Effects of Iron Ions on Rosmarinic Acid Production and Antioxidant System in *Melissa officinalis* L. Seedlings

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Authors' contributions

This work was carried out in collaboration between both authors. Author KES performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ARM designed the study, managed the analyses of the study. Both authors read and approved the final manuscript.

ABSTRACT

Aims: In this study, effects of various Fe^{2+} concentrations on the physico-chemical parameters including contents of rosmarinic acid, flavonoid and anthocyanin as well as antioxidant enzymes activity in *Melissa officinalis* seedlings were analyzed. Furthermore, some morphological parameters and contents of chlorophyll and carotenoid were investigated in 45-day-old *M. officinalis* seedlings in presence of various Fe^{2+} concentrations.
Study Design: completely random designs.
Place and Duration of Study: Department of Biotechnology, Institute of Science and High technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran.
Methodology: 45-day-old seedlings were treated with different Fe^{2+} concentrations and then physico-chemical parameters of them were evaluated after 8 and 16 hours incubation periods. Rosmarinic acid content was measured using HPLC and the flavonoid and anthocyanin contents were determined using a spectrophotometer. Morphological properties including seed germination, root and shoot elongation and dry biomass of the

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45-day-old Fe^{2+}-treated seedlings were analyzed.

Results: The results showed that the contents of rosmarinic acid, flavonoid and anthocyanin drastically decreased in all treatments compared to the control. In this condition, the activity of superoxide dismutase and peroxidase were increased more significantly after 8 hours treatment rather than 16 hours. The results revealed that changes in rate of germination, shoot elongation and dray biomass were not statically significant. Although, the root growth was decreased in presence of Fe^{2+} compared to control, that was significant at the level of 5% at the highest Fe^{2+} concentration.

Conclusion: Accumulation of free radicals under treatment conditions may lead to suppression of protein synthesis or degradation of them as indirectly confirmed by reduced protein content. Therefore, it may be concluded that the decrease in contents of rosmarinic acid, flavonoid and anthocyanin results from a reduction of enzyme levels in their biosynthesis pathways. The decrease in root growth can be due to exposition of the organ to the excess of Fe^{2+}, more increased uptake of the ion and triggering of free radicals. Furthermore, low rate in Fe^{2+} transportation to the shoots and elevated levels of chlorophyll and carotenoid contents may have roles in preventing damages to this organ.

1. INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is one of the most well-known medicinal plants belonging to the Lamiaceae family. The leaves of this plant were used as digestive, carminative, analgesic, anti-convulsant, diuretic and invigorating in traditional medicine [1]. The importance of this medicinal plant is due to possession of two groups of secondary metabolites; the essential oil and the phenylpropanoid derivatives [2]. Rosmarinic acid (RA)- an ester of caffeic acid and 3,4-dihydroxphenyllactic acid- is the main active phenolic compound in Lemon balm and many therapeutic properties of this plant are attributed to this phytochemical [3].

In plants, the biosynthesis of RA as well as flavonoids and anthocyanin takes place through phenylpropanoids pathway. A defensive role against pathogens and herbivores has been suggested for RA [4]. Flavonoids as the important phenolic compounds have antioxidant properties and play an important role in the development of color, protecting against pathogens attack as well as UV radiation, and also serve as an indicator of stress and accumulation of free radicals in plants [5]. Moreover, anthocyaninas the water-soluble compounds have also antioxidant activity [6].

So far, Different elicitors (stable molecules that influence defense responses in plant) including yeast extract, Ag^+, methyl jasmonate and jasmonic acid have been used to induce RA production in *Salvia miltiorrhiza*, *Solenostemon scutellarioides* and *Mentha χ piperita* plants [7,8,9]. In our knowledge no studies has ever been reported on the effects of Fe ions as an elicitor on RA production in *M. officinalis.*

Iron is an essential microelement for plants growth and development. It has critical roles in many vital processes such as photosynthesis, chloroplast development and chlorophyll biosynthesis. This element is a main component of the oxidation-reduction systems such as heme proteins, including cytochromes, catalase, peroxidase and leghemoglobin and iron-sulfur proteins, including ferredoxin, aconitase and superoxide dismutase [10]. However, excess iron in plants induces iron toxicity and produces the reactive oxygen species (ROS),

including superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($OH^\bullet$) and hydrogen peroxide that lead to oxidative stress in plants [11-13]. In plants, in order to prevent the possible damage caused by the ROS, two antioxidant systems were developed; non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherols and flavonoids) and enzymatic antioxidants (catalase, superoxide dismutase, peroxidase, ascorbate peroxidase and glutathione reductase) [14].

The effect of Fe^{2+} nutrient solutions were examined on growth and essential oil content in *Origanum vulgare* by Yeritsyan and Economakis, 2001. They reported that plant growth parameters and essential oil content of shoots decreased when iron concentration increased more than 11.0mg/mL in media [15]. In current study, we investigated the effects of various Fe^{2+} concentrations on non-enzymatic (RA accumulation, flavonoids and anthocyanin contents) and enzymatic (activity of superoxide dismutase, peroxidase and catalase) antioxidant systems in 45-day-old *M. officinalis* seedlings at different time intervals. Furthermore, some morphological properties and chlorophyll and carotenoid contents of plant which grow in presence of different Fe^{2+} concentrations were analyzed after 45 days.

2. MATERIALS AND METHODS

2.1 Materials

Seeds of Lemon balm (F1) were obtained from Pakanbazr, Esfahan, Iran. RA standard and methanol (HPLC grade) were purchased from Merck (Germany). Ethanol, orthophosphoric acid and acetic acid, purchased from Merck, were of analytical reagent grade. All other chemicals used were of analytical reagent grade and obtained from Sigma (United States).

2.2 Plant Culture

For surface sterilization, the seeds were exposed to 2% sodium hypochlorite solution for 5 min and then were rinsed several times using sterile distilled water. The disinfected seeds (30 numbers) were placed on the surface of solidified basal Murashige and Skoog (1962) (MS) medium [16] containing 0.8% agar. Then, they were incubated in a dark incubator under control temperature of 28±2ºC for 2 weeks. After germination, they were transferred to germinator at 28±2ºC, with a relative humidity of 60-65%, under photoperiodic condition of 8h:16h (dark : light).

2.2.1 Seedlings treatment

The 45-day-old seedlings were collected from their medium and rinsed thoroughly with sterile water. Final concentrations (0 (control), 10, 20, 30 and 40µM) of $FeSO_4.7H_2O$ were dissolved in 60mL of the MS solution into 250mL Erlenmeyer flasks. The seedlings (30 numbers) were transferred into the flasks and then the flasks were shaken at 100rpm on an orbital shaker at room temperature. After incubation for 8 and 16 hours, seedlings were harvested and washed using sterile distilled water in order to remove salt from their surface.

For measuring RA content, seedlings were dried in shade at 25ºC. In order to measure flavonoid and anthocyanin contents, as well as, antioxidant enzymes activity, seedlings were immediately frozen in liquid nitrogen, and were kept at -80ºC until used.

2.3 RA Identification and Quantification

Dried sample (100mg) was ground into powder and mixed with 25mL ethanol/water (30:70, v/v) solution and then sonicated for 10 min. The resulting mixture was centrifuged at 4500 rpm for 5 min at 4ºC. The volume of the supernatant was reached to 50mL by using sterile distilled water. The extract solution was filtered by using 0.2μM syringe-headed filter, prior to injected into column of High Performance Liquid Chromatography (HPLC, ZORBAX SB-C18 column, Agilent 1100 series, USA) [7,17].

The mobile phase containing; 40% solvent A (orthophosphoric acid in water, 1.0% v/v) and 60% solvent B (orthophosphoric acid in methanol, 1.0% v/v), was run at 1.0mL/min at room temperature. Identification of RA was achieved by comparing retention time with authentic standard that was detected at 330nm.

2.4 Measurement of Flavonoid and Anthocyanin Contents

To determine flavonoid content, 0.1g of fresh sample was extracted in 10mL solution containing ethylic alcohol 95% and glacial acetic acid (99:1, v/v).The mixture was centrifuged at 4000 rpm for 10 min, and then the supernatant was calmly heated for 10 min in water bath at 80ºC. Absorbance of the extract was measured at three wavelengths: 270, 300 and 330 nm using UV-Visible spectrophotometer (Varian cary 50, Australia). The flavonoid content was estimated cumulatively, considering the extinction coefficient of 33000 $M^{-1}cm^{-1}$ [18]. The results presented as μM/g fresh weight (fw).

Anthocyanin content was measured according to the method proposed by Krizek et al. (1993). In brief, 0.1g of fresh sample was ground to powder in 3mL acidified methanol (HCl:methanol, 1:99, v/v). The homogenate was centrifuged at 12000rpm for 20 min at 4ºC. The supernatant was filtered using Whatman paper prior to maintenance in darkness for 24 hours. Absorbance was measured at 550nm and the extinction coefficient of 33000 $M^{-1} cm^{-1}$ was used to calculate anthocyanin content and its concentration was expressed as μM/g fw [19].

2.5 Protein Extraction and Antioxidant Enzyme Activity

Protein extraction was done by homogenizing 0.5 g of fresh samples in 50mM potassium phosphate buffer (pH=7.0) containing 1% (w/v) Polyvinylpyrrolidone (PVP) and 1 mM EDTA. Then the homogenates were centrifuged at 11000 rpm for 20 min at 4ºC. The supernatants, as a crude enzyme were stored at -80ºC until used [20]. Concentration of protein was determined according to Bradford (1976) method, using Bovine Serum Albumin (BSA) as a standard [21].

2.5.1 Determination of antioxidant enzyme activity

Superoxide dismutase (SOD) (EC. 1.15.1.1) activity was measured based on its ability to inhibit the photochemical reduction of Nitroblue Tetrazolium (NBT) according to Giannopolitis and Ries (1977) method. One unit of enzyme was considered as the amount of enzyme needed for reducing photoreduction of NBT to blue formazan by 50%. The reaction mixture contained 50 mM potassium phosphate buffer (pH=7.8), 13mM methionine, 75 μM NBT, 2 μM riboflavin and 0.1mM EDTA. Absorbance was measured at 560nm [22]. SOD activity was presented as enzyme units per milligram of protein (U/mg protein).

The activity of catalase (CAT) (EC 1.11.1.6) was determined according to the method by Dhindsa et al. (1981). The reaction mixture (3mL) containing 50mM potassium phosphate buffer (pH=7.0) (2.87mL), 15mM H_2O_2 (30µL) and 100µL of enzyme extracted. Absorbance was measured at 240nm. One unit of the enzyme was considered as the amount of enzyme extract that degraded 1 mM of H_2O_2 during one min. Activity was calculated using the extinction coefficient of $0.036mM^{-1}cm^{-1}$ for H_2O_2 [23]. CAT activity was presented as enzyme units per milligram of protein (U/mg protein).

Peroxidase (POD) (EC.1.11.1.7) activity was assayed according to the Plewa et al. (1991) method. The reaction mixture contained 50 mM potassium phosphate buffer (pH=7.0), 1% H_2O_2 and 4% guaiacol. Changes in absorbance at 470nm were measured by determining the oxidation of guaiacol to tetraguaiacol for 3min. POD activity was measured using the extinction coefficient of 26.6 $mM^{-1}cm^{-1}$ for tetraguaiacol [24]. POD activity was presented as enzyme units per milligram of protein (U/mg protein).

2.6 Measurement of Morphological Properties

To measure effects of Fe^{2+} on morphological characteristics of *M. officinalis,* the disinfected seeds were cultured in MS medium containing different concentrations of Fe^{2+} (0 (control), 10, 20, 30 and 40µM) for 45 days under similar condition as mentioned at section 2.2.

2.6.1 Seed germination

The germination began on second day and continued until thirteenth day. The number of germinated seeds (the least of the radicals length was around 1 mm) were counted and noted every day. Seed germination was reported as the percentage of germinated seeds number on thirteenth day.

2.6.2 Measurement of root and shoot length

The 45-day-old seedlings (20 numbers) were harvested from their medium, randomly. Their root and shoot length were then measured and reported in millimeters (mm).

2.6.3 Dry biomass

The 45-day-old seedlings (15 numbers) were randomly collected from their medium and were washed thoroughly by water. The samples were dried at 70ºC for 24 hours in an oven and then were weighed. The weights were reported in milligrams (mg).

2.7 Measurement of Chlorophyll and Carotenoid Content

Chlorophyll and carotenoids contents of Fe^{2+}-treated seedlings were determined according to the method described by Lichtenthaler (1987). Fresh samples (0.1g) were grinded to powder in acetone (85%, v/v). Afterwards, the homogenate was centrifuged at 10000 rpm for 15 min at 4ºC and absorbance of supernatants was determined at three wavelengths: 470, 646.8 and 663.2 nm by spectrophotometer   (Varian Cary 50, Australia) [25]. Concentration of pigments was calculated following formulas below:

Chlorophyll a = $(12.25 \times OD_{663.2\ nm} - 2.79 \times OD_{646.8\ nm})$
Chlorophyll b = $(21.21 \times OD_{646.4\ nm} - 5.1 \times OD_{663.2\ nm})$
Total Chlorophyll = Chlorophyll a + Chlorophyll b
Carotenoids = $[(1000 \times OD_{470\ nm} - 1.8 \times \text{Chlorophyll a} - 85.02 \times \text{Chlorophyll b})/198]$

2.8 Statistical Analysis

Experiments were performed in completely random designs. Each treatment was repeated at least three times. One way analysis of variance (ANOVA) was utilized to analyze data using SAS 9.1.3 (service pack 4, version= 6.1.7601) software. Duncan's Multiple Range Test was utilized to determine significance of the difference between mean values at P≤0.05. The results are presented as mean values ± Standard Deviation (SD).

3. RESULTS

3.1 RA Content

RA content significantly decreased in the treated seedlings with all Fe^{2+} concentrations compared to control treatment (Fig. 1). As shown in Fig. 1, content of this phytochemical reduces by increasing Fe^{2+} concentrations in media as well as time of treatment.
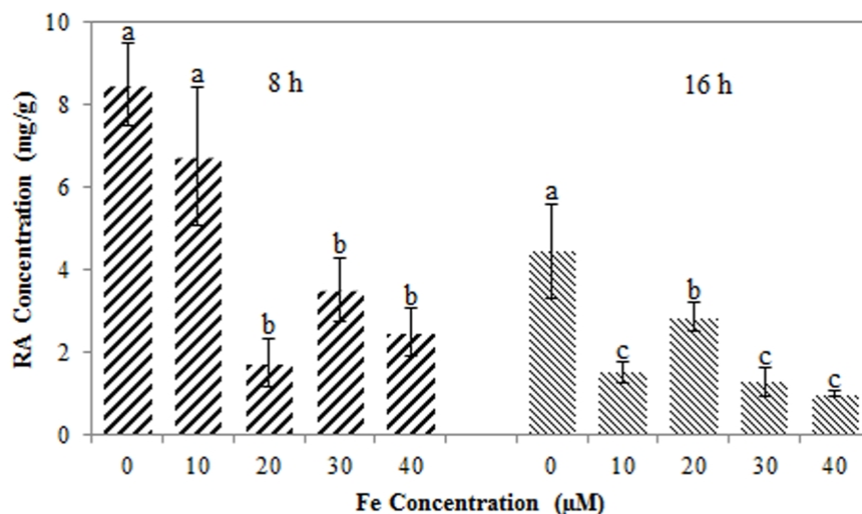

Fig. 1. RA content of control and Fe^{2+}-treated seedlings after 8 and 16 hours treatment
In each treatment time, signs with different letters indicate significant differences at P<0.05 according to Duncan's Multiple Range Test. Data are presented as the means ± SD (n=3)

3.2 Flavonoid Content

The flavonoid content of the Fe^{2+}-treated seedlings significantly decreased compared to control. Reduction of flavonoid content is more significant after 16 hours treatment compared to treatment for 8 hours (Fig. 2).

3.3 Anthocyanin Content

As seen in Fig. 3, anthocyanin content of Lemon balm treated seedlings with Fe^{2+} drastically reduced in compared to control at both time of treatment. The reduction of anthocyanin content was more remarked after 16 hours treatment compared to 8 hours.
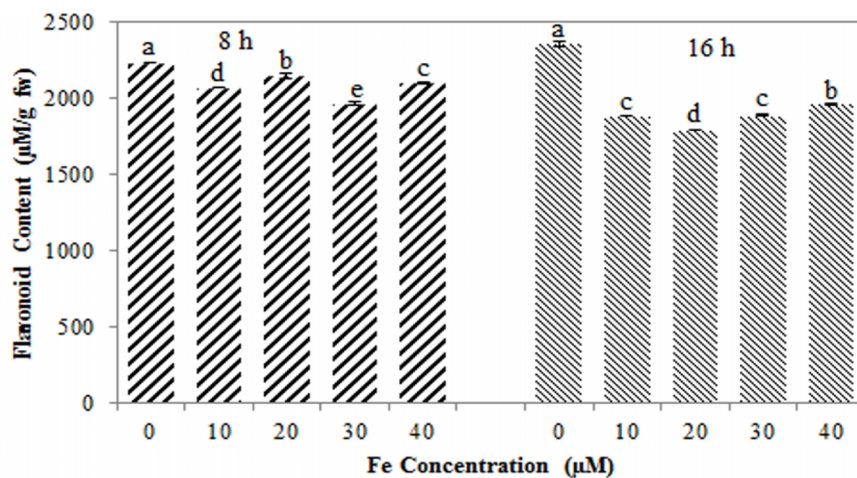

Fig. 2. Flavonoid content of Lemon balm seedlings in treatment with various Fe^{2+} concentrations at different time intervals (8 and 16 hours)
In each treatment time, signs with different letters indicate significant differences at P<0.05 according to Duncan's Multiple Range Test. Data are presented as the means ± SD (n=3)
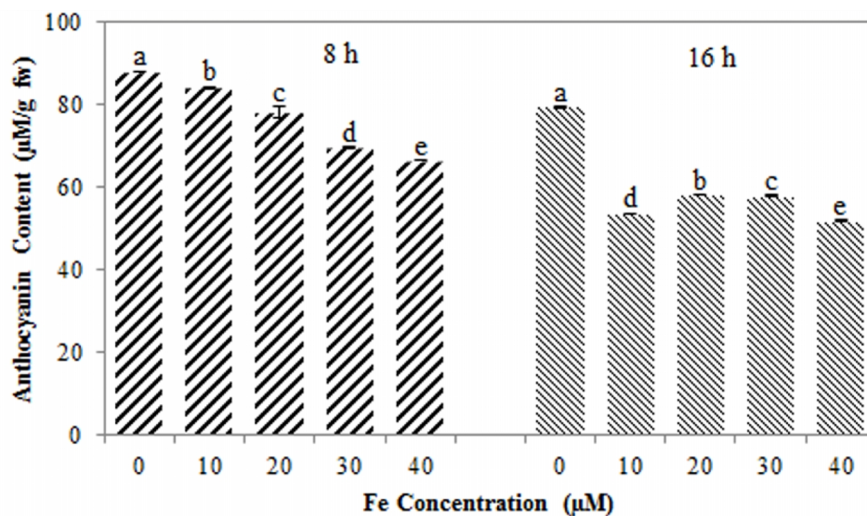

Fig. 3. Anthocyanin content of Lemon balm seedling in treatment with various Fe^{2+} concentrations at different time intervals (8 and 16 hours)
In each treatment time, signs with different letters indicate significant differences at P<0.05 according to Duncan's Multiple Range Test. Data are presented as the means ± SD (n=3)

3.4 Content of Total Protein

As seen in Fig. 4, the concentration of soluble protein in all treatments significantly decreased in comparison with the control at the both treatment times. Decreasing of total protein content was more remarked in treated seedlings after 16 hours treatment.
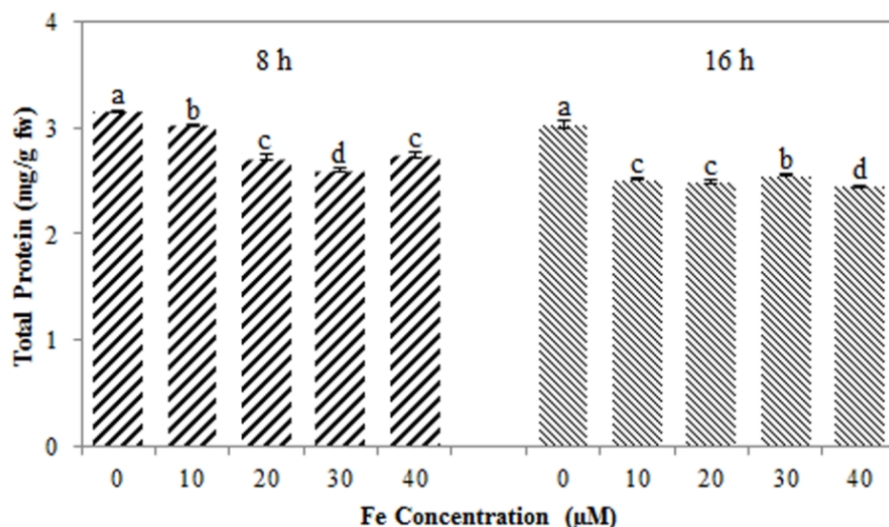

Fig. 4. Total protein content of treated Lemon balm seedlings with various concentrations of Fe^{2+} at different treatment times (8 and 16 hours)
In each treatment time, signs with different letters indicate significant differences at P<0.05 according to Duncan's Multiple Range Test. Data are presented as the means ± SD (n=3)

3.5 Antioxidant Enzymes Activity

The SOD enzyme activity was significantly promoted in all treated seedlings compared to the control at the both times of treatment. As shown in Table 1, the enzyme activity in presence of Fe^{2+} concentrations up to 20µM gradually increased after 8 hours treatment and thereafter decreased. Similar pattern was also revealed in the enzyme activity after treatment for 16 hours that the SOD activity gradually increased until 30µM of Fe^{2+} and thereafter decreased. It may be noticed that activity of this enzyme increased more significant at low Fe^{2+} concentrations after 8 hours treatment compared to the 16 hours.

The activity of CAT significantly decreased in Fe^{2+}-treated seedlings at both times of treatment compared to the control (Table 1). Considering the data, reduction of the enzyme activity was more significant during treatment with 10 and 20µM Fe^{2+} ions after 8 hours.

As reported in Table 1, POD activity was significantly promoted in Fe^{2+}-treated seedlings after 8 hours treatment compared to control (except for 30µM that was similar to the control). The highest POD activity was seen at concentration of 10µM and thereafter drastically decreased. But when the seedlings exposed to Fe^{2+} concentrations for 16 hours, a dramatic activity of the enzyme was seen only at 10µM Fe^{2+} concentration which at higher Fe^{2+} concentrations the POD activity decreased as the level of control.

3.6 Morphological Properties

As seen in Table 2, the germination rates were more than 85% in all treatments and there were no significant differences between treatments. Whereas shoot length of treated seedlings was similar to the control, root length decreased in presence of different concentrations of iron compared to the control. Decrease of root length was significant at the level of 5% at 20 and 40µM Fe^{2+} concentrations in comparison with control.

Table 1. SOD, CAT and POD activity of treated Lemon balm seedlings with various concentrations of Fe^{2+} at different treatment times (8 and 16 hours)

Fe Concentration (µM)	SOD (U/mg Protein)		CAT (U/mg Protein)		POD (U/mg Protein)	
	8 h	16 h	8 h	16 h	8 h	16 h
0	38.4±7.7[e]	51.6±17.2[d]	179.7±2.3[a]	161.9±1.8[a]	3.61±0.4[c]	3.51±0.3[c]
10	377.2±10.1[b]	232.5±17.5[b]	100.7±0.8[d]	119.9±1.5[c]	7.55±0.0[a]	4.44±0.2[a]
20	540.8±9.3[a]	246.1±6.7[b]	103.1±0.7[d]	137.8±2.0[b]	4.10±0.1[b]	3.81±0.4[bc]
30	90.9±6.8[d]	352.2±18.3[a]	137.7±0.5[c]	119.6±1.6[c]	3.51±0.1[c]	3.39±0.4[c]
40	155.2±4.3[c]	143.3±4.2[c]	159.6±1.1[b]	122.3±2.0[c]	4.49±0.2[b]	4.21±0.1[ab]

*Different letters in each group indicate significance at P≤0.05 according to Duncan's multiple range test. Data are presented as the means ± SD (n=3)

Table 2. Morphological properties of 45-day-old Lemon balm seedlings in treated with different concentrations of Fe^{2+}

Fe concentration (µM)	Germination (%)	Dry weight (mg)	Root length (mm)	Shoot length (mm)
0	91.1±0.57[a]	208±38.4[a]	74.2±8.01[a]	51.9±4.04[a]
10	86.6±1.00[a]	251±41.1[a]	68.4±1.44[ab]	50.8±4.11[a]
20	86.6±1.00[a]	239±14.0[a]	60.7±5.62[b]	57.0±0.92[a]
30	87.7±2.08[a]	200±31.5[a]	66.7±3.33[ab]	50.4±7.14[a]
40	88.8±1.15[a]	222±58.2[a]	64.6±3.11[b]	55.0±5.54[a]

*Different letters in each group indicate significance at P≤0.05 according to Duncan's multiple range test. Data are presented as the means ± SD (n=3)

3.7 Contents of Photosynthetic and Non-Photosynthetic Pigments

The contents of photosynthetic pigments including chlorophyll a, chlorophyll b and total chlorophyll; as well as, carotenoids content were drastically increased monotonically with increasing Fe^{2+} concentrations in media (Table 3).

Table 3. Chlorophyll and carotenoid contents of 45-day-old Lemon balm seedlings in treated with different concentrations of Fe^{2+}

Fe concentration (µM)	Chlorophyll a (mg/g fw)	Chlorophyll b (mg/g fw)	Total chlorophyll (mg/g fw)	Carotenoid (mg/g fw)
0	3.49±0.00[e]	1.34±0.01[d]	4.84±0.01[e]	1.04±0.00[e]
10	6.14±0.03[b]	2.36±0.00[b]	8.50±0.02[b]	1.79±0.00[c]
20	4.88±0.00[d]	1.88±0.00[c]	6.77±0.00[d]	1.45±0.00[d]
30	6.06±0.00[c]	2.34±0.01[b]	8.40±0.02[c]	1.83±0.01[b]
40	7.11±0.00[a]	2.61±0.00[a]	9.72±0.00[a]	2.08±0.00[a]

*Different letters in each group indicate significance at P≤0.05 according to Duncan's multiple range test. Data are presented as the means ± SD (n=3)

4. DISCUSSION

In the present study, antioxidant capacity including enzymatic (activity of SOD, POD and CAT) and non-enzymatic (RA, flavonoid and anthocyanin content) systems were investigated in Lemon balm seedlings in treatment with different concentrations of iron as an elicitor.

RA is the main active ingredient of the Lamiaceae family [26] with antioxidant activity [27] and numerous therapeutic properties [28]. Its biosynthesis as well as flavonoid and anthocyanin takes place through general phenylpropanoid pathway [29]. Based on the results, RA content is reduced in Fe^{2+}-treated seedlings compared to the control which accompanied with reduction of flavonoid and anthocyanin contents. Reduction of phenolic content is probably due to plant's defense system attempt to adjust the tension through enzymatic antioxidant systems. As suggested by Rajabbeigi et al. [30], the decrease in *Ocimum basilicum* L. essential oil content in treatment with iron, might be due to the reduction of phenolic compounds that were essential oil precursors or because the biosynthesis pathway of phenolic compounds are switched to produce other compounds such as malonyldialdehyde (MDA) in the presence of iron [30].

However, according to our results, enzymatic antioxidants system was more active than non-enzymatic antioxidants system. SOD activity that detoxify $O_2^{\bullet}$ and produce hydrogen peroxide [31], is more active in all treated seedlings (both treatment times 8 and 16 hours), especially at lower Fe^{2+} concentrations. H_2O_2 is a critical ROS molecule in induction of signaling network and a signal of oxidative stress [32]. It will be degraded by enzymes such as CAT and POD and produces H_2O and O_2. The activity of CAT tends to be reduced in Fe^{2+}-treated seedlings in comparison with the control. In contrast, the POD activity increased in treated seedlings compared to the control which was more significant at shorter treatment time (8 hours) and lower concentrations than longer time and higher concentrations of iron ions. As reported earlier, H_2O_2 scavenger enzymes act cooperatively, to keep it at normal levels [33]. Furthermore, several studies have suggested that CAT has a key role in degradation of H_2O_2 [34,35]. However, the POD has lower K_m to H_2O_2 than CAT [36]. These enzymes (POD and CAT) contain iron porphyrin [37], thus it seems that their activities is probably affected by iron concentrations [38]. Increase of antioxidant enzymes activity is a good proof for production of ROS in treated seedlings. It has been established that accumulation of superoxide radicals via the metal-catalyzed Haber-Weiss-type reaction leads to production of hydroxyl radicals [39]. These radicals can change enzymes activity; reduce gene expression, protein and soluble sugar content; release calcium from intracellular spaces; cause irreversible damages to biological membranes and nucleic acids as reported by many researchers [40,41].Therefore, it may be proposed that the inhibition of phenylpropanoid biosynthesis pathway (as revealed by the decline level of RA and anthocyanin and flavonoid contents) can be associated with the decrease in enzymes gene expression levels and/or inactivation or degradation of the enzymes involve in this pathway as clearly revealed by the decrease total protein content. Decrease of CAT activity at low concentrations (10 and 20µM) after 8 hours treatment may be attributed to high tendency of POD to H_2O_2 [42]. Overall, the decrease of CAT activity as well as POD and SOD activity at higher Fe^{2+} concentrations and longer time of treatment (16 hours) may be due to toxicity of ROS [42].

On the other hand, while the rate of germination and shoot growth was not affected by the iron, this element inhibited root growth in treated of Lemon balm seedlings. Therefore, it seems that the shorter roots can be related to the direct exposure of this organ to iron and

subsequent increase in uptake that lead to oxidative stress. The absence of any adverse effect of this ion on shoot growth can be attributed to its low mobility from root to shoot. Similar results were previously reported by Mehraban et al. [43]. They showed the shoot/root ratio increased by treatment of *Oriza sativa* L. with iron suggesting that iron toxicity affects root growth the most [43]. The elevated level of chlorophyll might be a result of its increased biosynthesis after uptake of iron [44]. While the enhancement of carotenoid content is a result of induce oxidative stress as reported by Munne-bosch and Alegre (2000) [45].

5. CONCLUSION

Overall, because the level of enzymatic antioxidant activity is promoted in Fe^{2+}-treated 45-day-old Lemon balm seedlings, it can be concluded that the ROS production level elevated. Accumulation of these radicals leads to oxidative damage as revealed by inhibition of phenylpropanoid biosynthesis pathway (reduction of RA, flavonoids, anthocyanin contents), reduction of CAT activity as well as total protein content. It seems that in Fe^{2+}-treated samples the oxidative stress induce suppression of proteins synthesis or degradation of them.

The inhibition of root growth in Lemon balm seedlings treated with iron for a long time (45 days) can be attributed to the induction of oxidative stress after uptake of this element. However, low rate of Fe^{2+} transport from root to the shoot and increased levels of photosynthetic and non-photosynthetic pigments may prevent further damages to this organ.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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