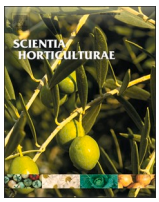
Scientia Horticulturae 329 (2024) 112980

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Phenolic compounds, enzymatic and non-enzymatic antioxidant activities of *Mentha piperita* modified by Zinc and methyl jasmonate concentrations

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ARTICLE INFO

Keywords:
Antioxidant activity
Malondialdehyde
Micronutrient
Peppermint
Phenolic profile
Phytohormone

ABSTRACT

This study aimed to assess the physiological characteristics, phenolic compounds (PCs), antioxidant enzymes, and antioxidant activity of *M. piperita* under different Zn concentrations (0, 0.025, 0.05, and 0.1 mgL^{-1}) and foliar spraying of methyl jasmonate (distilled water, distilled water + 1% ethanol and 1 and 2 mM of methyl jasmonate) under soilless culture system. According to the findings of this study, Zn omission and the highest Zn level (0.1mgL^{-1}) showed stress conditions on the plants and their effects on the studied traits was different from the other Zn concentrations (0.025 and 0.05mgL^{-1}). In addition, different Zn and MeJA concentrations had special effects on the treated plants. As 1 mM MeJA application significantly enhanced antioxidant activity and the accumulation of PCs of *M. piperita* in different Zn levels. Rosmarinic acid, as the main PCs, showed a higher content (34.34 mg g DW^{-1}) in 1 mM MeJA and 0.05 mgL^{-1} Zn application. In addition, a specific concentration of Zn could promote physiological characteristics such as antioxidant enzyme activities. These findings indicated that different Zn concentrations and exogenous MeJA might encourage the accumulation of PCs and enhanced antioxidant activity of peppermint. It also found that antioxidant enzyme activities modified with Zn and MeJA levels to decrease the injury produced by Zn stress.

1. Introduction

In a soilless agricultural system, the plants grow in nutrient solutions without soil. This technology is extensively used to study the nutrient effect on plant physiology. Zinc (Zn) is an essential micronutrient that plays multiple roles in cell division, photosynthesis, growth, supporting the integrity of cell membranes and function, protein synthesis, carbohydrates, auxin, and many biochemical pathways. In addition, for average plant growth, Zn acts as a functional structure in the metabolic processes of the plants and can modify the phenolic compounds (PCs) metabolism. In addition, it is a regulatory cofactor in various classes of enzymes, such as antioxidant enzymes (Białońska et al., 2007; Hafeez et al., 2013; Rezaeieh et al., 2016). To prevent the deleterious effects of oxidative stress, two antioxidant systems include in the plant stress responses comprising enzymatic (e.g., superoxide dismutase (SOD), peroxidase (POX), and non-enzymatic (e.g., PCs) (Del Rio, 2015). Zn displayed in the several antioxidant enzyme activities involved in the superoxide anion radical (O^{2-}), singlet oxygen (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (*OH) scavenging (Hafeez et al., 2013). Reactive oxygen species (ROS) are naturally produced by plant cell metabolism through respiration. They are toxic to cells, so non-enzymatic and enzymatic systems have evolved to neutralize and prevent the oxidation of cellular components. Moreover, in the roots of Zn-deficient growth and development of plants, plant leakage of solutes is increased by interfering with specific critical metabolic processes resulting from excessive production of reactive ROS (Del Rio, 2015).

Abbreviations: ANOVA, Analysis of variance; CA, Caffeic acid, DPPH, Diphenyl-1-picrylhydrazyl; EC, Electric conductivity; EDTA, Ethylene diamine tetra acetic acid; FRAP, Ferric reducing antioxidant power; GA, Gallic aid; GAE, Gallic acid equivalent; H_2O_2, Hydrogen peroxide; HPLC, High-performance liquid chromatography; JA, Jasmonic acid; MDA, Malondialdehyde; MeJA, Methyl jasmonate; MSI, Membrane stability index; NBT, Nitro blue tetrazolium; PGRs, Plant growth regulators; PCs, Phenolic compounds; POX, Peroxidase; PPO, Polyphenol oxidase; PVP, polyvinyl polypirrolidone; QE, Quercetin equivalent; RA, Rosmarinic acid; ROS, Reactive oxygen species; RWC, Relative water content; SE, Standard error; TBA, Thiobarbituric acid; TW, Turgor weigh; TPTZ, 2,4,6-tripyridyl-S-triazine; Zn, Zinc.

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https://doi.org/10.1016/j.scienta.2024.112980
Received 15 June 2023; Received in revised form 1 February 2024; Accepted 5 February 2024
Available online 23 February 2024

Plants have developed different mechanisms to decrease injury to O_2^- cellular components, including membrane lipids and proteins by the free radical oxygen molecules (Afkar et al., 2013). Furthermore, oxidative stress induction in plants due to Zn deficiency has been reported previously (Gupta et al., 2011).

Plant growth regulators (PGRs) are synthetic chemicals that act similarly to plant hormones and play an individual role as chemical signals in growth regulation, plant development, and metabolism causing physiological responses in the plants. one new method to confirm plant yield and quality is the PGRs application (Anjum et al., 2011b). Jasmonates, as a family of phytohormones, are important PGRs. Methyl jasmonate (MeJA) is a volatile ester form of Jasmonic acid (JA) that is widely distributed in several plants to improve defense and regulate growth as well as play a vital role in different physiological procedures in plants, comprising seed germination, flowering, fruit ripening, nutrient storage, and senescence (Wasternack and Strnad, 2017). Furthermore, elicitors like MeJA act as biotic and abiotic stresses and can stimulate secondary metabolite synthesis in plants. MeJA was applied to increase PCs content in medicinal plants (Xing et al., 2018; Fatemi et al., 2019). An important class of secondary metabolites is PCs regarded as utilizing different medicinal and pharmacological properties. Several studies have indicated that MeJA as a signaling molecule and second messenger in secondary metabolite production by stimulating the specific gene expression plays a vital role in plants (Xing et al., 2018; Fatemi et al., 2019).

The *Mentha* genus (Lamiaceae family) consists of about 25 species of perennial herbaceous plants which is one of the most widely cultivated aromatic plants in the world (Tuckerand Naczi, 2007). Aerial parts of different *Mentha* species have been significantly used as traditional medicine for colds, sinusitis, and bronchitis treatment. Moreover, they have carminative, expectorant, diuretic, antitussive, and antioxidant properties (Ahmed, 2016, 2023). *Mentha piperita* L. (peppermint) is one of the most critical plants from this genus with anti-inflammatory, antimicrobial, and antioxidant activity (Afkar et al., 2013). All these properties have related to the polyphenol derivatives combination. Plants from the Lamiaceae family are rich in polyphenolic compounds and famous for their antioxidant properties (Brahmi et al., 2015). *Mentha* species are a source of PCs. Based on the results of previous studies, spearmint leaves are a rich source of rosmarinic acid (RA), caffeic acids (CA), and chlorogenic acids (Kivilompolo and Hyötyläinen, 2007), also peppermint leaves comprised high levels of RA and CA (Dorman et al., 2009; Farnad et al., 2014). *Mentha* species are potent sources of polyphenols and are mentioned as suitable primary natural antioxidants and free radical scavengers that probably react with free radicals and ROS attacks on food and biological systems (Rita et al., 2016).

Despite global Zn deficiency problems in different agroclimatic regions, the information on the associated role of enzymatic and non-enzymatic antioxidants in the defense mechanisms against oxidative injuries in Zn-omitted or extra plants is deficient. In addition, MeJA is a chemical signal that affects plant metabolism causing physiological reactions in the plants. Therefore, due to the crucial effects of Zn omission, concentration, and toxicity on the antioxidative defense system, and the importance of PCs in plant antioxidant activity, the Zn tolerance capacity of *M. piperita*, and the interaction effect of different Zn levels and MeJA application on physiological characteristics of *M. piperita* examined. However, no such experiment has been directed toward the antioxidant activity and phenolic content and PCs of the peppermint leaves under different Zn and MeJA concentrations. Hence, this study aimed to assess the PCs, antioxidant enzymes, and antioxidant activity of *M. piperita* under different Zn and MeJA levels.

2. Materials and methods

2.1. Plant material and experimental design

The greenhouse soilless factorial experiment was performed based on the randomized complete block design (RCBD) with four replications in the controlled conditions, including temperature 25/18 ± 2 °C (day and night respectively) and relative humidity 60 ± 2% in the research greenhouse at the Ferdowsi University of Mashhad, Iran in 2021.

2.2. Growth conditions

In this experiment, sand was used as growth media. The plastic pots (40 × 30 cm) were filled with sand (0.05–0.1 mm) that was washed several times (Pourranjbari Saghaiesh et al., 2019; Safari et al., 2019). Peppermint plants were grown from rhizomes (obtained from the research field of the Ferdowsi University of Mashhad). In each pot, five similar rhizomes of 5–6 cm in length were cultivated. The pots were put under controlled conditions during the growth period in the greenhouse and irrigated with deionized water to adjust; the rhizomes were established entirely and enabled the improvement from transplant stress. At the 4–6 leaves stage, three more vigorous seedlings were kept, and two others were immediately removed. After that, the Hoagland formula was used to prepare the nutrient solution with distilled water. The Zn concentrations varied as Zn omission (0), 0.025, 0.05, and 0.1 mgL^{-1}, while all other nutrients were kept constant. Then the stock solutions were ready and diluted up to the suitable concentrations based on Hoagland and Arnon's (1950) method. Then, the plants were fed with the Hoagland solution and different Zn concentrations three times a week. The solution was modified every week to resupply nutrients that might have run out. The original EC and pH of the target nutrient solution were kept between 1–1.3 dSm^{-1} and 5.8–6.5, respectively. The pH of the solution was regulated as required (due to the alkalinity of water) by using H_2SO_4 (5% vv^{-1}) every second day. All chemical reagents applied in this experiment were bought from Merck (Germany) (Maggini et al., 2012; Abbasi Khammar et al., 2021).

On the other hand, to prepare the MeJA solution for foliar application, MeJA (from Sigma Aldrich Company) was dissolved in 1% ethanol and ready with several desired concentrations. Experimental MeJA treatments included: I) Distilled water foliar application (control), II) Distilled water + 1% ethanol foliar application (as solvent), III) MeJA at 1 mM, and IV) MeJA at 2 mM. The distilled water, ethanol 1% as a solvent, and prepared concentrations were sprayed at a dew point. Moreover, the MeJA solutions were applied every ten days until one week before harvesting. The treatment with no Zn and distilled water foliar application was considered as a control. Finally, after one week from foliar spraying, at the flowering stage, the plants were harvested, and their characteristics measurements were done.

2.3. Leaf fresh and dry weight

The fresh and dry weight of the leaves was evaluated at the flowering stage by selecting three plants from each pot. The leaves were weighed on a digital scale.

2.4. High-performance liquid chromatography (HPLC) analysis

To estimate seven PCs of treated *M. piperita* an analytical HPLC system (Shimadzu Nexera X2 LC-30AD, Japan) was applied (Gharibi et al., 2019). To prepare the extracts of the samples, 2.5 g of dried ground leaves was extracted in methanol (HPLC grade,Merck) used as solvent that was later removed from the combined extracts by evaporation under pressure to dryness. The extract was filtered through a 0.22 μm nylon acro disk filter (Hodaei et al., 2021). All used standards purchased from Sigma-Aldrich with high purities including rosmarinic acid, gallic acid, caffeic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid,

and tannic acid, dissolved in HPLC-grade methanol before the injection. After that, the filtered methanolic extracts (20 µl from each one) were used for analysis. The stationary phase dominated a 150 mm × 4.6 mm (5 µm) Nucleodur C18 Gravity-SB column (Macherey–Nagel (Germany)), and the mobile one included 0.1% formic acid in acetonitrile (flow rate of 0.8 mL min^{-1}) with the wavelength between 200 and 400 nm with 25 °C temperature. Firstly, as solvent A water-formic acid (0.1%) was used, whereas 0.1% of B for 70 min, and finally 100% of solvent B for 75 min was applied. To evaluate PCs, the peak areas were compared with the retention times. Finally, the results were published as mg g DW^{-1}.

2.5. Methanolic extract preparation

To prepare the extract for determining antioxidant activity of peppermint leaves in each treatment, 0.5 g of the finely ground dried leaves were extracted with 5 mL methanol (99% vv^{-1}) and they put in the shaker for 48 h Then the mixture was centrifuged for 20 min at 4500 rpm at room temperature. Finally, the prepared extracts were stored at 4 °C in a refrigerator before further analysis.

2.6. Antioxidant activity

Antioxidant activity of the extracts was reported by the ability to scavenge Diphenyl-1-picrylhydrazyl (DPPH) radical, Ferric reducing antioxidant power (FRAP), and phosphomolybdenum complex methods.

2.6.1. DPPH assay

The free radical-scavenging activity of the extracts was evaluated with the modified DPPH assay (Wang et al., 2008). For each sample, different concentrations ranging from 500 to 8000 μgmL^{-1} were prepared with methanol (99%). The reaction mixtures (in the 96-well plates) consisted of a 100 µL sample and 100 µL of 0.2 mM DPPH radical, dissolved in methanol. The mixture was then shaken vigorously and then kept in the dark at room temperature for 15 min. Then, the absorbance of the resulting solutions was measured by using a Bio Quest C2502 Spectrophotometer at 517 nm, against a blank. All determinations are performed in triplicates. GA is used as a standard indicator of antioxidant activity. The antioxidant activity of the extracts was evaluated by using 1 mL DPPH (500 mM), which was added to the 1:10 diluted methanolic extracts. Then the mixture shakes vigorously and is kept for 30 min at room temperature in the dark. Finally, the absorbance was reported at 517 nm. The inhibition percentage of free radical DPPH (IP%) is calculated by using the following formula:

$$IP\% = [(Ablank - -Asample) / Ablank] \times 100$$

Where Ablank is the absorbance of the control reaction; A sample is the absorbance in the presence of plant extract.

2.6.2. FRAP assay

The FRAP reagent contained 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mM HCl (2.5 mL), 20 mM $FeCl_3$ (2.5 mL), and 300 mM acetate buffer (25 mL, pH = 3.6). Standard solutions or samples were mixed with FRAP reagent (2850 µL) and for 30 min kept in the dark. The absorbance of the complex was measured at 593 nm. Results report as µmol ASC g^{-1} DW (Thaipong et al., 2006).

2.6.3. Phosphomolybdenum complex method

The antioxidant activity of the extracts was evaluated based on the phosphomolybdenum complex assay. The reaction mixture included a sample solution (0.1 mL), a reagent solution comprised of sulfuric acid (0.6 mM), sodium phosphate (28 mM), and ammonium molybdate (4 mM). After that, put them in hot water (95 °C) for 90 min (Prietoet al., 1999). Then cool them at room temperature and was reported the absorbance at 695 nm. The antioxidant activity was reported as mg ASC g^{-1} DW.

2.7. Determination of antioxidant enzymes activity

2.7.1. Preparation of enzyme extraction

Enzymatic extraction was prepared by extracting fresh leaf samples (0.5 g) in 50 mM potassium phosphate buffer (5 mL, pH = 7.5), including 1 mM ethylene diamine tetra acetic acid (EDTA) and 1% (wv^{-1}) polyvinyl polypirrolidone (PVP). The homogenate was centrifuged at 12,000 rpm, for 20 min at 4 °C, and the supernatant fraction was used as the extract for protein and enzyme determination.

2.7.2. Total soluble protein extraction and assay

The amount of total soluble protein content in the extract was evaluated by Bradford's (1976) method, and absorbance was reported at 560 nm. Bovine serum albumin (BSA) was applied as the standard.

2.7.3. Peroxidase (POX) activity

The activity of POX was evaluated by guaiacol and H_2O_2 substrates as described by Chance and Maehly (1955). The reaction mixture was composed of 25 mM potassium phosphate buffer (pH = 6.8) (2.7 mL), 20 mM guaiacol (0.1 mL), and 40 mM H_2O_2 (0.1 mL). The reaction was begun by adding H_2O_2 and enzyme extract (0.1 mL). The POX activity was reported as mmol-produced tetra guaiacol per min per mg soluble protein (Unit mg^{-1} protein) using the tetra guaiacol extinction coefficient of 26.6 mM^{-1} cm^{-1}. The oxidation of guaiacol to tetra guaiacol was determined based on the increase in absorbance at 470 nm.

2.7.4. Polyphenol oxidase (PPO) activity

The assay mixture was composed of 0.2 M potassium phosphate buffer (2.5 mL) (pH = 6.8), 20 mM pyrogallol (0.2 mL), and 200 µL of enzyme extract. The absorbance modification at 420 nm was described for 4 min (Raymond et al., 1993). The PPO activity was quantified as pyrogallol oxidized after 4 min per mg protein [Unit mg^{-1} (protein)].

2.8. Determination of H_2O_2

Hydrogen peroxide levels were determined according to the method of Velikova et al. (2000). Leaf tissues (0.5 g) were homogenized with 5 mL 0.1% (wv^{-1}) TCA. The homogenate was centrifuged at 12,000 g for 15 min. Subsequently, 0.5 mL of the supernatant was added to 0.5 mL 10 mM potassium phosphate buffer (pH = 7.0) and 1.0 mL 1.0 M KI. The absorbance of the solution was recorded at 390 nm. The content of H_2O_2 is described using a standard curve.

2.9. Lipid peroxidation (malondialdehyde (MDA)) content

Lipid peroxidation of the leaves was evaluated by measuring the malondialdehyde (MDA) content, as reported by Heath and Packer (1968), using thiobarbituric acid (TBA). Leaves (0.3 g) homogenized in 5 mL 0.1% (wv^{-1}) TCA solution. After centrifuging at 10,000 g for 15 min, 1 mL of the supernatant was added to 4 mL 0.5% (wv^{-1}) TBA in 20% TCA. This mixture was incubated for 30 min at 95 °C, then cooled to room temperature and centrifuged at 10,000 g for 10 min. The supernatant was exposed to analysis through a spectrophotometer (Jasco 7800 Japan), and the absorbance was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted from absorbance at 532 nm. The MDA–TBA complex (red pigment) concentration was recorded using the extinction coefficient 155 mM^{-1} cm^{-1}.

2.10. Membrane stability (MSI)

MSI concluded by reporting the electric conductivity (EC) of leaves at 40 and 100 °C in double-distilled water (Sairam, 1994). 0.1 g of leaf samples were cut into discs of uniform size and put in test tubes, including double-distilled water (10 mL). One set was put at 40 °C for 30

min, and the other was kept in a boiling water bath (100 °C) for 10 min. Their respective EC_1 and EC_2 were measured by a conductivity meter (AD330, Adwa, Szeged, Hungary). The MSI reported by using the following formula:

$$MSI = [1 - (EC1 / EC2)] \times 100$$

2.11. Determination of relative water content (RWC)

To estimate RWC, one healthy leaf from each treatment was selected and cut. Firstly, their fresh weight (FW) was estimated; then, they were kept in distilled water at room temperature for 24 h Afterward, their turgor weight (TW) was measured. Finally, the samples were dried at 72 °C for 48 h in the oven (Binder, Germany), and their dry weight (DW) was evaluated (Sánchez et al., 1998). The following formula is used for calculating RWC:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

2.12. Statistical analysis

All analytical data indicated as mean values ± Standard Error (SE). Statistical analysis was done with Minitab 17 software. The interaction effects of Zn and MeJA application were analyzed by analysis of variance (ANOVA), with a probability defined at $p \leq 0.05$. Tukey, as a post hoc test, was used to compare the means. Microsoft Excel software is utilized for drawing figures.

3. Results

3.1. Leaf fresh and dry weight

Based on the findings of this study showed that the fresh and dry weight of peppermint leaves was influenced by Zn concentrations and MeJA application (Fig. 1a, b). Foliar spraying by 1 mM MeJA at 0.025 mgL^{-1} Zn treatment increased fresh and dry weight about 2.21 and 4.63 folds, respectively, compared to the control plants (Fig. 1a, b), whereas application of 2 mM MeJA decreased them.

3.2. HPLC assay

The stimulatory effect of elicitation with MeJA on the production of seven PCs including RA, CA, ferulic acid, tannic acid, GA, chlorogenic acid, and *p*-coumaric acid in the leaves of *M. piperita* were examined by the HPLC method (Table 1). The results showed that the content of RA

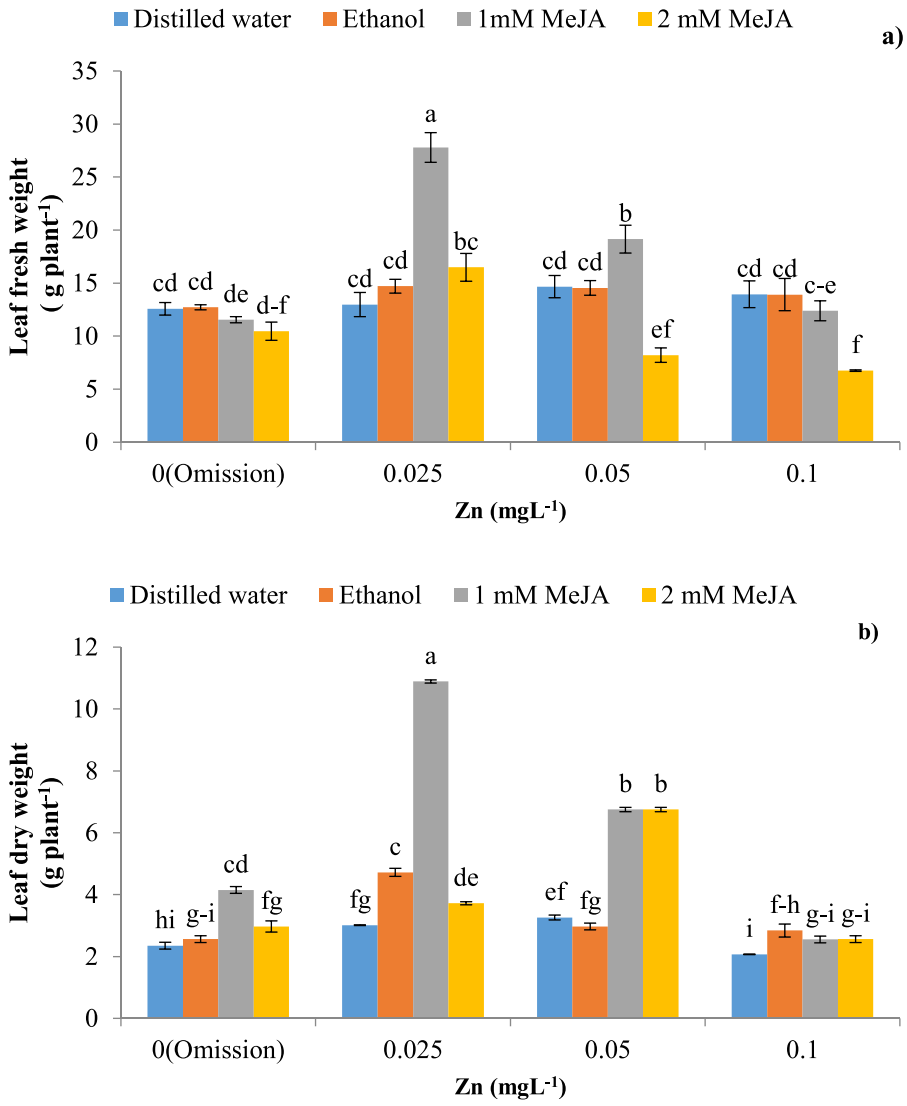

Fig. 1. a) Fresh and b) dry weight of peppermint leaves at flowering stage exposed to different levels of Zn and methyl jasmonate application. Values are mean ± SE ($n = 3$). Different lowercase letters indicate a significant difference among the treatments at $p < 0.05$.

Table 1
Chromatographic profile of phenolic compounds identified in extract prepared from peppermint (*Mentha piperita*) leaves treated with different Zn and MeJA levels.

Zn (mgL^{-1})	MeJA (mM)	Rosmarinic acid (mg gDW^{-1})	Caffei acid (mg gDW^{-1})	Gallic acid (mg gDW^{-1})	Ferulic acid (mg gDW^{-1})	Chlorogenic acid (mg gDW^{-1})	*p*-Coumaric acid (mg gDW^{-1})	Tannic acid (mg gDW^{-1})
	Distilled water (Control)	1.20 ± 0.05°	1.40 ± 0.05^{j}	5.60 ± 0.06^{i}	1.80 ± 0.06^{e-g}	3.80 ± 0.06^{i}	0.87 ± 0.02 g^{h}	4.52 ± 0.04^{kl}
	Ethanol	1.38 ± 0.05^{n}	1.60 ± 0.06^{i}	9.00 ± 0.06f^{g}	2.84 ± 0.02^{b}	4.93 ± 0.12^{ef}	1.73 ± 0.02^{b}	5.55 ± 0.03^{f}
0 (Omission)	1	32.08 ± 0.04^{b}	3.15 ± 0.03^{d}	10.04 ± 0.09^{d}	3.20 ± 0.06^{a}	5.22 ± 0.06^{de}	2.08 ± 0.02^{a}	6.35 ± 0.09^{d}
	2	19.30 ± 0.06^{k}	2.48 ± 0.03^{f}	9.30 ± 0.06f^{g}	1.51 ± 0.04^{h}	3.45 ± 0.03^{j}	0.46 ± 0.04 ^{j}	5.19 ± 0.04h
	Distilled water	18.77 ± 0.04^{l}	2.08 ± 0.04 g^{h}	7.05 ± 0.03^{i}	1.98 ± 0.03^{de}	3.60 ± 0.05^{ij}	0.93 ± 0.03^{fg}	4.50 ± 0.06^{l}
	Ethanol	22.89 ± 0.06^{h}	2.76 ± 0.03^{e}	8.50 ± 0.06^{h}	1.98 ± 0.03^{de}	4.50 ± 0.01 g^{h}	1.04 ± 0.02^{ef}	5.28 ± 0.05 g^{h}
0.025	1	32.29 ± 0.05^{b}	3.32 ± 0.02^{c}	9.78 ± 0.05^{de}	2.09 ± 0.06^{d}	4.75 ± 0.03^{fg}	1.13 ± 1.13^{de}	4.71 ± 0.03^{j}
	2	23.11 ± 0.06^{h}	3.13 ± 0.05^{d}	9.26 ± 0.11^{fg}	1.77 ± 0.04^{-g}	4.48 ± 0.05 g^{h}	1.24 ± 0.02c ^{d}	5.36 ± 0.09 g
	Distilled water	22.87 ± 0.04^{i}	2.65 ± 0.03 g	8.50 ± 0.05^{h}	1.83 ± 0.04^{ef}	3.58 ± 0.06^{j}	0.80 ± 0.02 g^{-i}	4.65 ± 0.09^{jk}
	Ethanol	26.63 ± 0.04^{f}	2.87 ± 0.05^{e}	9.35 ± 0.03^{fg}	2.24 ± 0.05^{c}	5.38 ± 0.03^{d}	1.14 ± 0.02 ^{de}	4.86 ± 0.03^{i}
0.05	1	34.34 ± 0.09^{a}	3.86 ± 0.02^{b}	13.14 ± 0.11^{b}	2.65 ± 0.03^{bc}	8.16 ± 0.04^{a}	1.07 ± 0.02 ^{e}	5.28 ± 0.05 g^{h}
	2	30.60 ± 0.06^{c}	3.06 ± 0.03^{d}	9.39 ± 0.06^{f}	1.53 ± 0.04^{h}	5.9 ± 0.02^{b}	0.75 ± 0.03^{hi}	10.22 ± 0.04^{b}
	Distilled water	25.18 ± 0.05 g	2.12 ± 0.02 g	9.85 ± 0.03^{de}	1.58 ± 0.02 g^{h}	5.45 ± 0.03^{c}	0.86 ± 0.02^{hi}	6.19 ± 0.06^{e}
	Ethanol	27.5 ± 0.06^{d}	3.32 ± 0.02^{c}	10.65 ± 0.03 ^{c}	2.11 ± 0.04^{d}	6.05 ± 0.03^{b}	1.04 ± 0.02^{ef}	7.83 ± 0.04^{c}
0.1	1	21.58 ± 0.04^{j}	5.14 ± 0.03^{a}	15.00 ± 0.06^{a}	1.60 ± 0.06 g^{h}	4.48 ± 0.01 g^{h}	1.28 ± 0.05 ^{c}	10.37 ± 0.06^{a}
	2	11.69 ± 0.07^{m}	1.95 ± 0.03^{h}	9.61 ± 0.14^{e}	1.62 ± 0.7^{f-h}	4.40 ± 0.06^{h}	0.72 ± 0.02 ^{i}	5.67 ± 0.05^{f}

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

was the highest among all the PCs detected in the peppermint leaves, followed by GA, tannic acid, chlorogenic acid, CA, ferulic acid, and *p*-coumaric acid (Table 1). Foliar application by 1 mM MeJA increased the accumulation of all the studied PCs of peppermint in different Zn concentrations (Table 1). Using 1 mM MeJA at 0.05 mgL^{-1} Zn treatment enhanced RA and chlorogenic acid by about 28.66 and 2.15 times, respectively, compared to the control (Table 1). In addition, the highest CA, GA, and tannic acid were obtained in 1 mM MeJA and 0.1 mgL^{-1} Zn levels which were 3.76, 2.68, and 2.29-fold higher than those of the control, respectively. Whereas, the highest content of ferulic and *p*-coumaric acid in the leaves was detected in the control plants (Table 1).

3.3. Antioxidant activity

The antioxidant activity of peppermint extracts was evaluated by three different methods, including DPPH, FRAP, and Phosphomolybdenum complex methods. All the extracts exhibited a noticeable effect that varied significantly among treatments (Table 2).

3.3.1. Radical scavenging activity

The DPPH radical scavenging capacity of *M. piperita* extract was evaluated in terms of the percent reduction of the initial DPPH absorption, and the results were expressed as relative activities against control (Table 2). The DPPH scavenging assay of the methanolic extracts obtained from different treatments ranged from 26.42 ± 0.03% to 44.43 ± 0.36% (Table 2). It showed that 44.43% of DPPH radical scavenged at Zn omission and 1 mM MeJA which increased by about 25.84% compared with the control (Table 2). Also, 1 mM MeJA application increased antioxidant activity in all Zn concentrations (Table 2).

3.3.2. FRAP assay

The FRAP value of the peppermint leaves ranged from 4.59 ± 0.38 to 10.23 ± 0.19 μmol ASC g DW^{-1}. 1 mM MeJA application in all Zn levels except the highest Zn concentration (0.1 mgL^{-1}) increased FRAP value (Table 2). The highest FRAP value was obtained in Zn omission and 1 mM MeJA application which increased by 10.71% compared to the control (Table 2). The lowest FRAP value was reported at the highest Zn (0.1 mgL^{-1}) and distilled water application, which decreased by 50.33% compared to the control (Table 2).

3.3.3. Phosphomolybdenum complex method

The results from the phosphomolybdenum complex method for methanolic extracts are presented in Table 2. Significantly, the highest value (5.04 ± 0.09 mg ASC g DW^{-1}) of antioxidant activity (according to this method) obtained at the highest Zn level (0.1 mgL^{-1}) and 1 mM

Table 2
Antioxidant activity of *M. piperita* by different assays affected by Zn and methyl jasmonate concentrations.

Zn (mgL^{-1})	MJA (mM)	DPPH radical scavenging activity (%)	FRAP (μmol ASC g DW $^{-1}$)	Phosphomolibden (mg ASC g Dw $^{-1}$)
	Distilled water (Control)	35.22 ± 0.36^{*d}	9.24 ± 0.63^{a-d}	3.24 ± 0.37^{cd}
	Ethanol	32.58 ± 0.29^{e}	9.69 ± 0.21^{a-c}	3.81 ± 0.20^{a-d}
0 (Omission)	1	44.30 ± 0.36^{a}	10.23 ± 0.19^{a}	4.96 ± 0.20^{ab}
	2	29.07 ± 0.25 g	6.56 ± 0.92^{d-g}	4.11 ± 0.40^{a-d}
	Distilled water (Control)	31.26 ± 0.25^{f}	8.54 ± 0.42^{a-e}	3.23 ± 0.15^{cd}
	Ethanol	32.21 ± 0.26^{ef}	9.19 ± 0.63^{a-d}	4.38 ± 0.26^{a-d}
0.025	1	39.54 ± 0.08^{bc}	9.81 ± 0.63^{ab}	4.77 ± 0.23^{a-c}
	2	27.24 ± 0.03^{hi}	6.94 ± 0.31^{c-f}	3.51 ± 0.47^{cd}
	Distilled water (Control)	28.34 ± 0.23 g^{h}	7.14 ± 0.67^{b-e}	2.99 ± 0.10^{d}
	Ethanol	32.04 ± 0.29^{ef}	8.13 ± 0.19^{a-e}	3.77 ± 0.15^{a-d}
0.05	1	38.48 ± 0.03^{c}	8.40 ± 0.38^{a-e}	4.71 ± 0.23^{a-c}
	2	26.42 ± 0.03^{i}	6.36 ± 0.67^{e-g}	3.49 ± 0.29^{cd}
	Distilled water (Control)	29.27 ± 0.2^{3} g	4.59 ± 0.38 g	3.59 ± 0.17^{b-d}
	Ethanol	31.70 ± 0.336^{ef}	5.36 ± 0.59^{fg}	3.46 ± 0.22^{cd}
0.1	1	40.61 ± 0.00^{b}	4.98 ± 0.66^{fg}	5.04 ± 0.09^{a}
	2	24.29 ± 0.24^{j}	4.95 ± 0.14^{fg}	3.48 ± 0.23^{cd}

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

MeJA treatment (Table 2) which improved antioxidant activity about 55.55% compared with the control (Table 2).

3.4. Total soluble protein content

Total soluble protein content increased by raising Zn concentrations, but MeJA application decreased it (Table 3). The highest total soluble protein content (8.83 ± 0.2 mg g^{-1} FW) was observed in the highest Zn concentration (0.1 mgL^{-1}) with distilled water spraying; however, there was no significant statistical differentiation between this Zn level and 0.05 mgL^{-1} (Table 2). The total soluble protein content increased by 26.32% and 25.61% in 0.05 and mgL^{-1} with distilled water spraying, in comparison with the control (Table 3).

3.5. Antioxidant enzyme activities

The results of the present experiment indicated that the foliar application of MeJA and Zn had significant effects on the antioxidant enzyme activities of peppermint.

3.5.1. POX activity

Our findings showed that treatment of *M. piperita* plants with Zn (up to 0.025 mgL^{-1}) leads to a significant increase in POX activity and a significant decrease in its activity in the treated plants with higher Zn levels (Table 3). In addition, the MeJA application increased POX activity (Table 3). By application of 2 mM MeJA under 0.025 mgL^{-1} Zn, POX activity increased 2.62-fold in comparison with the control (Table 3).

3.5.2. PPO activity

Raising in Zn concentration leads to a significant increase in PPO activity (Table 3). Moreover, 1 mM MeJA application increased PPO activity, while it increased PPO activity in 2 mM concentration (Table 3). PPO activity showed a prominently about 5.20-fold increase in the highest Zn concentration (0.1 mgL^{-1}) and 1 mM MeJA compared with the control (Table 3). Moreover, by 2 mM MeJA application in Zn-omitted plants, PPO activity decreased by 11.75% in comparison with the control (Table 3).

3.6. H_2O_2 content

In this study, H_2O_2 concentration has increased in parallel with the level of Zn omission and excessive (Table 3). In addition, MeJA application increased H_2O_2 concentration in all Zn levels (Table 3). The lowest H_2O_2 contents were observed in 0.025 and 0.05 mgL^{-1} Zn with distilled water spraying (Table 3). The application of 2 mM MeJA in the treatment with no Zn, increased H_2O_2 content by 12.13% compared with the control (Table 3).

3.7. MDA content

Based on the results of this study, MDA concentration significantly increased by foliar application of MeJA. However, it decreased by raising Zn concentrations to 0.1 mgL^{-1} and then increased similarly to Zn omission treatment (Table 3). MDA content increased by 22.22% in the 1 mM MeJA application with Zn omission treatment compared with the control (Table 3). At the highest Zn level (0.1 mgL^{-1}), 2 mM MeJA application decreased MDA content by 49.61% compared with the control (Table 3).

3.8. MSI

According to the results, MSI was enhanced by increasing Zn concentration to 0.1 mgL^{-1} and then decreased. Also, applying MeJA

Table 3
Physiological properties in *M. piperita* affected by different levels of Zn and foliar spraying of methyl jasmonate.

Zn (mgL−1)	MJA (mM)	Total soluble protein (mg g^{-1} FW)	POX activity (Unit mg protein−1)	PPO Activity (Unit mg protein−1)	H2O2 content (μmol g^{-1} FW)	MDA (nmol g^{-1} FW)	MSI (%)	RWC (%)
	Distilled water (Control)	6.99 ± 0.03^{bc}	0.64 ± 0.22^{bc}	9.45 ± 0.16^{ij}	3.38 ± 0.08^{a-e}	6.39 ± 0.22^{a-d}	83.71 ± 1.18^{e}	64.64 ± 1.64^{b}
	Ethanol	6.63 ± 0.07^{b-d}	0.34 ± 0.07^{bc}	16.49 ± 1.07^{f-h}	3.59 ± 0.14^{a-e}	6.60 ± 0.22^{a-c}	90.12 ± 0.96^{a-d}	77.55 ± 5.95^{ab}
0 (Omission)	1	6.54 ± 0.39^{b-d}	0.87 ± 0.01^{bc}	17.68 ± 0.98^{fg}	3.69 ± 0.08^{a-c}	7.81 ± 0.68^{a}	90.94 ± 1.07^{ab}	86.61 ± 5.05^{ab}
	2	4.39 ± 0.34^{e}	0.89 ± 0.03^{b}	8.34 ± 0.77^{j}	3.79 ± 0.06 ^{a}	4.33 ± 0.84^{c-f}	85.24 ± 0.87^{b-e}	75.91 ± 4.52^{ab}
	Distilled water	7.22 ± 0.17^{b}	0.81 ± 0.01^{b}	18.59 ± 2.58^{e-g}	3.15 ± 0,02^{e}	6.09 ± 0.75^{a-e}	83.89 ± 0.92 ^{de}	71.46 ± 1.76^{b}
	Ethanol	5.49 ± 0.39^{c-e}	0.37 ± 0.06^{bc}	23.13 ± 0.31^{d}	3.24 ± 0.03^{c-e}	6.59 ± 0.24^{a-c}	89.89 ± 1.54 $^{a-e}$	85.30 ± 1.10^{ab}
0.025	1	5.45 ± 0.40^{c-e}	0.90 ± 0.11^{b}	34.31 ± 0.55^{b}	3.41 ± 0.09 $^{a-e}$	6.60 ± 0.22^{a-c}	89.94 ± 1.77^{a-e}	97.07 ± 3.99^{a}
	2	2.49 ± 0.39^{f}	1.68 ± 0.11^{a}	16.81 ± 1.45^{f-h}	3.49 ± 0.02^{a-e}	5.01 ± 0.02^{b-f}	91.79 ± 0.92^{a}	69.46 ± 5.97^{b}
	Distilled water	8.78 ± 0.17^{a}	0.54 ± 0.19 ^{bc}	19.29 ± 1.24^{ef}	3.16 ± 0.02^{e}	3.85 ± 0.86^{ef}	87.67 ± 1.62^{a-e}	74.79 ± 7.73^{ab}
	Ethanol	6.71 ± 0.24^{bc}	0.27 ± 0.01^{bc}	28.17 ± 0.55^{c}	3.21 ± 0.08^{de}	4.24 ± 0.84^{d-f}	90.72 ± 0.76^{a-c}	85.62 ± 3.21^{ab}
0.05	1	6.62 ± 0.43^{b-d}	0.90 ± 0.12^{b}	29.39 ± 0.98^{c}	3.41 ± 0.09^{a-e}	6.69 ± 0.74^{ab}	91.15 ± 0.34^{ab}	87.78 ± 4.01^{ab}
	2	5.11 ± 0.48^{de}	0.92 ± 0.20^{b}	12.33 ± 2.43 g	3.64 ± 0.09^{a-d}	3.29 ± 0.56^{fg}	90.95 ± 1.13^{ab}	75.14 ± 6.04^{ab}
	Distilled water	8.83 ± 0.20^{a}	0.49 ± 0.14^{bc}	19.68 ± 0.98^{ef}	3.39 ± 0.09^{a-e}	6.30 ± 0.23^{a-d}	84.62 ± 1.52^{c-e}	69.79 ± 5.12^{b}
	Ethanol	7.53 ± 0.17^{ab}	0.22 ± 0.008^{c}	25.62 ± 0.54^{cd}	3.55 ± 0.13 $^{a-e}$	6.62 ± 0.17^{a-c}	87.75 ± 1.62^{a-e}	85.09 ± 3.57^{ab}
0.1	1	6.22 ± 0.15^{b-d}	0.71 ± 0.21^{bc}	49.08 ± 0.55^{a}	3.61 ± 0.14^{a-e}	6.86 ± 0.32^{ab}	91.57 ± 0.36^{a}	87. 14 ± 3.29^{ab}
	2	5.54 ± 0.07^{c-e}	0.76 ± 0.001^{bc}	14.08 ± 0.17 g^{-i}	3.74 ± 0.05^{ab}	3.22 ± 0.59 g	90.22 ± 1.22^{a-c}	80.62 ± 6.57^{ab}

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

improved cell membrane stability (Table 3). Compared to the control, MSI increased by 9.65% with 2 mM MeJA application in 0.025 mgL^{-1} Zn treatment (Table 2). The lowest MSI was reported in the control (Table 3).

3.9. RWC

Our findings indicated that RWC increased by raising Zn concentration to 0.1 mgL^{-1} and then decreased (Table 2). Applying 1 mM MeJA increased the RWC of the treated plants, but 2 mM MeJA decreased it (Table 1). Applying 1 mM MeJA in 0.025 mgL^{-1} Zn treated plant, increased RWC by 50.17% compared to the control (Table 3). Furthermore, the Zn omission plant showed the lowest RWC (64.64%) (Table 3).

4. Discussion

Zn is a compound of many structural substances in plants and works as an activator of many enzymes. It plays multiple roles in plant metabolism, including plant photosynthesis, cell membrane structure, cell defense against ROS, and the synthesizing of hormone precursors (Hafeez et al., 2013). MeJA application in plants stimulated the molecules and genes that encoded defense proteins, cell wall formation, and stress protection (Afkar et al., 2013). Based on the results of the present study, we found that the physiological characteristics, antioxidant activity, and PCs of peppermint are influenced by different concentrations of Zn and foliar application of MeJA in addition to enzymatic and non-enzymatic antioxidant activity. The content of PCs in the plants depends on various factors such as climatic, genetic, cultivation factors, and stresses as well as harvesting time (Khan et al., 2012). The effect of Zn on PCs was studied before in *Vaccinium myrtillus* (Białońska et al., 2007). Zn treatments on the plants had particular effects which raised the content of PCs (Białońska et al., 2007) which is in conformity with the results of the present study and PCs increased by increasing Zn levels. For instance, some PCs, such as CA, GA, and tannic acid increased by raising Zn concentration and showed the highest content at the highest Zn level (0.1 mgL^{-1}). Supra-optimal Zn concentration causes oxidative stress, so PC synthesis is obtained through indirect mechanisms that influence their interactions with the antioxidative defense system against ROS (Flora, 2009). Plants can accumulate more PCs to scavenge free radicals or binding heavy metals. Therefore, in the present experiment, the amount of some PCs, like ferulic acid and *p*-coumaric acid, increased under stress conditions like Zn omission to defense plants against ROS. However, *Mentha* species PCs were reported in the previous papers, but the available data are often difficult to compare because of the variation in the methodology (Benabdallah et al., 2016). Different studies have been performed to determine the exogenous chemical treatment which may improve the PCs production supplies (Szymanowska et al., 2015). An effective method to improve herb quality is elicitor treatment. Jasmonates application has long been known to increase secondary metabolites such as PCs in aromatic and medicinal plants (Wasternack and Strnad, 2017). In conformity with the findings of the present study, PCs content increased by MeJA application in different plant species (Attaran Dowom et al., 2017; Xing et al., 2018; Pesaraklu et al., 2021). According to the previous reports, treatment with elicitors such as JA-induced polyphenols synthesis, especially RA (Szymanowska et al., 2015). In addition, the positive effect of elicitation by MeJA on RA accumulation reported in the previous experiments (Attaran Dowom et al., 2017; Xing et al., 2018; Fatemi et al., 2019) conforms with the findings of this study. PCs accumulation in MeJA-treated plants are probably due to the increase in the phenylpropanoid pathway (Tassoni et al., 2012). PCs of the plants are mainly produced through the phenylpropanoid pathway, which is started by the two primary enzymes: Phenylalanine ammonia-lyase (PAL) and Tyrosine ammonia-lyase (TAL). The effect of plant elicitation on polyphenol production and the role of PAL in the biosynthetic pathway has previously been investigated by most researchers (Kim et al., 2006; Gholizadeh and Kohnehrouz, 2010). Overproduction of PCs in basil by methyl jasmonate elicitation has been reported before (Kim et al., 2006). The effects similar to these findings obtained in the present study and PCs increased by 1 mM MeJA application, but higher concentration (2 mM) decreased them. So far, there are no studies concerning the impact of MeJA on peppermint PCs under different Zn concentrations. MeJA induces the PCs accumulation by the enzyme activity modification that is responsible for PC biosynthesis. The Plant elicitation influence on polyphenol production is focused on the role of PAL in the biosynthetic pathway (Kim et al., 2006; Gholizadeh and Kohnehrouz, 2010). The secondary metabolite amount is affected by the duration, concentration of exposure, and stage of the culture at the treatment time. PCs are the influential plant secondary metabolites and essential constituents of medicinal plants with high antioxidant potential (Adhikari et al., 2018). There are different methods to evaluate antioxidant activity. Depending on several generators of free radicals acting via different mechanisms to protect all properties of antioxidant capacity, each method has its advantages and limitations (Viuda-Martos et al., 2010). So, to determine the antioxidant activity, using different substrates and analytical methods for measuring different antioxidant characteristics is useful. In the present study, three different methods were performed to determine the antioxidant activity of peppermint leaves in each treatment. Usually, mixted methods should be applied to evaluate the antioxidant activity to cover all features of antioxidant effects (Viuda-Martos et al., 2010). DPPH and FRAP methods commonly used to estimate the antioxidant capacity of the plants or free radical-scavenging potentials of PCs originating from the cells are helpful in understanding their adapting mechanism to different adverse conditions (Gülçin, 2020). The antioxidant potential of plants is related to the existence of phytochemical compounds, mainly PCs. In the antioxidant activity of peppermint extract, differences between DPPH and FRAP methods observed that indicated bioactive compounds of peppermint have different abilities to reduce Fe^{3+} ions and neutralize DPPH radicals, which may be related to high PCs content (Gülçin, 2020). The antioxidant activity of peppermint leaves due to PCs content which is in line with a previous report in spearmint (Rita et al., 2016). Moreover, antioxidant potential is related to other PCs besides RA and non-PCs with synergistic effects (Gülçin, 2020). In several genera of the Lamiaceae family, there are different PCs, such as GA, RA, and CA confirmed the findings of this study. They are involved in plant physiology and responsible for their antioxidant activities (Kwee and Niemeyer 2011). Accordingly, antioxidant activity variation between the treatments, observed in this study could be described by their differentiations in their individual PCs, such as RA, and CA. The RA may be responsible for the antioxidant activity of the plant extract (Kim et al., 2006). Furthermore, antioxidant capacities might be ascribed to the chemical structure of PCs, as well as the synergistic or antagonistic influence of the compounds exhibited in the extract (Adhikari et al., 2018). In agreement with the present study, strong correlations reported in the previous studies between total PCs and the antioxidant activity of different plant species (Dorman et al., 2009; Benabdallah et al., 2016; Adhikari et al., 2018; Li et al., 2018). Phenols are responsible for the antioxidant and free radical scavenging effects of plants because of their ability to act as radical scavengers, hydrogen donors, and reducing agents (Dorman et al., 2009; Benabdallah et al., 2016). In addition, the antioxidant and, or pro-oxidant activity of PCs depends on different factors, including solubility, chelating behavior, metal-reducing potential, and pH (Wasternack and Strnad, 2017). The antioxidant activity induction in the plants may be caused by the RA and CA accumulation in sweet basil by MeJA foliar application (Kim et al., 2006), as this effect was observed in the present study. However, there is still uncertainty regarding the PCs functions in response to Zn and MeJA concentrations.

Antioxidant compounds play essential roles in preventing ROS formation in organisms. In scavenging or neutralizing ROS, PCs are effective and can delay, inhibit, or prevent oxidizable matter oxidation. Therefore, oxidative damage to cells and cell components like proteins,

DNA, and lipids are reduced (Gawlik-Dziki et al., 2012). PCs act as an antioxidant because of their hydroxyl groups. The synthesis of these compounds is accomplished through indirect mechanisms, which comprise their interactions with the antioxidative defense system versus the ROS and their behaviors as antioxidants and chelators of redox-active metals (Flora, 2009). PCs have a high propensity to chelate heavy metals. Therefore, they prevent ROS generation and ROS reduction once they are formed (Wei and Guo, 2014).

The plants use different developed enzymatic and non-enzymatic systems to minimize the harmful effects of ROS, which are produced under optimal conditions and normal metabolic function. Production and destruction of ROS modulated in the cell metabolism, as a result of different environmental stresses, such as mineral nutrient deficiency or toxicity, ROS increases, which is directly or indirectly connected with oxidative stress (Del Rio, 2015). The redox reactions, especially those associated with electron transfer in chloroplasts, mitochondria, and cell membranes, lead to the superoxide radical (O_2^-), and other ROS such as hydroxyl radical (OH), and H_2O_2 production (Del Rio, 2015). An antioxidative system produces low-molecular-weight antioxidants and defensive enzymes to remove ROS (O^{2-}, OH, O_2, H_2O_2) effectively (Del Rio, 2015). POX activity increments directly connected to ROS detoxification. Plants produce antioxidant enzymes to scavenge guaiacol-dependent peroxidase, detoxifying results, to reduce stress and keep the ROS lower than the toxic limit (Del Rio, 2015). According to the result of the preceding experiment in *M. piperita* (Afkar et al., 2013), increasing POX activity was observed due to the high requirement for relieving H_2O_2. Based on the results, MeJA mediated the stimulation of antioxidant enzymes, including POX, which conforms with a previous report in barley (Popova et al., 2004).

Zn has a crucial function as a metal component of enzymes. It is needed as a catalytic and structural regulatory component of protein (Marschner, 2012). Zn is a defensive factor for different targets of oxidation like proteins, membrane lipids, and the constitutive enzymes POX involved in ROS detoxification (Del Rio, 2015). In addition, for average plant growth and development, Zn acts as a functional-structural or regulatory cofactor in various classes of enzymes, such as antioxidant enzymes, including POX, which is involved in the detoxification of ROS (Hafeez et al., 2013; Rezaeieh et al., 2016). Also, antioxidant enzymes and compounds cleanse free radicals to inhibit their harmful effects on plant growth (Babaei et al., 2017). In conformity with the results of this experiment, preceding reports on the antioxidative activity of Zn showed that it is directly or indirectly involved in the detoxification of ROS by preserving the proper levels of antioxidative enzyme activities. PCs levels are often used to assess environmental stress effects on plants. This could be the reason why PCs content increased in Zn omission and higher Zn concentrations (such as 0.1 mgL^{-1} in this experiment) treatment that showed stress conditions for the plants in the present study. In this case, PCs became involved in one of the defensive systems that the plants used against Zn stress. In addition, PCs can chelate heavy metals (Sharma et al., 2019), and capture heavy metals by polymerized phenols, which are biosynthesized by PPO and POX (Cristina Sgherri, 2003).

In this experiment, MeJA application caused a senescence-like symptom as shown by a significant decline in photosynthesis and an increase in antioxidant enzyme activities which are in agreement with the findings of a previous report in *Arabidopsis thaliana* (Jung, 2004). Furthermore, in the present study, plant senescence was followed by protein content reduction, which aligns with the preceding report on peppermint (Afkar et al., 2013). Antioxidant enzyme activities of PPO or POX which Zn is not known as an activator or constituent, change differently under different Zn concentrations. In this study, POX activity increased with the application of 0.025 mgL^{-1} Zn, but in higher concentrations, it decreased. In this study, improved stress tolerance at higher Zn concentrations is often related to an increment in antioxidant enzyme activity, such as PPO activity. An increase in PPO activity probably plays a vital role in the development and growth of stress plants against oxidative destruction and inhibition (Aziz et al., 2017). Generally, in plants, PCs synthesis and accumulation are influenced by different stress conditions, and PPO is the most crucial enzyme that is responsible for the PCs oxidation and oxidizes some phenols to chinone (Tarchoune et al., 2012). So, peppermint could be considered as a tolerant plant against Zn omission showing higher PCs and antioxidant activity with lower damage. Moreover, PPO activity decreased significantly in Zn omission treatment, whereas it increased in excess Zn concentration (0.1 mgL^{-1}). Under stress conditions, the most significant factor of total phenolic oxidation in the plants may be due to an increment in PPO activity (Tarchoune et al., 2012). In the present study, PPO activity significantly decreased in Zn omission treatment. It may be indicated that PPO was powerless to oxidize and reduce the toxic material and also did not have a role in the TPC adjustment of this treatment.

On the other hand, MeJA acts like biotic or abiotic stress that induces ROS in cells. The exogenous application of MeJA protects the plants from induced damage by increasing the antioxidant enzyme activities such as POX (Anjum et al., 2011b). In addition, MeJA helps in maintaining the antioxidant enzyme combination and relieving oxidative stress (Jung, 2004). Under our findings, exogenous MeJA application increased POX activity as well as PCs *M. piperita* L. (Afkar et al., 2013). Furthermore, MeJA causes the induction of defense genes such as PPO in responses to stress (Aziz et al., 2017), and alleviates the adverse effect of stress in peppermint leaves.

The protein content is indicated as the main index of the physiological condition of plants. We can demonstrate the distraction in protein content as a reason for reducing the total soluble protein amount of the plants (Fan et al., 2021). Proteins are critical osmoregulatory substances that protect enzymes by osmotic pressure regulation in the cytoplasm (Fan et al., 2021). Based on the findings of this study, we found that an increase in Zn concentration led to an increase in soluble protein content in line with a previous study in *Dendrobium nobile* (Fan et al., 2021). Zn is involved in RNA metabolism and ribosomal content in plant cells, causing to stimulate proteins, and DNA formation (Aziz et al., 2010). During leaf senescence, protein degradation was observed in a previous study (Afkar et al., 2013). In the present study, *M. piperita* senescence was followed by protein content reduction. It is clear that MeJA application significantly increases peppermint leaf senescence, which is in line with the preceding study (Afkar et al., 2013).

MDA, produced by lipid peroxidation of the cell membrane, is often used as an indicator of oxidative damage, and its level can show the extent of oxidative stress (Afkar et al., 2013). Based on the results of the present study, the MDA content of leaves under excess Zn increased similarly to that of the leaf during treatment with no Zn application. These results indicated that excess Zn application showed stress effects. Zn deficiency or excesses cause oxidative stress (ROS and lipid peroxidation), which reported before in legumes (Gupta et al., 2011). Thus Zn is an essential element in cell membrane structure. ROS production under Zn deficiency conditions affects the membrane integrity pathways (Hafeez et al., 2013).

In addition, the accumulation of H_2O_2 is one of the reasons for lipid peroxidation that influences lipid membranes. The results of the preceding report, Zn deficiency increased H_2O_2 concentration in pecan trees (Castillo-Gonzalez et al., 2019), which conforms with the findings of this study. In addition, H_2O_2 accumulation under higher Zn levels can result in antioxidant enzyme activities increment to protect plants from oxidative stress caused by excess Zn concentration.

According to the results of a previous study, MeJA causes lipid peroxidation products in plant cells (Hung and Kao, 2004), which is in line with the findings of this experiment. Thus, MeJA leads to oxidative stress in plant cells. Based on the results of this study, in treated plants with 1 mM MeJA, MDA content significantly increased compared with the control. A smaller amount of MDA was observed by the application of 2 mM MeJA in this study, showing that 2 mM concentration had better efficiency in tolerating the cellular membrane injuries than 1 mM

MeJA concentration. In a previous experiment, such results were reported in peppermint (Afkar et al., 2013). On the other hand, the reduction in MDA content by 2 mM MeJA application may be due to the antioxidant enzyme activities that can help to clean up ROS and modify the ratio of membrane fatty acids as a primary source of ROS production (Afkar et al., 2013). In previous reports, it was found that MeJA stimulates the H_2O_2 generation (Hung and Kao, 2004), leading to oxidative stress in plant cells, which is in agreement with the findings of this experiment. Reduced oxygen species such as hydrogen peroxide (H) and superoxide radicals (O_2^-) are formed due to oxidative stress, and they can produce free radicals, inducing lipid peroxidation and protein denaturation (Afkar et al., 2013). In this study, RWC and MSI in the leaves decreased under Zn stress and increased by MeJA application compared with control plants. Moreover, our findings indicated that RWC is at high levels in the plants, whereas the membrane lipid peroxidation is low in the plants subjected to the highest Zn concentration and MeJA application compared with control plants. In contrast, MeJA secretes osmolytes, such as proteins, in response to Zn stress conditions, which stimulates plant growth and acts synergistically with plant-produced osmolytes (Moreira et al., 2012).

5. Conclusion

Zn is an essential micronutrient for plant growth and development, as it is required in several metabolic processes. It is involved in antioxidant enzymes and free radical removal. In addition, elicitors such as MeJA play several roles in the plants. The results of the present study demonstrated that Zn omission and, or excess had individual influences on the studied traits because they have shown some stress effects on the plants. Moreover, the foliar application of MeJA and distinctive concentrations of Zn differently affected the PCs, enzymatic and non-enzymatic antioxidant activities, and physiological characteristics of peppermint. Changing physiological characteristics and antioxidant enzyme activities can mitigate the harmful effects of Zn omission or high Zn concentration. Finally, foliar MeJA spraying and suitable concentrations of Zn can enhance the antioxidant activity, and biosynthesis of some secondary metabolite classes, such as PCs of peppermint. Based on the results, the highest RA and chlorogrnic acids were observed in the treated plants with 0.05 mgL^{-1} Zn and 1 mM MeJA application, whereas treatment with 0.1 mgL^{-1} and 1 mM MeJA showed the highest CA, GA, and tannic acid. On the other hand, the most favorable combination to increase productivity of peppermint was 0.025 mg L^{-1} Zn and 1 mM MeJA which shown the highest leaf fresh and dry weight. Further research is needed to develop appropriate growing procedures (approporiate Zn and MeJA concentrations) and to select high-producing secondary metabolites including phenolic compounds in order to combine high productivity plants with satisfactory phenolic compounds accumulation in the hydroponic culture of this important medicinal plant.

CRediT authorship contribution statement

Leila Mehdizadeh: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Mohammad Moghaddam:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Ali Ganjeali:** Writing – review & editing, Methodology, Formal analysis. **Mehdi Rahimmalek:** Writing – review & editing, Software, Formal analysis, Data curation.

Declaration of competing interest

We declare that there is no conflict of interest.

Data availability

Data will be made available on request.

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