyl jasmonate.

Zinc (mgL^{-1})	Methyl jasmonate (mM)	SPAD index	Anthocyanin (mg g^{-1} FW)	Total soluble sugar (mg g^{-1} DW)	Total phenolic content (mg GAE g^{-1} DW)	Total flavonoids (mg QE g DW^{-1})	Total flavones (mg QUE g^{-1} DW)	Total flavonol (mg QUE g^{-1} DW)	IC_{50} (mgL^{-1})
	Distilled water (Control)	$42.10 \pm 0.02^{h*}$	0.040 ± 0.000^{g}	47.59 ± 3.51^{a}	34.87 ± 2.3^{ab}	2.97 ± 0.73^{f}	1.80 ± 0.09^{ij}	1.56 ± 0.26^{g}	$\mathbf{1.29 \pm 0.05^{ab}}$
	Ethanol (as solvent)	42.10 ± 0.02^{h}	$0.095 \pm 0.004^{c-e}$	$29.19 \pm 3.03^{b-e}$	42.69 ± 2.9^{a}	4.66 ± 0.92^{e}	$1.84 \pm 0.29^{h-j}$	3.07 ± 0.55^{e}	$\mathbf{1.11 \pm 0.05^{a-c}}$
0	1	$42.63 \pm 1.29^{f-h}$	0.126 ± 0.002^{a}	$25.70 \pm 0.49^{c-f}$	46.75 ± 0.9^{a}	8.10 ± 0.14^{cd}	$2.32 \pm 0.06^{f-i}$	4.09 ± 0.72^{c}	$\mathbf{0.52 \pm 0.03^{f}}$
	2	$44.40 \pm 0.79^{d-g}$	0.081 ± 0.001^{de}	$22.94 \pm 2.86^{c-f}$	23.84 ± 2.72^{bc}	11.29 ± 0.31^{b}	$2.38 \pm 0.02^{e-h}$	3.48 ± 0.38^{de}	$\mathbf{1.05 \pm 0.02^{b-d}}$
	Distilled water	42.18 ± 0.12^{h}	0.037 ± 0.000^{g}	13.15 ± 2.82^{f}	20.29 ± 1.4^{c}	2.63 ± 0.21^{f}	1.12 ± 0.08^{k}	2.17 ± 0.29^{f}	$\mathbf{1.09 \pm 0.02^{b-d}}$
	Ethanol	42.35 ± 0.69^{gh}	0.106 ± 0.003^{bc}	$20.05 \pm 2.49^{d-f}$	24.79 ± 0.05^{bc}	3.70 ± 0.07^{ef}	3.13 ± 0.19^{d}	3.09 ± 0.93^{e}	$\mathbf{0.97 \pm 0.02^{b-e}}$
0.025	1	$44.68 \pm 1.07^{d-f}$	0.119 ± 0.001^{ab}	$20.19 \pm 2.30^{d-f}$	29.7 ± 1.61^{b}	11.28 ± 0.09^{b}	4.75 ± 0.04^{c}	3.35 ± 0.32^{de}	$\mathbf{0.86 \pm 0.03^{c-f}}$
	2	60.61 ± 0.97^{a}	0.100 ± 0.003^{bc}	$17.95 \pm 0.22^{d-f}$	14.75 ± 1.81^{de}	9.74 ± 0.07^{c}	7.80 ± 0.42^{b}	4.95 ± 0.45^{bc}	$\mathbf{1.53 \pm 0.16^{a}}$
	Distilled water	$45.15 \pm 0.83^{c-e}$	0.047 ± 0.002^{fg}	16.91 ± 2.66^{ef}	15.22 ± 0.09^{de}	3.71 ± 0.84^{ef}	1.59 ± 0.05^{jk}	2.22 ± 0.26^{f}	$\mathbf{1.45 \pm 0.02^{ab}}$
	Ethanol	47.15 ± 0.97^{bc}	0.059 ± 0.004^{f}	$19.35 \pm 1.49^{d-f}$	17.98 ± 1.79^{d}	8.29 ± 0.37^{cd}	$2.04 \pm 0.10^{g-i}$	3.71 ± 0.57^{d}	$\mathbf{0.77 \pm 0.14^{d-f}}$
0.05	1	47.69 ± 0.97^{b}	0.097 ± 0.001^{cd}	$31.67 \pm 3.18^{b-d}$	42.81 ± 2.16^{a}	10.33 ± 0.22^{bc}	2.90 ± 0.15^{e}	3.97 ± 0.84^{d}	$\mathbf{0.72 \pm 0.15^{d-f}}$
	2	47.82 ± 0.84^{b}	0.057 ± 0.005^{f}	$24.05 \pm 3.11^{c-f}$	13.86 ± 2.35^{e}	11.32 ± 0.64^{b}	10.37 ± 0.15^{a}	5.59 ± 0.89^{b}	$\mathbf{0.57 \pm 0.14^{ef}}$
	Distilled water	$43.45 \pm 0.69^{e-h}$	0.048 ± 0.004^{fg}	$20.03 \pm 2.80^{d-f}$	8.47 ± 0.09^{f}	2.89 ± 0.64^{f}	$2.53 \pm 0.03^{e-g}$	2.23 ± 0.58^{f}	$\mathbf{1.28 \pm 0.00a^{b}}$
	Ethanol	$43.35 \pm 0.70^{e-h}$	0.087 ± 0.002^{de}	$34.32 \pm 3.42^{a-c}$	21.73 ± 1.58^{bc}	6.64 ± 0.24^{de}	2.74 ± 0.04^{ef}	3.19 ± 0.7^{e}	$\mathbf{1.01 \pm 0.07^{b-d}}$
0.1	1	$43.26 \pm 0.70^{e-h}$	0.115 ± 0.005^{ab}	40.54 ± 2.13^{ab}	45.96 ± 2.62^{a}	14.54 ± 0.50^{a}	3.88 ± 0.01^{d}	8.08 ± 0.32^{a}	$\mathbf{0.92 \pm 0.09^{b-e}}$
	2	$45.99 \pm 0.97^{b-d}$	0.107 ± 0.005^{bc}	$24.35 \pm 3.02^{c-f}$	16.72 ± 0.50^{de}	13.62 ± 1.86^{a}	10.44 ± 0.03^{a}	7.89 ± 0.56^{ab}	$\mathbf{1.31 \pm 0.04^{ab}}$

*Data are mean ± standard Error (SE). Values with different letter(s) in a column are statistically significant at $p < 0.05$ according to Tukey test.

(47.59 mg g^{-1} DW) were obtained due to the stress conditions that occurred in the Zn omission treatment (Table 1). The lowest total soluble sugars (13.15 mg g^{-1} DW) were obtained in the 0.025 mgL^{-1} Zn treatment with distilled water spraying, which decreased by approximately 72.37% compared with that of the control (Table 1). The total soluble sugar content in MeJA-treated plants in the Zn omission treatment group was significantly lower than that in the control group (Table 1), whereas at other Zn concentrations, the total soluble sugar content increased in response to 1 mM MeJA, although it decreased in response to 2 mM MeJA (Table 1).

Total phenolic content

At all Zn concentrations, the total phenolic content significantly ($p \leq 0.05$) increased in the leaves of peppermint after MeJA application up to a 1 mM concentration and then decreased (Table 1). Its maximum level reached 46.75 ± 0.9 mg GAE g^{-1} DW (23.45% higher than that of the control) in the Zn omission and 1 mM MeJA treatment. However, there was no significant statistical difference between this treatment and some other treatments, as shown in Table 1. The lowest total phenolic content (8.47 ± 0.09 mg GAE g^{-1} DW) was detected at 0.1 mgL^{-1} Zn and distilled water spraying, which decreased by 75.71% compared to that of the control (Table 1).

Total flavonoid content

The highest total flavonoid content (14.54 ± 0.50 mg QE g DW^{-1}) was detected in the plants treated with 0.1 mgL^{-1} Zn and 1 mM MeJA (Table 1), in which the total flavonoid content increased by 389.56% in comparison with that in the control plants (Table 1).

Total flavone and flavonol contents

The total flavone and flavonol contents in the leaves separated after treatment with various Zn concentrations and MeJA application, calculated as the QE equivalent, varied from 1.12 to 10.44 mg QE g^{-1} DW and from 1.56 to 8.08 mg QE g^{-1} DW, respectively (Table 1). Applying 2 and 1 mM MeJA at the highest Zn concentration (0.1 mgL^{-1}) increased the total flavone and flavonol contents by 82.76% and 80.7%, respectively, compared with those of the control (Table 1).

Free radical-scavenging activity (IC_{50})

The antioxidant activity of peppermint extract is indicated as the IC_{50}. The IC_{50} is a suitable measure of oxidation development in extracts. Therefore, it is regarded as a good index for the practical evaluation of antioxidants. The mean values of the IC_{50} (mg mL^{-1}) determined by the DPPH assay of peppermint for each treatment are shown in Table 1. The IC_{50} values ranged between 0.52 ± 0.03 and 1.53 ± 0.16 mg mL^{-1} (Table 1). Significant differences between the extracts of different treatments were observed (Table 1). A lower IC_{50} indicates better radical scavenging activity. According to the results, the interaction effect of Zn and MeJA increased the inhibitory effects. Compared to the control plants, the greatest increase (59.69%) in radical scavenging activity was detected in the plants treated with 1 mM MeJA under the Zn omission condition (Table 1).

EO content and composition

The foliar application of MeJA and Zn affected the EO content and composition of peppermint in the present study (Figure 3 and Table 2). Some Zn and MeJA levels had stimulatory effects on

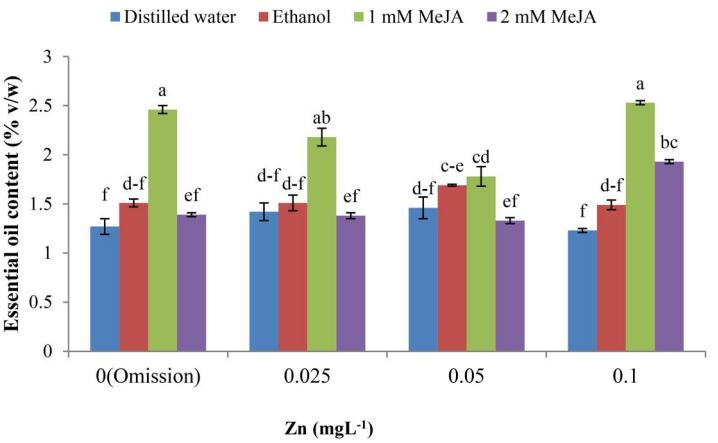

Figure 3. Essential oil content of peppermint at flowering stage exposed to different levels of zinc and methyl jasmonate (MeJA) application. Values are mean ± SE ($n = 4$). Different lowercase letters indicate a significant difference among the treatments at $p < 0.05$.

the EO content and constituents, while others had inhibitory effects (Figure 3 and Table 2). Variation in EO content was detected in peppermint after foliar application of different concentrations of MeJA and Zn levels. The interaction effect of Zn × MeJA on the EO content of peppermint was significant. Each Zn level and MeJA application resulted in different EO contents ranging from 1.23 to 2.53% v/w (Figure 3). Zn omission and the highest Zn level (0.1 mgL^{-1}) occurred under plant stress conditions, which increased the EO content, and the application of 1 mM MeJA increased the EO content, but 2 mM MeJA decreased it. The highest (2.53% v/w) and lowest (1.23% v/w) EO contents were obtained with 0.1 mgL^{-1} Zn with 1 mM MeJA and distilled water, respectively (Figure 3). At the highest Zn level (0.1 mgL^{-1}), 1 mM MeJA application increased the EO content by approximately 99.21% compared with that of the control (Figure 3).

The qualitative and quantitative data for the EO compounds obtained from the aerial parts of peppermint *via* GC and GC–MS analysis are presented in Table 2. Based on the findings of this research, the peppermint EO composition was altered by MeJA spraying at different Zn levels (Table 2). In total, 27 constituents were identified by GC and GC-MS, representing 92.14–99.98% (at 0.025 mgL^{-1} Zn and distilled water spraying and Zn omission with 1 mM MeJA application, respectively) of all the constituents in the EO (Table 2).

EO analysis at different levels of Zn and MeJA application revealed that the major components included menthol (32.38 to 50.10%), menthone (16.96 to 24.43%), menthofuran (5.02 to 11.63%), and 1,8-cineole (5.05 to 8.52%). Peppermint EO was overcome by aromatic constituents (which were symbolized exclusively by menthol). Our findings showed that peppermint EO had different responses to Zn and MeJA concentrations. Therefore, each concentration of Zn and MeJA represented a different type and amount of constituents. The findings of this study indicated that the EO of peppermint had different responses to different Zn and MeJA concentrations. The levels of menthol and menthone, the major constituents of peppermint, increased in response to the application of 1 mM MeJA at different Zn concentrations (Table 2 and Figure 4a). With increasing MeJA concentrations at different Zn concentrations, some of the major compounds, such as menthone and menthofuran, increased. The highest menthol content (50.10%) was detected in the plants treated with 1 mM MeJA and 0.025 mgL^{-1} Zn (Table 2). The highest contents of menthone (24.43%) and menthofuran (11.63%) were obtained in the 0.1 mgL^{-1} and 2 mM MeJA treatment groups, respectively (Table 2). Some major compounds, including menthol, menthone, menthofuran, and 1,8-cineole, were reported

Table 2. The chemical composition and content of the EOs from peppermint (*Mentha piperita*) under different levels of foliar application and zinc.

			Zinc (mgL^{-1})															
			0				0.025			0.05				0.1				
			Foliar spraying treatments															
No	Compound	RI*	Distilled water	Ethanol (as solvent)	1 mM Methyl jasmonate	2mM Methyl jasmonate	Distilled water	Ethanol (as solvent)	1 mM Methyl jasmonate	2mM Methyl jasmonate	Distilled water	Ethanol (as solvent)	1 mM Methyl jasmonate	2mM Methyl jasmonate	Distilled water	Ethanol (as solvent)	1 mM Methyl jasmonate	2mM Methyl jasmonate
No	Monoterpenes hydrocarbons		5.04	5.89	6.44	6.48	7.3	7.92	7.06	4.4	9.94	9.73	9.9	9.35	9.7	8.57	8.89	8.88
1	α-Pinene	936	0.58 ± 0.03**	0.62 ± 0.01	0.70 ± 0.03	1.27 ± 0.11	1.29 ± 0.14	0.92 ± 0.03	0.2 ± 0.02	0.1 ± 0.02	1.12 ± 0.06	1.16 ± 0.06	1.30 ± 0.11	1.35 ± 0.11	1.20 ± 0.11	1.37 ± 0.11	1.74 ± 0.07	1.65 ± 0.11
2	Camphene	951	0.40 ± 0.01	0.40 ± 0.02	0.42 ± 0.01	0.35 ± 0.11	0.19 ± 0.05	0.25 ± 0.1	0.30 ± 0.11	0.25 ± 0.1	0.55 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.65 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3	Sabinene	971	0.55 ± 0.03	0.60 ± 0.02	0.68 ± 0.04	0.65 ± 0.11	0.50 ± 0.03	0.62 ± 0.03	0.70 ± 0.02	0.51 ± 0.03	0.60 ± 0.01	0.57 ± 0.02	0.55 ± 0.02	0.72 ± 0.03	0.44 ± 0.02	0.55 ± 0.03	0.80 ± 0.03	0.97 ± 0.01
4	β-Pinene	978	1.11 ± 0.06	1.17 ± 0.05	1.20 ± 0.11	1.06 ± 0.05	0.60 ± 0.11	1.00 ± 0.09	1.04 ± 0.05	0.15 ± 0.06	2.41 ± 0.04	2.31 ± 0.02	1.22 ± 0.01	1.06 ± 0.06	1.32 ± 0.02	1.34 ± 0.02	1.79 ± 0.11	2.76 ± 0.02
5	β-Myrcene	990	0.25 ± 0.06	0.24 ± 0.01	0.28 ± 0.08	0.13 ± 0.01	0.09 ± 0.01	0.13 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.42 ± 0.06	0.43 ± 0.04	0.62 ± 0.07	0.55 ± 0.06	0.51 ± 0.02	0.38 ± 0.03	0.28 ± 0.01	0.24 ± 0.02
6	α-Terpinene	1016	0.23 ± 0.02	0.22 ± 0.01	0.30 ± 0.11	0.27 ± 0.11	0.16 ± 0.06	0.22 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.44 ± 0.03	0.45 ± 0.06	0.43 ± 0.01	0.45 ± 0.06	0.20 ± 0.03	0.27 ± 0.01	0.30 ± 0.03	0.24 ± 0.02
7	*p*-Cymene	1021	0.18 ± 0.04	0.24 ± 0.02	0.34 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.26 ± 0.03	0.30 ± 0.03	0.00 ± 0.00	0.45 ± 0.0.06	0.51 ± 0.02	0.25 ± 0.04	0.15 ± 0.03	0.04 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
8	Limonene	1031	2.07 ± 0.04	2.12 ± 0.06	2.30±	1.93 ± 0.03	3.25 ± 0.02	3.30 ± 0.17	3.50 ± 0.14	2.55 ± 0.02	2.64 ± 0.02	3.50 ± 0.11	3.70 ± 0.11	2.35 ± 0.06	3.55 ± 0.05	3.74 ± 0.11	3.70 ± 0.11	2.78 ± 0.06
9	(*Z*)-β-ocimene	1036	0.08 ± 0.01	0.1 ± 0.02	0.15 ± 0.03	0.31 ± 0.02	0.09 ± 0.01	0.10 ± 0.01	0.13 ± 0.00	0.12 ± 0.01	0.21 ± 0.02	0.65 ± 0.03	0.80 ± 0.03	0.90 ± 0.01	0.55 ± 0.03	0.21 ± 0.01	0.09 ± 0.01	0.08 ± 0.00
10	α-Terpinolene	1088	0.13 ± 0.02	0.14 ± 0.01	0.00 ± 0.00	0.16 ± 0.01	0.76 ± 0.01	0.87 ± 0.00	0.89 ± 0.03	0.72 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.01	0.96 ± 0.07	0.53 ± 0.06	0.19 ± 0.05	0.12 ± 0.01	0.15 ± 0.06
11	*Trans*-sabinene hydrate	1102	0.04 ± 0.00	0.04 ± 0.01	0.07 ± 0.00	0.11 ± 0.00	0.15 ± 0.06	0.25 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	1.10 ± 0.09	0.15 ± 0.06	0.23 ± 0.01	0.26 ± 0.01	0.71 ± 0.02	0.52 ± 0.02	0.07 ± 0.01	0.01 ± 0.00
	Oxygenated monoterpenes		**82.77**	**84.65**	**86.99**	**82.1**	**78.07**	**80.73**	**85.06**	**83.53**	**74.09**	**77.21**	**80.5**	**81.12**	**80.02**	**80.85**	**81.12**	**81.44**
12	1,8-Cineole	1033	6.55 ± 0.11	6.74 ± 0.02	7.45 ± 0.06	8.52 ± 0.03	5.05 ± 0.04	5.16 ± 0.07	5.60 ± 0.17	6.41 ± 0.03	6.58 ± 0.17	6.86 ± 0.03	7.17 ± 0.08	7.27 ± 0.16	6.01 ± 0.2	6.49 ± 0.06	8.44 ± 0.06	8.51 ± 0.03
13	Menthone	1153	18.27 ± 0.03	19.084 ± 0.05	19.35 ± 0.11	19.53 ± 0.04	18.28 ± 0.02	18.49 ± 0.12	18.66 ± 0.06	18.64 ± 0.05	16.96 ± 0.00	19.96 ± 0.01	20.01 ± 0.08	20.09 ± 0.03	19.07 ± 0.01	20.56 ± 0.06	21.20 ± 0.11	24.43 ± 0.06
14	Menthofuran	1166	8.09 ± 0.04	8.36 ± 1.10	8.60 ± 0.05	8.95 ± 0.06	5.02 ± 0.02	5.05 ± 0.03	5.70 ± 0.11	5.75 ± 0.11	8.14 ± 0.05	8.19 ± 0.06	9.59 ± 0.05	10.13 ± 0.06	5.12 ± 0.04	5.36 ± 0.05	7.99 ± 0.03	11.63 ± 0.05
15	Menthol	1169	46.71 ± 0.49	47.12 ± 0.04	48.10 ± 0.44	42.32 ± 0.06	44.85 ± 0.31	46.74 ± 0.49	50.10 ± 0.09	49.10 ± 0.32	37.72 ± 0.1	37.18 ± 0.1	38.30 ± 0.14	39.78 ± 0.06	45.96 ± 0.50	44.08 ± 0.31	38.80 ± 0.14	32.83 ± 0.02
16	Terpinene-4-ol	1180	1.42 ± 0.09	1.54 ± 0.06	1.62 ± 0.03	1.33 ± 0.06	2.20 ± 0.11	2.32 ± 0.15	2.46 ± 0.06	2.16±	1.60 ± 0.11	1.70 ± 0.11	1.85 ± 0.06	1.35 ± 0.05	1.65 ± 0.05	1.69 ± 0.06	1.72 ± 0.05	1.8 ± 0.06
17	α-Terpineol	1188	0.62 ± 0.04	0.64 ± 0.06	0.68 ± 0.05	0.42 ± 0.06	0.47 ± 0.05	0.57 ± 0.06	0.60 ± 0.05	0.16 ± 0.05	1.81 ± 0.02	1.18 ± 0.01	0.99 ± 0.04	0.88 ± 0.06	0.85 ± 0.05	0.83 ± 0.05	0.77 ± 0.06	0.51 ± 0.03
18	Pulegone	1235	0.68 ± 0.11	0.71 ± 0.06	0.79 ± 0.05	0.67 ± 0.05	0.50 ± 0.06	0.60 ± 0.05	0.64 ± 0.06	0.54 ± 0.06	0.34 ± 0.05	0.91 ± 0.01	1.04 ± 0.03	1.58 ± 0.01	0.91 ± 0.01	0.93 ± 0.03	0.98 ± 0.00	1.04 ± 0.03
19	Piperitone	1252	0.34 ± 0.12	0.38 ± 0.06	0.40 ± 0.05	0.31 ± 0.05	1.05 ± 0.03	1.11 ± 0.06	1.20 ± 0.11	0.71 ± 0.04	0.88 ± 0.06	0.80 ± 0.05	0.72 ± 0.04	0.69 ± 0.03	0.01 ± 0.00	0.36 ± 0.05	0.47 ± 0.06	0.43 ± 0.06
20	Piperitenone	1345	0.09 ± 0.01	0.08 ± 0.01	0.00 ± 0.00	0.05 ± 0.01	0.65 ± 0.11	0.69 ± 0.11	0.1 ± 0.03	0.06 ± 0.01	0.06 ± 0.00	0.38 ± 0.05	0.75 ± 0.06	0.44 ± 0.06	0.44 ± 0.06	0.55 ± 0.06	0.75 ± 0.06	0.26 ± 0.05
	Sesquiterpene hydrocarbons		**5.45**	**6.14**	**6.55**	**5.47**	**6.77**	**7.58**	**7.85**	**5.58**	**10.43**	**10.23**	**9.54**	**9.2**	**9.24**	**9.42**	**9.81**	**7.62**
21	Menthyl acetate	1296	1.27 ± 0.06	1.28 ± 0.11	1.31 ± 0.06	1.63 ± 0.11	1.95 ± 0.04	2.07 ± 0.04	2.10 ± 0.05	1.12 ± 0.05	3.59 ± 0.31	3.25 ± 0.12	1.16 ± 0.05	1.21 ± 0.05	2.10 ± 0.05	2.19 ± 0.1	2.35 ± 0.11	2.36 ± 0.02
22	β-Bourbonene	1390	1.24 ± 0.11	1.55 ± 0.06	1.60 ± 0.11	1.10 ± 0.05	1.14 ± 0.06	1.51 ± 0.06	1.61 ± 0.11	0.84 ± 0.05	2.13 ± 0.07	2.23 ± 0.12	2.98 ± 0.03	2.27 ± 0.06	1.48 ± 0.05	1.55 ± 0.06	1.59 ± 0.11	1.00 ± 0.06
23	β-Elemene	1394	0.24 ± 0.11	0.32 ± 0.05	0.35 ± 0.06	0.55 ± 0.05	0.61 ± 0.02	0.63 ± 0.06	0.68 ± 0.05	0.94 ± 0.02	0.64 ± 0.03	0.66 ± 0.03	0.85 ± 0.02	0.92 ± 0.01	0.94 ± 0.01	0.97 ± 0.01	0.96 ± 0.01	0.45 ± 0.06
24	*Trans*-β-Caryophyllene	1420	1.04 ± 0.03	1.10 ± 0.06	1.19 ± 0.08	0.55 ± 0.05	1.37 ± 0.11	1.45 ± 0.05	1.50 ± 0.29	1.00 ± 0.17	1.57 ± 0.05	1.75 ± 0.06	2.26 ± 0.12	2.57 ± 0.11	2.95 ± 0.16	2.55 ± 0.11	2.39 ± 0.06	1.66 ± 0.12
25	(*E*)-β-farnesene	1459	0.15 ± 0.06	0.25 ± 0.05	0.30 ± 0.06	0.18 ± 0.05	0.15 ± 0.06	0.24 ± 0.06	0.20 ± 0.05	0.12 ± 0.06	0.34 ± 0.05	0.36 ± 0.06	0.39 ± 0.06	0.27 ± 0.11	0.28 ± 0.06	0.24 ± 0.05	0.20 ± 0.03	0.15 ± 0.02
26	Germacrene D	1489	0.90 ± 0.03	1.00 ± 0.29	1.10 ± 0.04	1.05 ± 0.44	1.17 ± 0.09	1.32 ± 0.05	1.36 ± 0.06	1.21 ± 0.06	1.17 ± 0.09	1.21 ± 0.12	1.35 ± 0.06	1.29 ± 0.15	1.15 ± 0.08	1.37 ± 0.16	1.58 ± 0.06	1.33 ± 0.06
27	Bicyclogarmacrene	1502	0.61 ± 0.11	0.64 ± 0.06	0.70 ± 0.11	0.41 ± 0.02	0.38 ± 0.06	0.36 ± 0.04	0.40 ± 0.03	0.35 ± 0.02	0.99 ± 0.01	0.77 ± 0.12	0.55 ± 0.05	0.47 ± 0.06	0.34 ± 0.06	0.55 ± 0.05	0.74 ± 0.05	0.67 ± 0.06
	Total		93.26	96.68	99.98	94.05	92.14	96.23	99.97	93.51	94.46	97.12	99.86	99.96	98.96	98.84	99.82	97.94

*RI: Retention Index.

**Data are mean ± standard Error (SE).

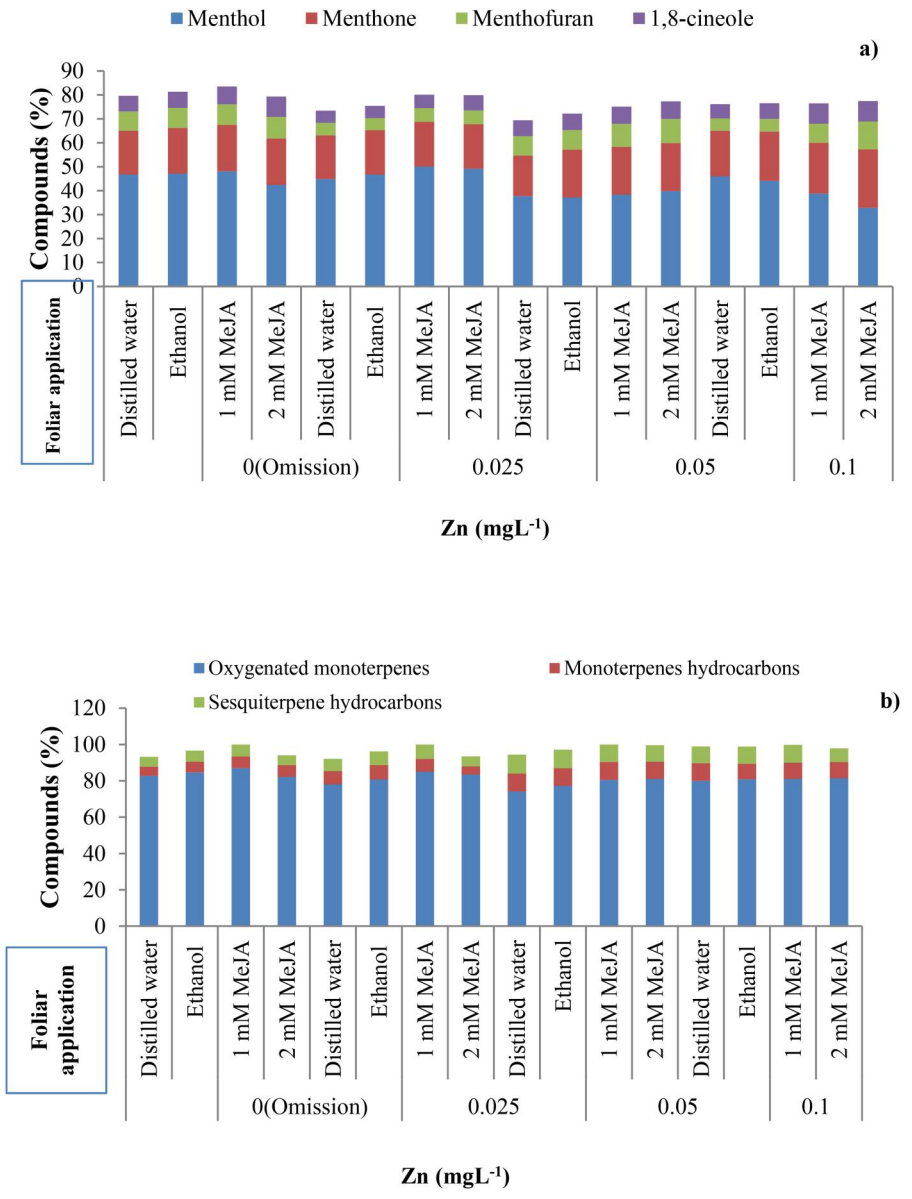

Figure 4. Percentage of (a) major compounds and (b) major component classes of constituents of peppermint at flowering stage exposed to different levels of zinc and methyl jasmonate (MeJA) application.

in all the treatments (Figure 3a). On the other hand, the minor compounds had different responses to Zn and MeJA (Table 2). Additionally, the major component classes of compounds were oxygenated monoterpenes (74.09%–86.99%), sesquiterpene hydrocarbon (5.45%–10.43%), and monoterpene hydrocarbons (4.40%–9.94%) of total compounds, respectively (Table 2 and Figure 3b). The highest percentage of oxygenated monoterpenes (86.99%) was detected in the Zn omission and 1 mM MeJA treatment groups (Table 2 and Figure 4b). Therefore, MeJA application increased the percentage of the main compounds in peppermint EO at different Zn levels (Table 2). Sesquiterpene hydrocarbons such as menthyl acetate, β-bourbonene, and *trans*-β-caryophyllene were the second most common class of EO compounds in peppermint. The contents of monoterpene hydrocarbons such as limonene and β-pinene were lower than those of the other component classes (Table 2).

Discussion

Micro- and macronutrient applications improve nutrient use efficiency, growth characteristics, biomass, and Chl content (Sidhu et al. 2019). After the application of fertilizers, a greater availability of nutrients could increase the growth parameters (Marschner 2012). The influence of Zn application on plant growth may be ascribed to its carbonic anhydrase function, in addition to the production of several auxins and dehydrogenases (Behera et al. 2021). Zn is an important factor in the metabolism of tryptophan and, as a result, affects the auxin content of plants; Zn has also been detected in the phosphoenolpyruvate carboxylase structure (Afsahi et al. 2020). In the present study, the growth and biochemical characteristics of peppermint were influenced by different concentrations of Zn and foliar applications of MeJA. Zn is involved in the photosynthetic enzymatic process and is essential for root cell membrane integrity through cell division, both of which are necessary for plant growth. The effects of different concentrations of Zn on *Ocimum basilicum* were evaluated in a previous study (Hanif et al. 2017). Furthermore, the improvement in plant growth is likely a consequence of the stimulatory effects of micronutrient fertilizer on Chl generation, the biosynthesis of hormones (JA, gibberellic acid (GA), and ethylene) and the enhancement of mitochondrial respiration and photosynthesis (Marzouk et al. 2019).

MeJA is an important phytohormone and a natural compound with a broad range of activities that is completely metabolized by plants and thus safe for the environment (Javadipour et al. 2019). MeJA is involved in different plant growth and developmental processes through long-distance signaling (Jeyasri et al. 2023). In this study, 1 mM MeJA increased the aboveground biomass and DM, but 2 mM MeJA decreased them. The results showed that the highest levels of MeJA reduced aboveground biomass and DM, which was in line with the findings of previous studies (Kandoudi et al. 2021; Hamidian et al. 2023). The mechanisms by which MeJA inhibits plant growth are not well understood. Perhaps jasmonate can prevent the cell division cycle, reducing cell number and size through the jasmonate receptor COI1 (Wasternack and Strnad 2017; Jeyasri et al. 2023). Moreover, since MeJA is an important factor in the control of plant senescence, a high concentration of MeJA reduces plant growth by triggering plant senescence (Miladinova-Georgieva et al. 2022). Therefore, because MeJA application improves plant defense systems and high MeJA concentrations cause a decrease in plant biomass and an increase in LA, this adverse effect should be carefully considered and estimated (Hamidian et al. 2023).

On the other hand, the availability of macro- and micronutrients, particularly N, Mn, and Zn, can significantly influence the Chl content of plants (White and Brown 2010). Zn is essential for chl and carbohydrate synthesis and formation (Behera et al. 2021). In the present study, the greater SPAD index (Chl content) in the 0.025 mgL^{-1} Zn treatment group than in the Zn omission treatment group could be attributed to nutrient enhancement, which improved the photosynthetic rate, RUBISCO activity, and growth parameters of the plants (Marschner 2012; Abbasifar, Shahrabadi, and ValizadehKaji 2020). In addition, the synthesis of growth substances, protein, and Chl increased in response to Zn fertilizer application (Babaei et al. 2017). Therefore, the lower SPAD index in the Zn-omitted or lower Zn treatments can be attributed to the role of Zn in protein synthesis. Moreover, ROS are expected to accumulate in Zn-stressed plants, leading to membrane lipid peroxidation and thus Chl co-oxidation (Castillo-Gonzalez et al. 2019).

In the case of the effect of MeJA on photosynthetic pigment value, different results, such as a reduction or increase in Chl concentration, have been reported for different plant species. In line with the results of this study, MeJA application increased the SPAD index in *Hesperantha coccinea* (Salachna et al. 2020). Furthermore, in some cases, low concentrations of jasmonates repaired photosynthetic pigments (Sarabi and Arjmand-Ghajur 2021). The results of this study showed that foliar application of MeJA increased the SPAD index. MeJA may inhibit Chl degradation in leaves, reducing the expression of chlorophyllase and delaying their aging. These results showed that MeJA could induce photosynthetic pigment synthesis (Salachna et al. 2020). In addition, MeJA influences the electron transport of photosystem II and is involved in the expression of a

series of key enzymes involved in Chl biosynthesis through aminolevulinic acid (ALA) formation (Salachna et al. 2020). In this study, MeJA application caused senescence-like symptoms, as shown by a great decrease in photosynthesis and Chl content as well as an increase in anthocyanins, which is in agreement with the findings of a previous study in *Arabidopsis thaliana* (Sarabi and Arjmand-Ghajur 2021).

From another point of view, total soluble sugars also act as a carbon source and are important for osmotic regulation. A higher content of soluble sugars in plant tissue fluid can reflect the adaptability of plants to stress and stronger resistance. Total soluble sugars are important osmoregulatory substances that protect proteins and enzymes by regulating osmotic pressure in the cytoplasm (Fan et al. 2021). The total soluble sugars were greatest in the leaves of the plants in the control group. This is probably because photosynthesis was the highest in this treatment, which led to the extreme consumption of soluble sugars as a carbon source. Its utilization was greater than its production for osmoregulation (Marschner 2012; Castillo-Gonzalez et al. 2019; Fan et al. 2021). The stimulatory effect of Zn on carbohydrate content may be ascribed to its role in the activation of the enzymes responsible for photosynthesis, biosynthesis and transformation of carbohydrates, regulation of sugars, and starch formation. Moreover, Zn is involved in RNA metabolism and ribosomal content in plant cells, stimulating protein, carbohydrate, and DNA formation (Marschner 2012; Afsahi et al. 2020).

As shown in the results, the total soluble sugars in the leaves increased under Zn stress conditions, but the SPAD index in the leaves decreased under Zn stress and increased in response to MeJA application compared with that in the control plants. Therefore, a significant increase in total soluble sugars is related to a reduction in the SPAD index. Moreover, MeJA application in plants stimulated the expression of molecules and genes encoding defense proteins, genes involved in cell wall formation, and genes involved in stress protection. The results of the present research indicated that the total soluble sugar content in MeJA-treated peppermint in the Zn omission treatment group decreased compared with that in the control group, but it increased at other Zn concentrations. In addition, MeJA secretes osmolytes such as total soluble sugars in response to Zn stress conditions, which act synergistically with plant-produced osmolytes and stimulate plant growth (Moreira, Zas, and Sampedro 2012).

Phenolic compounds (PCs), including phenols and flavonoids, are influential plant secondary metabolites with hydroxyl groups that act as antioxidants. The synthesis of these compounds is accomplished through indirect mechanisms that involve their interactions with the antioxidative defense system versus the ROS and their behaviors as antioxidants and chelators of redox-active metals (Tungmunnithum et al. 2018). The antioxidant and/or pro-oxidant activity of PCs depends on different factors, including solubility, chelating behavior, metal-reducing potential, and pH (Tungmunnithum et al. 2018). However, there is still uncertainty regarding the functions of PCs in response to Zn concentrations. Zn treatment of the plants had specific effects on the PC content. A supra-optimal Zn concentration causes oxidative stress, so PC synthesis occurs through indirect mechanisms that influence PC interactions with the antioxidative defense system against ROS (Alara, Abdurahman, and Ukaegbu 2021; Fan et al. 2021). PCs such as anthocyanins have a high propensity to chelate heavy metals. Therefore, they prevent ROS generation and ROS reduction once they are formed (He et al. 2010). Antioxidant activity is another important characteristic for reducing oxidative stress in plants. DPPH is a common method used to estimate the antioxidant capacity of plants or free radical-scavenging potential of PCs (Tungmunnithum et al. 2018; Babenko et al. 2019; Zaid, Mohammad, and Fariduddin 2020). In a previous study, the external application of PGRs and mineral nutrients had a synergistic effect on mitigating the negative effect of heavy metal stress on mint plants (Zaid, Mohammad, and Fariduddin 2020). Moreover, in line with a previous report in spearmint (Rita et al. 2016), the antioxidant activity of peppermint leaves in this study was reported to be due to the chemical structure of PCs, which had synergistic or antagonistic effects on the compounds in the extract (Rita et al. 2016). In this

study, peppermint extract had high PCs and FCs, and with increasing PCs, the antioxidant activity of the leaves significantly increased. In agreement with the results of this study, a previous report indicated a close relationship between the antioxidant activity of medicinal plants and the PC content of plants from different natural sources (Gülçin 2020).

Jasmonate application has long been known for increasing secondary metabolites such as PCs in aromatic and medicinal plants (Jeyasri et al. 2023). The modulation of phytohormone levels could balance oxidative stress conditions by affecting plant metabolism by controlling vital plant physiological processes such as antioxidant defense system stimulation (Zaid, Mushtaq, and Wani 2021).

According to the findings of this research, the contents of PCs and flavonoid compounds in *Thevetia peruviana* (Mendoza et al. 2018) and of anthocyanins in *M. piperita* (Khalvandi et al. 2019) increased in response to MeJA application. PC accumulation in MeJA-treated plants is likely due to an increase in the activity of the phenylpropanoid pathway (Tassoni, Durante, and Ferri 2012). MeJA, as an external stimulus, activates signal transduction pathways, so oxidative stress is decreased by increasing antioxidant capacity (Khalvandi et al. 2019).

In addition, the most important factor in EO biosynthesis and quality is nutrient availability. In addition, EO content and composition depend on the type of nutrients that are available for plants (Khalid 2015). Consistent with the findings of this study, the application of different fertilizers and micronutrients caused a pronounced increase in the EO content of *Apium graveolens* fruit (Khalid and Hussein 2012) in previous research. In addition, micronutrient treatments resulted in higher concentrations of the main components of *Coriandrum sativum* (Khalid 2015). The results of the present study are in good agreement with those of a previous study that reported a significant increase in the EO content of *Ocimum basilicum* (Hanif et al. 2017) in response to different concentrations of Zn fertilizer. The increase in the EO content in the plants treated with Zn could be related to its important role in the development and division of new EO-containing cells, secretion tubes, EO channels, and tuber hairs (Butnariu and Sarac 2018). On the other hand, our findings also showed that the EO content is not directly associated with growth parameters because the highest aboveground fresh biomass and DM were obtained at 0.025 and 0.05 mgL^{-1} Zn with 1 mM MeJA, respectively, while the highest EO content and the lowest LA were obtained at 0.1 mgL^{-1} Zn with 1 mM MeJA treatment. A lower LA consequently has a greater density of EO-secreting glands, which causes the EO content to be more concentrated (Lange et al. 2011). A similar effect of Zn supply on growth parameters and EO content was reported for *Salvia farinacea* and *Ocimum basilicum* (Hanif et al. 2017).

The EO content and composition can be affected by PGRs by modifying terpenoid biosynthesis pathways, the LA index, the number of EO storage structures, and some enzymatic processes (Jahani et al. 2021). In previous reports, foliar JA application increased the EO production and quality of *Satureja hortensis* (Ghasemi Pirbalouti et al. 2014), *Thymus vulgaris* and *T. daenensis* (Alavi-Samani, Kachouei, and Pirbalouti 2015), *Ocimum basilicum* (Złotek, Michalak-Majewska, and Szymanowska 2016), and *Melissa officinalis* (Ghasemi Pirbalouti et al. 2019). Similarly, in previous reports, foliar application of MeJA increased the EO content in peppermint (Khanam and Mohammad 2017). In addition, peppermint exposed to MeJA improved the concentrations of different EO components, such as menthol (Khanam and Mohammad 2017; Cappellari et al. 2019; 2020), menthone, menthofuran, limonene, 1,8-cineole, and pulegone (Cappellari et al. 2019; Cappellari et al. 2020), which partially conforms with the findings of this study.

Menthol is a famous cooling odor and taste. Peppermint EO with a high menthol concentration is valuable for commercial applications, different industries, and international business (Zhao et al. 2022). Menthone is a monoterpene ketone that is important for aromatic and minty odors in the cosmetics and perfumery industries (Kamatou et al. 2013). Pulegone is utilized by different industries as a base molecule for the synthesis of menthol and is used in the cosmetic and

fragrance industries (Malekmohammad et al. 2021; Zhao et al. 2022). Pulegone is biosynthetically regarded as the central intermediate depending on environmental and developmental conditions modified to menthol through menthone, or it may be directly converted to menthofuran (Malekmohammad et al. 2021). Thus, decreasing or increasing the pulegone, menthone, and menthofuran concentrations may be related to these modifications (Zhao et al. 2022). The EO content and monoterpenes in aromatic and medicinal plants are enhanced with JA treatment due to the monoterpenes accumulating in glandular trichomes (Złotek et al. 2016). The accumulation of EO constituents was synergistic with the increase in glandular trichome density, and this synergistic effect occurred *via* the expression of genes linked to the biosynthesis of these genes in response to MeJA application (Cappellari et al. 2019; 2020).

Conclusion

Zinc (Zn) omission and/or excess Zn concentration had individual influences on plant growth, phytochemical characteristics, and the antioxidant activity of peppermint because they have shown some stress effects on plants. The findings of the present study indicated that suitable concentrations of Zn and MeJA can enhance the growth, photosynthetic pigments, biosynthesis of some biochemical compounds, and quality and quantity of essential oils (EOs) in peppermint. To obtain high growth indexes with high-quality, environmentally friendly and human-safe plant production, nutrient management and some elicitors are useful. Field studies are required to further confirm the effectiveness of different Zn and MeJA concentrations in obtaining high-quality crops.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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