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Effects of iron nanoparticles on *Mentha piperita* L. under salinity stress

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The progress of nanotechnology presents many nanoparticles that are important in medicine, agriculture and industry. Quickly and entirely absorbed by plants, nano-compounds and remedy their nutrient deficiency and satisfy this need. Iron oxide nanoparticles with suitable surface chemistry can be used as a rich source of iron for plants due to its gradual release of Fe in a wide pH range (pH 3 to 11). The present study investigated the impact of iron oxide nanoparticles (Fe_2O_3NPs in 0, 10, 20 and 30 μM concentrations) on physiological parameters of peppermint (*Mentha piperita*) under salt stress (0, 50, 100 and 150 mM concentrations of NaCl). Fe_2O_3NPs caused increases in leaf fresh weight and dry weight, phosphorus, potassium, iron, zinc, and calcium contents of the peppermint under salinity stress but did not have an effect on the sodium element. 30 μM concentration of Fe_2O_3NP was more impressive. Lipid peroxidation and proline contents of the peppermint under salinity decreased significantly by applying Fe_2O_3NPs. The maximum activities of total antioxidant enzymes (I %), catalase, superoxide dismutase, and guailcol peroxidase were observed in plants treated with 150 mM of NaCl, but application of Fe_2O_3NPs declined these antioxidant activities. The results suggest that the appropriate concentration of iron nanoparticles could be used for stress resistance of the peppermint.

Keywords: antioxidants, Fe_2O_3 nanoparticles, peppermint, salinity

INTRODUCTION

Mentha piperita L. (peppermint) is a natural interspecific (*M. aquatic* × *M. spicata*) hybrid. It is an important medicinal and aromatic herb worldwide, in addition to its potential uses as a flavoring agent, in cosmetics, and pharmaceutical products among others (Rita, Animesh, 2011). The peppermint is widely used in medical and food industries (Roodbari et al., 2013). Some medicinal plants contain essential oils elicited from the upper parts of the flowering stems, dried leaves, and the whole plant (Singh et al., 2015). Abiotic environmental stresses, particularly salinity and drought, have the highest effect on medicinal plants (Heidari et al., 2008). Salt stress is one of the most important restricting factors for plant growth and production in dry lands. About 23% of the world's cultivated lands are saline and 37% aresodic (Jouyban, 2012). One of the major problems of saline and alkaline soils is iron deficiency due to high pH of the soil (Ksouri et al., 2007). Iron is an important element

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in crops because it is essential for many enzymes including cytochromes, which is involved in the electron transport chain, chlorophyll synthesis, and maintains the structure of chloroplasts (Mamatha, 2007). Iron is the fourth most abundant element on the Earth, but its amount is low or not accessible for the needs of plants and microorganisms because of low solubility of minerals containing iron in many places of the world, particularly in dry regions with alkaline soils. Iron is mostly found in the form of Fe^{3+} under aerobic conditions. It has little solubility and in many instances there is not enough iron to meet the needs of a plant. Fe^{+3} solubility decreases with increasing pH values (Eskandari, 2011). One of the solutions to address iron deficiency is the use of nanoparticles.

Nowadays, nanoparticles (NPs) of metals are widely used in many sections, such as medicine, agriculture, and industry (Paresh et al., 2009). Iron oxide nanoparticles (Fe_2O_3 NPs) have a large surface area and high reactivity. Moreover, when compared to many other metallic NPs, the iron oxide nanoparticles are constant, less expensive, and less toxic (Wannoussa et al., 2015). The Fe_2O_3 NPs have high magnetization amounts, a size smaller than 100 nm and a thin particle size distribution. These particles also have a special surface cover of magnetic particles, which has to be harmless and biocompatible (Wannoussa et al., 2008). In this study, the effects of different concentrations of Fe_2O_3 NPs on some physiological parameters and antioxidant activities of the peppermint under salinity were investigated.

MATERIALS AND METHODS

Plant material and treatments

The rhizomes of the peppermint were prepared at the Arak Agricultural Research Center. The rhizomes were cultured in plastic vases with a mixture of soil and perlite (1:1). The plants were maintained under 25/20 °C day/night temperatures with 14/10 hours (light/dark) photoperiods. Irrigation was done weekly with 100 ml complete Hoagland solution (containing iron-chelate for control plants), or 100 ml Hoagland solution without iron-chelate and containing different concentrations of Fe_2O_3NPs (0, 10, 20 and 30 µM) (Adamski et al., 2012). Different concentrations of Fe_2O_3 nanoparticles were prepared by the Prasad et al. (2012) method. Sodium chloride (NaCl) was added to the irrigation water in different concentrations of 0, 50, 100, and 150 mM, 18 and 21 days after planting.

Leaf fresh and dry weights

After 60 days of planting, leaf fresh weight was measured. Leaf dry weight was obtained by drying samples in an oven for 24 h at 75 °C until constant weight.

Element content evaluations

Potassium and sodium levels were measured using the method of Wang and Zhao (1995) based on flame photometry. K_2O and NaCl were used as potassium standard and sodium standard, respectively. The level of phosphorus was measured by spectrophotometry (Creus et al., 2004), while the calcium content was determined based on the complex ometric titration method (uses the EDTA for titration and murexide as an indicator) (Bao, 1981). Contents of iron and zinc were determined by flame-atomic absorption spectroscopy (Celik et al., 2004).

Proline and lipid peroxidation

Proline colorimetric determination was performed according to Bates et al. (1973) based on the reaction of proline with Ninhydrin. Lipid peroxidation was obtained as the amount of malondialdehyde (MDA) was determined by the thiobarbituric acid (TBA) reaction (Heath, Packer, 1969).

Antioxidant activity

Leaf fresh material (0.1 g) was powdered by liquid nitrogen and homogenized in 1 ml of 50 mM phosphate buffer (pH = 7), which contained 1 Mm Ethylene Diamine Tetra Acetic Acid (EDTA) as a homogenizer added to microtubes. Insoluble materials were removed by a refrigerated centrifuge at 13000 g for 20 min at 4 °C and the supernatant was used as

the source of enzyme extraction. The catalase (CAT) activity was evaluated by the Cakmak and Marschner (1992) method, and the activity determined that it would decrease in the absorbance at 240 nm following the decomposition of H_2O_2. The SOD activity was measured by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium according to the Giannopolitis and Ries (1977) method. The guaiacol peroxidase (GPOX) activity was measured based on the approach of Polle et al. (1994) and by monitoring guaicol oxidation at 470 nm.

Total antioxidant activity was evaluated as the scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. To determine this radical activity, the method of Abe et al. (1998) was used. Leaf fresh material (100 mg) was powdered by liquid nitrogen, homogenized in 1ml of 90% ethanol and then maintained at 4 °C for 24 h. Insoluble materials removed by centrifuge at 3500 g for 5 min. 20 µl of extracting solution was mixed in 800 µl of DPPH (0.5 mM in ethanol). The absorbance of the resulting solution was measured at 517 nm after 30 min in darkness. The ability to scavenge the DPPH radical was expressed by inhibition percentage (I %).

RESULTS

The XRD results revealed the presence of magnetite nanoparticles and indicated their sizes varied between 30 to 40 nm (Figure 1a). The SEM micrograph clearly illustrates that the magnetite nanoparticles sizes varied differed between 27.3 to 34.62 nm (Fig. 1b).

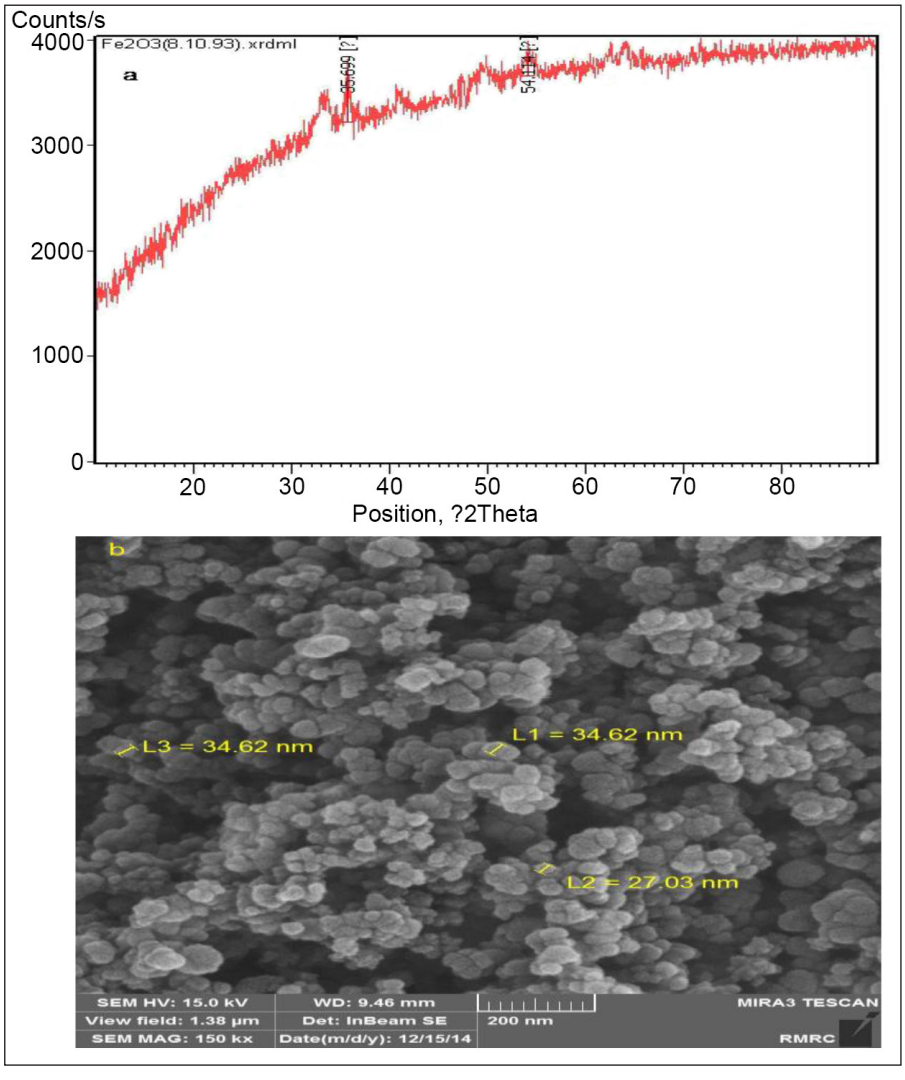

Fig. 1. (a) Representative XRD pattern of the α-Fe_2O_3 nanoparticles. (b) SEM image of Fe_2O_3

Variance analysis shows that the effects of salinity, of Fe_2O_3 nanoparticles, and of the interaction of both of them on the total of measured factors (except the effects of Fe_2O_3 nanoparticles on the sodium content) are statistically significant (Tables 1, 2). Our results indicated that leaf fresh weight (FW) and dry weight (DW) of peppermint leaves changed under different levels of salinity (0, 50, 100 and 150 mM). Fresh and dry weights of leaves were gradually decreased with increasing NaCl concentration (Table 1). Maximum amounts of decrease were obtained at 150 mM NaCl. Application of 10 µM Fe_2O_3 NPs and subtraction of FW and DW were changed to 76.98% in 150 mM NaCl. The highest values of leaf FW and DW at all concentrations of NaCl were recorded in 30 µM of Fe_2O_3 NPs. In this concentration of nanoparticles, the decrement both of FW and DW changed to 68.25% in 150 mM salinity (Table 3).

Salinity effects on the element content of *M. piperita* leaves were significant. With the increase of salinity levels (of 0 to 150 mM NaCl), sodium and phosphorus content of leaf increased, while the levels of potassium, iron, zinc and calcium decreased (Fig. 2). In 150 mM NaCl, 5.8- and 4-fold increases of sodium and phosphorus contents, respectively, were seen in comparison to the control plants. Minimum quantities of potassium (16.31 mg/g DW), iron (322.16 µg/g DW), zinc (1.91 ppm), and calcium (1.41 ppm) were found in plants treated with 150 mM salt and no iron. In 150 mM NaCl, the amounts of K, Zn, Fe, and Ca of leaves decreased by 67.31%, 62.54%, 63.12%, and 75.31%, respectively, in comparison to the control plants. The amounts of phosphorus, potassium, iron, zinc, and calcium enhanced by 68.63%, 29.80%, 22.75%, 42.11%, and 23.89%, respectively, in plants under treatments of

Table 1. **Results of variance analysis of the effects of salinity and Fe_2O_3 NPs levels on leaves**

Treatment	Index							
	FW	DW	Proline	MDA	CAT	SOD	GPOX	I%
Salinity	136.96**	118.27**	974.73**	126.86**	893.41**	258.47**	901.86**	624.12**
Fe_2O_3 NPs	28.16**	40.27**	25.16*	25.56*	17.93*	5.84*	7.65*	12.41*
Interaction between salinity and Fe_2O_3 NPs	54.35**	28.03**	9.23**	8.95**	5.91*	12.13*	8.26*	17.58*

*, ** = significant in probability value of $P \leq 0.05$ and $P \leq 0.01$, respectively; ns = not significant

Abbreviations: FW – leaf fresh weight, DW – dry weight, MDA – proline, malondialdehyde CAT – activity of catalase, SOD – superoxide dismutase, GPOX – guaiacol peroxidase, I% – total antioxidant activities of peppermint leaves

Table 2. **Results of variance analysis of the effects the levels of salinity and Fe_2O_3 NPs on sodium (N), phosphorus (P), potassium (K), iron (Fe), zinc (Zn), and calcium (Ca) of peppermint leaves**

Treatment	Index					
	N	P	K	Fe	Zn	Ca
Salinity	278.146**	129.107**	455.91**	77.62**	12.43**	455.91**
Fe_2O_3 NPs	2.54^{ns}	29.80**	25.82**	143.81**	32.48**	55.39**
Interaction between salinity and Fe_2O_3 NPs	74.08**	41.53**	14.67**	89.68**	8.71**	21.58**

*, ** = significant in probability value of $P \leq 0.05$ and $P \leq 0.01$, respectively; ns = not significant

Table 3. **Effects of different concentrations of NaCl, Fe-chelate and Fe_2O_3NPs on leaf fresh weight (FW) and dry weight (DW). Means followed by similar letters did not differ significantly when determined by the Duncan test**

		Leaf weight	
NaCl (mM)	Fe_2O_3 NPs (µM)	FW (g)	DW (g)
	0	$1.26^{e} \pm 0.008$	$0.26^{e} \pm 0.001$
0	C	$1.38^{d} \pm 0.037$	$0.13^{d} \pm 0.002$
	10	$1.56^{c} \pm 0.028$	$0.15^{c} \pm 0.02$
	20	$1.86^{b} \pm 0.011$	$0.18^{b} \pm 0.001$
	30	$2.12^{a} \pm 0.04$	$0.21^{a} \pm 0.004$
	0	$0.94^{i} \pm 0.02$	$0.094^{i} \pm 0.002$
50	C	$0.99^{hi} \pm 0.009$	$0.09^{hi} \pm 0.001$
	10	$1.03^{h} \pm 0.012$	$0.103^{h} \pm 0.001$
	20	$1.09^{g} \pm 0.008$	$0.109^{g} \pm 0.001$
	30	$1.18^{f} \pm 0.015$	$0.118^{f} \pm 0.01$
	0	$0.45^{m} \pm 0.015$	$0.045^{m} \pm 0.001$
100	C	$0.52^{l} \pm 0.015$	$0.052^{l} \pm 0.002$
	10	$0.68^{k} \pm 0.003$	$0.068^{k} \pm 0.001$
	20	$0.73^{k} \pm 0.025$	$0.073^{k} \pm 0.001$
	30	$0.88^{j} \pm 0.017$	$0.088^{j} \pm 0.01$
	0	$0.26^{n} \pm 0.005$	$0.026^{n} \pm 0.01$
150	C	$0.27^{n} \pm 0.003$	$0.027^{n} \pm 0.002$
	10	$0.29^{n} \pm 0.003$	$0.029^{n} \pm 0.003$
	20	$0.31^{n} \pm 0.008$	$0.031^{n} \pm 0.002$
	30	$0.4^{m} \pm 0.008$	$0.04^{m} \pm 0.003$

30 µM of Fe_2O_3 NPs and 0 mM of NaCl. The leaf sodium content was not changed by application of Fe_2O_3 NPs. The highest amounts of potassium (63.73 mg/g DW), iron (984.73 µg/g DW), zinc (6.75 ppm), and calcium (6.69 ppm) were obtained in plants with 30µM iron oxide nanoparticles with no salinity.

Fe_2O_3 NPs treatment caused an increase in phosphorus, potassium, iron, zinc, and calcium contents of peppermint leaves under salinity (Fig. 2). In 150 mM salinity with application 30 µM of Fe_2O_3 NPs, the levels of K (67.31%), Zn (62.54%), Fe (63.12%), and Ca (75.31%) changed to 52.88%, 48.04%, 47.58%, and 62.52%, respectively.

The increase in NaCl concentrations changed significantly the proline content and lipid peroxidation (MDA content) in leaf. Enhancement of NaCl levels increased proline and MDA amounts (Fig. 3). They came up to 9.98-fold increase for proline and 3.07-fold increase for MDA in relation to control plants. In addition, application of 0, 10, 20, and 30 µM Fe_2O_3 NPs varied proline and MDA amounts significantly. Proline and MDA levels decreased under different concentrations of Fe_2O_3 NPs (Fig. 3).

Antioxidant enzyme activities and I% (ability to scavenge the DPPH radical) were significantly changed under salinity stress. CAT, SOD and GPOX activities increased remarkably with different levels of NaCl stress. Moreover, I% enhanced significantly with the increase of NaCl from 0 to 150 mM. The highest percentage of I% was seen in plants treated with 150 mM NaCl. This value accounted for 45.60%, or 5.62-fold increase in relation to control plants. The lowest percentage of I% (7.07%) was obtained in 0 mM of salt and 10 µM of Fe_2O_3 NPs. Treatments with

iron oxide nanoparticles changed the levels of antioxidant enzymes. Plants treated with Fe_2O_3 NPs had lower activities of CAT, SOD, GPOX, and I% under salinity stress (Figure 4).

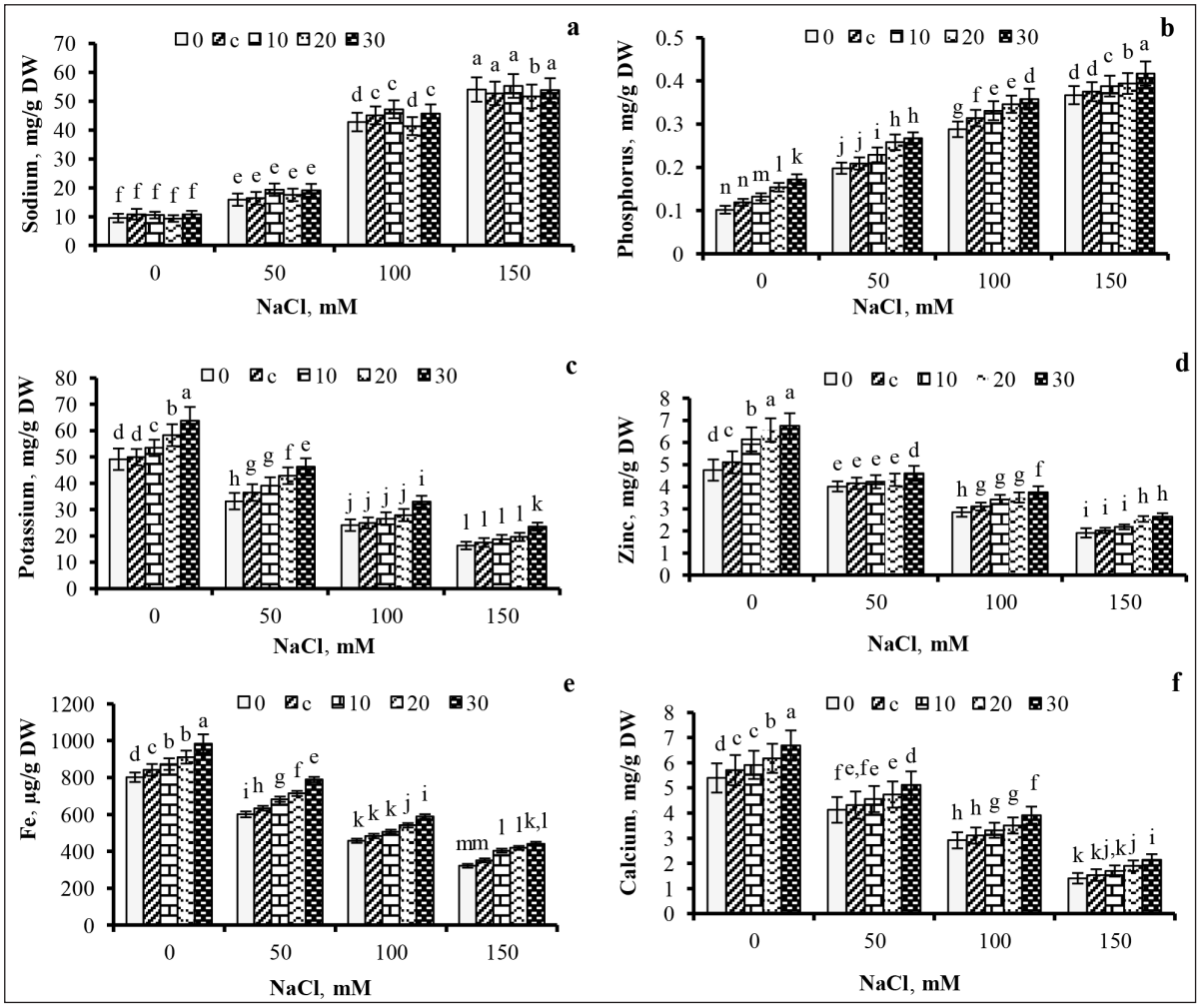

Fig. 2. Effects of different concentrations of NaCl, Fe-chelate, and Fe_2O_3 NPs on sodium (a), phosphorus (b), potassium (c), zinc (d), Fe (e), and calcium (f). Means followed by different letters differ significantly when determined by the Duncan test

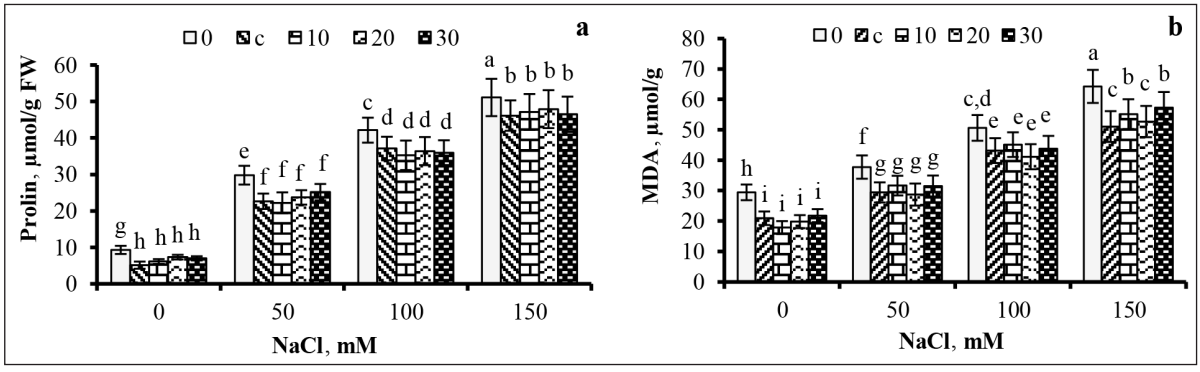

Fig. 3. Effects of different concentrations of NaCl, Fe-chelate, and Fe_2O_3 NPs on priline (a), MDA (b). Means followed by different letters differ significantly determined by Duncan test

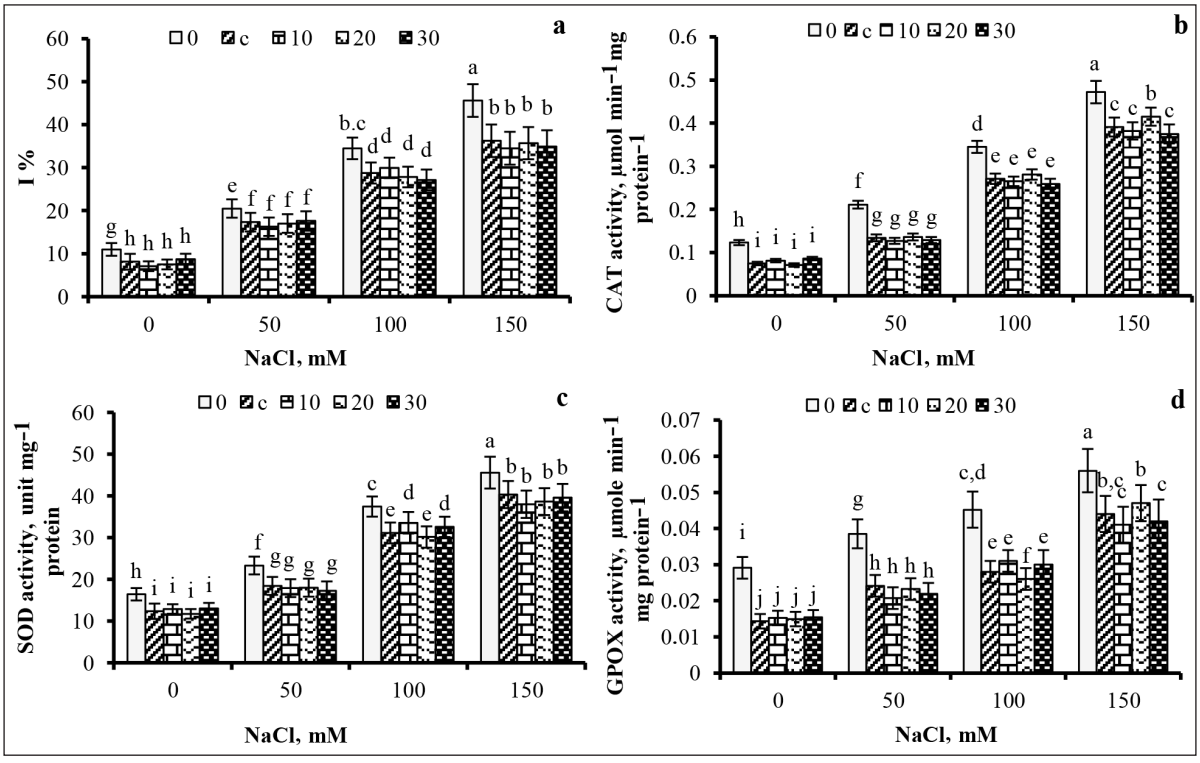

Fig. 4. Effects of different concentrations of NaCl, Fe-chelate and Fe_2O_3 NPs on I% (a), CAT (b), SOD (c) and GPOX (d). Means followed by different letters differ significantly when determined by the Duncan test

DISCUSSION

FW and DW of peppermint leaves decreased in different concentrations of NaCl. Several factors, such as reduction of photosynthesis, degradation of cell membranes, reduction of water available to plants, and Na^+ accumulation in the leaves were the main causes of weight loss under the salt stress (Hajiaghayi-Kamrani et al., 2013). Also, results showed that Fe_2O_3 NPs treatments increased the FW and DW values as compared to control plants. Iron was directly involved in the photosynthetic activity of plants and, consequently, their growth and productivity. Typically, approximately 80% of iron was found in photosynthetic cells where it was essential for the biosynthesis of cytochromes and other heme-containing molecules, including chlorophyll, the electron transport system, and the construction of Fe-S clusters (Briat et al., 2007). Nano-compounds are quickly absorbed by plants and supply the plants with required nutrients. Therefore, an increase in plant growth occurs with the use of nanomaterials (Mohammadipour et al., 2013). Shankramma et al. (2015) reported that Fe_2O_3 magnetic nanoparticles enhanced the growth of *Solanum lycopersicum* plant. They also showed that Fe^{2+}/Fe_{total} increased with the enhancement of NPs concentration and >45% ferrous iron. In Alidoust and Isoda (2013) research, Fe_2O_3 NPs were given to soybean by foliar application and soil route. Growth parameters and photosynthetic potential significantly enhanced by foliar application of NPs but this enhancement was far lesser when NPs were given to the plant via soil route which may be due to the precipitation of Fe ions. In our study, the leaf content of sodium and phosphorus increased and potassium, iron, zinc, and calcium of the peppermint decreased with rising salinity levels. Increased production of reactive oxygen species (ROS) under the NaCl stress may result in plasma membrane peroxidation, thereby affecting cell membrane integrity. These changes modulate the pattern of ion

leakage and uptake (Ashraf, Ali, 2008). The cellular membrane dysfunction due to the salt stress leads to non-selective uptake of Na^+ by plants. Shoot Na+ concentration was positively correlated to membrane permeability indicating that membrane damage resulted in excessive Na^+ uptake (Hejazi-Mehrizi et al., 2011). The increase of salinity level decreased shoot K^+ and Ca^{2+} concentrations due to Na^+/K^+ and Na^+/Ca^{2+} antagonism (Tuna et al., 2008). The rise of the P concentration caused by salinity may be due to the plant losing control of phosphate uptake and transport to the shoot. The increased P accumulation in the shoot is likely controlled at the root and is free of the salt element. Redondo-Gomez et al. (2011) reported that Fe content in *Spartina densiflora* diminished by application of salinity stress. Fe_2O_3 NPs treatment resulted in increased levels of phosphorus, potassium, iron, zinc, and calcium of the peppermint under salinity. The leaf sodium content was not affected by salinity. Phosphorus, potassium, iron, zinc, and calcium enhanced in plants under treatments of 30 µM of Fe_2O_3 NPs and 0 mM of NaCl. An increase in the calcium uptake by iron spraying was reported (Ravi et al., 2008).

Proline and lipid peroxidation (MDA content) enhanced by the increase of NaCl concentrations. As a compatible molecule in the cell, proline mediates osmotic adjustments, stabilizes sub-cellular structures, and scavenges free radicals. In addition, proline reposition may decrease stress-induced cellular acidification or first oxidative respiration to provide energy for recovery (Tan et al., 2008). MDA is a genotoxic yield of enzymatic and ROS-induced lipid peroxidation. When plant tissues are damaged under stress, the activity of the membrane would be disturbed and electrolytes inside the cell would leak outward. Changes in membrane permeability involve changes in the chemical combination of membranes including lipid peroxidation caused by ROS (Takáč, 2004). Determining the proline and MDA amounts has often been used as a tool to assess the degree of plant sensitivity to the oxidative damage (Ahmad et al., 2008). Treatment with Fe_2O_3 NPs reduced proline and MDA contents in peppermint plants significantly. Researchers have shown that micronutrients can reduce the impressions of environmental stresses such as salinity stress (Wang et al., 2011), and the application of Fe_2O_3 NPs can supply these micronutrients. Remarkable increases in CAT, SOD, and GPOX activities were observed in different concentrations of NaCl. Samples treated with iron oxide nanoparticles demonstrated significantly lower CAT, SOD and GPOX activities compared with the controls under the salinity stress. I% enhanced significantly under salinity stress. The increase of I% means that more antioxidants have been produced. Treatment with Fe_2O_3 NPs decreased I% levels in the treated plants. It is known that plant responses to the salt stress are multigenic, involving both osmotic and ionic homeostasis, as well as cell detoxification. The efficiency of the latter process is dependent upon the plant antioxidant defense mechanisms. The scavenging system forms the primary defense line in protecting the pea (*P. sativum*) cultivars against superoxide radicals (Ahmad et al., 2008). Our results were in agreement with other studies that demonstrated that total antioxidant activity and activities of CAT, SOD, and GPOX enzymes increased under salinity stress (Singh et al., 2015). The biotic and/or abiotic stress conditions over crop systems accelerate the formation of ROS resulting in the oxidative damage at the cellular level. Thus, antioxidant enzymes function to intercept the cascades of unconstrained oxidation in some organelles. These antioxidative enzymes play a role in eliminating H_2O_2 and are dispersed in at least four separate cell partitions (Gunjan et al., 2014).

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GELEŽIES NANODALELIŲ POVEIKIS *MENTHA PIPERITA* L. ESANT DRUSKINGUMO SUKELTAM STRESUI

Santrauka

Nanotechnologijų nanodalelės yra svarbios medicinai, žemės ūkiui ir pramonei. Tam tikro paviršiaus geležies oksido nanodalelės gali būti naudojamos kaip geležies šaltinis, nes nuo jų palaipsniui atsiskiria Fe plačiame pH intervale (ph 3–11). Šiame straipsnyje pateikiami skirtingų geležies oksido nanodalelių (Fe_2O_3 nanodalelių koncentracijos – 0, 10, 20, ir 30 μM) poveikio fiziologiniams pipirmėtės (*Mentha piperita*) parametrams, esant druskingumo sukeltam stresui (NaCl koncentracijos – 0, 50, 100 ir 150 mM), tyrimų rezultatai. Nustatyta, kad Fe_2O_3 nanodalelės didina pipirmėtės šviežių lapų ir sausą svorį, taip pat fosforo, kalio, geležies, cinko ir kalcio kiekį, bet neturi įtakos natriui. Pipirmėtės lipidų peroksidacija ir prolino kiekis gerokai sumažėja naudojant Fe_2O_3 nanodaleles. Gauti rezultatai rodo, kad atitinkamos koncentracijos geležies nanodalelės gali būti naudojamos didinant pipirmėčių atsparumą druskingumo sukeltam stresui.

Raktažodžiai: antioksidantai, Fe_2O_3 nanodalelė, pipirmėtė, druskingumas