Selenium enhances nutrient uptake and rosmarinic acid biosynthesis in *Melissa officinalis L.* under salinity stress

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Abstract

Salt stress is a serious problem facing plant growth and development. Selenium (Se) could improve plant growth and reduce stress. Hence, the aim of this study was to determine whether Se application could alleviate negative effects of salinity stress on *Melissa officinalis* L. Different salinity levels in this research were control (0), 50, 100, and 200 mM. Simultaneously, sodium selenate (Se) (0 and 50 mg L^{-1}) was applied. Salinity showed adverse effects on different plant growth parameters as well as pigment content. Se at 50 mg L^{-1} increased the vegetative growth of *M. officinalis* plants under different salinity levels. Salt induced oxidative stress conditions by increasing malondialdehyde and proline content, and Se foliar spraying enhanced antioxidative enzymes activity such as superoxide dismutase and catalase. Compared with control group, selenium accumulation in shoot and root significantly increased with Se levels increment. Selenium application increased N, Ca, K, and Mn accumulation. The foliar spray of Se increased rosmarinic acid compared to salt and non-salt treated plants. It is concluded that the application of Se can mitigate salt stress damages on *M. officinalis* plants and enhance mineral uptake.

Keywords: *Selenium; rosmarinic acid; Melissa officinalis; salinity*

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Introduction

Melissa officinalis L. is a perennial herbaceous plant known as lemon balm and belongs to the mint family or Lamiaceae. *M. officinalis* is native to Iran, Central Asia, the Mediterranean Basin, and South-central Europe. Essential oil and extract of this plant is used in various food and beverage products (Sharopov and Setzer, 2018). It was reported that *M. officinalis* had medicinal compounds that can be used to treat different illnesses. Properties like antioxidant, anti-mutagen, anti-inflammatory, anti-hyperglycemic, and memory impairment are some of the biologically important functions which have been confirmed in several experimental methods (Eudes Filho et al., 2017; Hasanein and Riahi, 2015; Jahanban-Esfahlan et al., 2017; Shinohara et al., 2015).

Worldwide, soil salinity is one of the most crucial environmental problems. Sodium chloride (NaCl) is the most soluble and abundant constituent, causing salt stress by negatively

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affecting all the physiological processes in the plants. Excess ions, such as Na^+ and Cl^-, lead to osmotic stress and ionic toxicity; consequently, inhibiting the plant growth and development by disrupting numerous physiological processes (Liu et al., 2016) such as water uptake, metabolic processes, nutrient composition, osmotic adjustment and hydraulic conductivity (Hashem et al., 2016). Although it has been demonstrated that salinity to somewhat can create soil structure improvement, it can also result in unsolicited effects on photosynthetic pigment formation, proline content and rosmarinic acid biosynthesis (Akcin and Yalcin, 2016). Amelioration of negative effects of salt by rosmarinic acid was reported by a study on potato explants (Solanum tuberosum L.) (Eskandari et al., 2018). Another recent report suggests that the effect of salt stress at a moderate level of 200 mM on Spinach (Spinacia oleracea L.) shoot cultures significantly accumulate 20-hydroxyecdysone, a chemical that plants synthesize for defense against phytophagous (plant-eating) insects as compared to untreated samples (Muchate et al., 2019).

Selenium (Se) is an essential element for humans and animals. This trace element also appears to be a substantial element for many plants. There is some evidence indicating that Se at low concentrations may create resistance in plants to environmental stresses (Ghazi, 2018). While there is no evidence for the need for Se in higher plants, Se exerts beneficial effects on plant growth at low concentrations. Se may alter different physiological and biochemical traits. Thus, plants physiologically and biochemically response to Se (El-Ramady et al., 2016).

Plant species have the capacity to accumulate Se and thus they can provide the adequate Se of the human body. In other words, the application of exogenous Se could improve the nutritional value of plants (El-Ramady et al., 2016). Se exhibited a remarkable role in mitigating the tolerance of salt in tomato (*Solanum lycopersiucm*), strawberry, garlic (*Allium sativum*), and maize (*Zea mays*) by enhancing the enzymatic activities of antioxidants and functioning in maintaion of the photosynthetic system (Ashraf et al., 2018; Astaneh et al., 2018; Diao et al., 2014; Jiang et al., 2017; Zahedi et al., 2019). It seems that Se could induce the contents and activity of RA-associated genes. In their study, Babajani et al. (2019) reported that the exposure of *M*. *officinalis* to Se supplementations in a dose-dependent manner could significantly induce the RAS expression gene (Babajani et al., 2019).

The aim of this study was to evaluate the effect of selenium on mineral uptake and rosmarinic acid content in *M. officinalis* under salt stress conditions. We also wished to know whether Se can ameliorate the negative effects of salt.

Materials and Methods

Plant material, culture condition, and treatment

Seeds of *M. officinalis* (Mashhad Seedlings and Seeds Co.) were cultivated on the sand culture media. Pots containing seeds were transferred to the greenhouse with a 16/8 light/dark cycle (irradiance: 120-150 µM m^{-2} s^{-1}). After germination, the seeds were irrigated with different concentrations of Hoagland solution 25%, 50%, and 100%. On the 17th day, the saline stress was exerted at different concentrations of 0, 50, 100, and 200 Mm every other day. Also, Se (0 and 50 mg L^{-1}) was sprayed to plants every two days. Four weeks after the start of the treatments, the plants were harvested.

Chlorophyl assay

Chlorophyll a and b were measured by lichtenthaler's method (1978). For this purpose, 0.5 g of leaf tissue was weighed and then rubbed out with 4 ml of 80% acetone. The mixture was centrifuged at 4000 rpm for 5 minutes to smooth and remove additional parts of the plant. The supernatant was then transferred to another test tube, to which was added another 14 ml of acetone 80%. To measure chlorophyll a and b a spectrophotometer (T80 model) was calibrated and zeroed using acetone 80% at 664, 647, and 470 nm wavelengths. The absorbance of the solutions was then read and the concentration of chlorophylls was calculated (lichtenthaler and Wellburn, 1983).

RA assay

The plant tissue (0.5 g) and 20 ml of ethanol 50% were mixed. The mixture was transferred to a 70 °C water bath. The solvent of ethanol was evaporated using rotary apparatus. The extract was dissolved in 70% ethanol. The dissolved extract was transferred to -20 °C refrigerator for 24 hours. Then, it was passed through the Whatman paper and was stored in the refrigerator until the assay day. The absorbance of samples was gained using 327 nm wavelength (Tepe 2008).

Proline content

The Bates et al. (1973) technique with slight modification was applied to analyze the proline content. First, 0.2 g of frozen leaf tissues were homogenized in 3% sulfosalicylic acid solution (4 ml) and then Whatman filter paper was used to filter the solution. Two ml of a solution of Ninhydrin and 2 ml of acetic acid along with 2 ml of the extract were placed at 100 °C for one hour. The cooling process was done using an ice container to stop the reaction. The toluene solution (4 ml) was added to the test tube and agitated quickly for 30 seconds. The supernatant absorbance was read at 520 nm after 20 minutes. The proline content was calculated using the standard curve (range of 20-100 mg ml^{-1}) (Bates et al., 1973).

Catalase and superoxide dismutase activities

Pereira et al. (2002) method was used to measure the activity of catalase (CAT) enzyme. Potassium phosphate buffer pH 7 (15 mM) and hydrogen peroxide (30 mM) was used. Ten µl enzyme extract was necessary in a final volume of 3 µl to start the assay. The absorbance was immediately recorded at 25 °C by 240 nm for 2 minutes and once every half minute with a spectrophotometer. Based on the breakdown of a mole of peroxide per gram fresh weight, the enzyme activity was analyzed in different treatments (Pereira et al., 2002). To prepare the enzyme extract to determine the activity of antioxidant enzymes of superoxide dismutase enzyme (SOD) and CAT, Sairam et al (2002) method was used.

Beauchamp and Fridovich (1971) method were used to determine SOD activity. The optical reduction of Nitro Blue Tetrazolium (NBT) was used to evaluate the activity of the SOD enzyme at wavelength 560 nm. To do this, 50 mM phosphate buffer solution (pH 7.5) was prepared at the beginning. Then, Riboflavin 4 mM, EDTA 0.1 M, methionine 13 mM, and NBT 75 mM were added to prepare the reaction mixture. The container was well covered with aluminum foil to avoid light intensity, and then potassium phosphate buffer was added. Forty µl of the enzyme extract was added to the final volume of 3 ml and then the tube was placed near a light bulb (40 W) (distance: 50 cm). The absorbance recording was performed at 560 nm, and one unit of enzyme activity was considered as that amount of enzyme, which declined the absorbance reading to 50% in comparison with tubes without enzyme (Beauchamp and Fridovich, 1971).

Malondialdehyde content

Hess and picker (1969) method was used to determine membrane lipid peroxidation which was performed based on the formation of a Malondialdehyde (MDA) complex. First, 0.5 grams of fresh leaf tissue was rubbed with 5 mL trichloroacetic acid (TCA) 0.1%. Then, the homogenized solution was centrifuged for 5 min at 15000 rpm. One ml of the supernatant was mixed with 3 ml of TCA 20% containing thiobarbituric acid (TBT) 0.5%. The resulting solution was placed in a 90 °C water bath for 30 min. The solution was placed into ice and then centrifuged for 5 min at 15,000 RPM at 4 °C, and the absorbance was measured at 532 nm. The absorbance of compounds other than MDA was also read at 600 nm. In the end, the OD's difference was calculated using subtraction of absorbance at 600 nm and 532 nm (Heath and Packer, 1968).

Estimation of mineral nutrient concentrations

Shoot dried samples were acid digested and Na, K, Mn, and Ca were determined using the method of Wolf (1982) utilizing a flame photometer. Nitrogen content was determined in

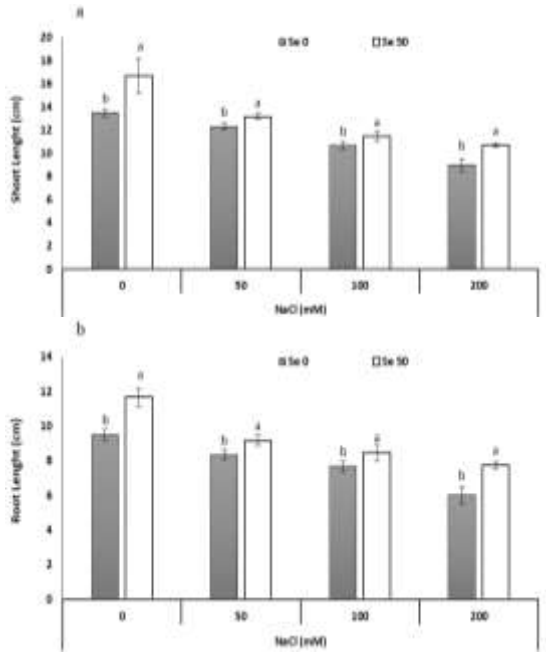

Fig. I. Selenium effect on shoot length (a) and root length (b); data are the means of three independent replicates ± SD; columns followed by the same letter are not significantly different, according to LSD, p<0.05.

dried shoot colorimetrically as described by Lueck and Boltz (1958). Selenium content was determined by addition of 1 M hydrochloric acid to the processed dried shoot powder. Concentrations of Se were analyzed by atomic absorption spectrophotometer.

Statistical Analysis

Data analysis was carried out in a completely randomized design with three replications and statistical analysis was performed using SPSS software version 16. One-way analysis of variance (ANOVA) and Fisher's Least Significant Difference test (LSD) were performed to compare different saline treatments after slicing. The probability level p<0.05 was used to determine which means are significantly different. Excel software was used for drawing charts.

Results

The results of growth characterizations of shoot length (SL), root length (RL), shoot dry weight (SDW), and root dry weight (RDW) are depicted in Fig. I. The SL, RL, RDW, and SDW of *M. officinalis* were significantly reduced by salinity.

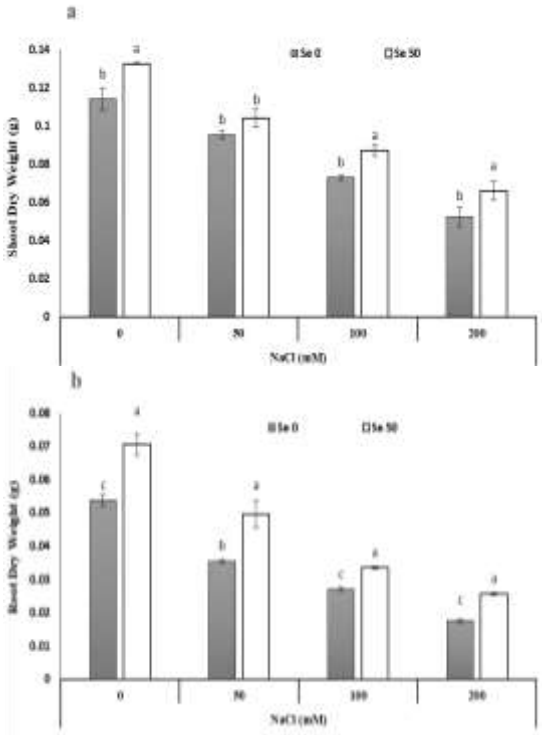

Fig. II. Selenium effect on shoot dry weight (a) and root dry weight (b). Data are the mean of three independent replicates ± SD; columns followed by the same letter are not significantly different, according to LSD, p<0.05.

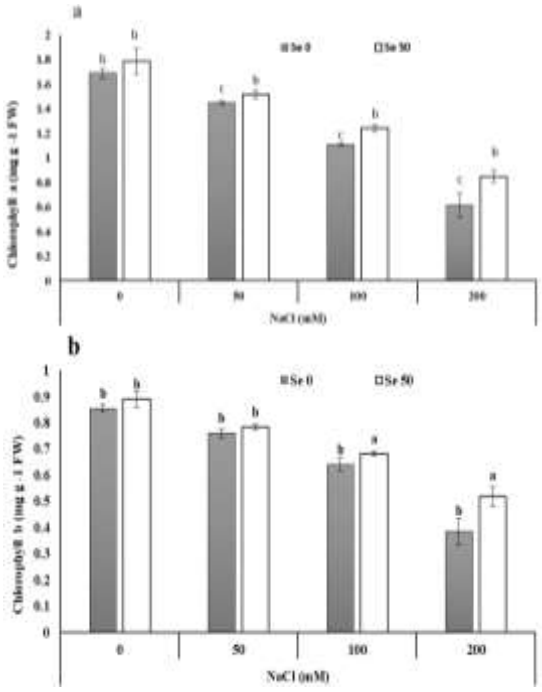

Fig. III. Selenium effect on chlorophyll a (a) and b (b) contents in *M. officinalis*; columns followed by the same letter are not significantly different according to LSD (p<0.05).

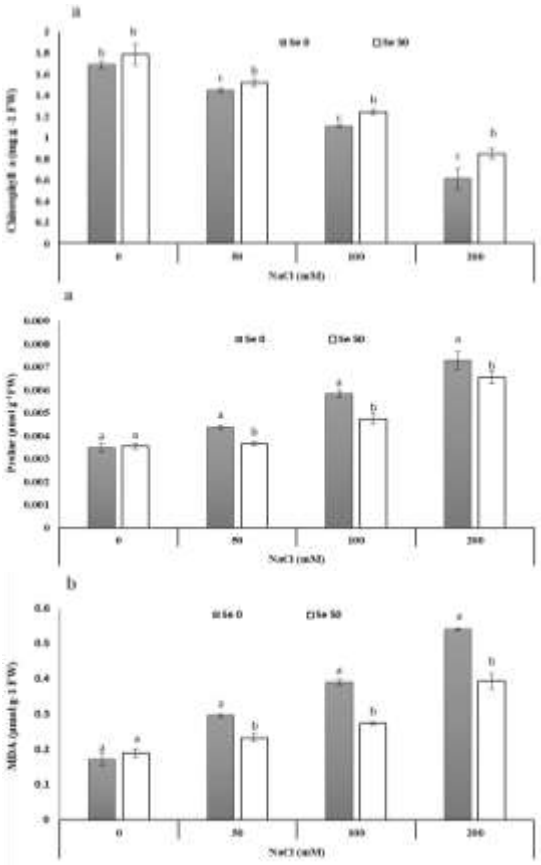

Fig. IV. Selenium effect on proline and lipid peroxidation (MDA) content; columns followed by the same letter are not significantly different according to LSD ($p<0.05$).

Data analysis also revealed that SL and RL of *M. officinalis* treated with 50 mg L^{-1} Se had significantly more length versus the non-saline control ranging from 2.6 to 0.4 cm for SL and 3.2 to 0.8 cm for RL. Similarly, a remarkable decrease was recorded in SDW and RDW after severe salinity treatment and the results after applying the Se treatments were also the same as that of SL and RL (Fig. II).

Chlorophyll (a and b) decreased significantly after treatment of the plants with different levels of salt concentration ranging from 0 to 200 Mm. In addition, the foliar spraying of the Se significantly increased the pigment content in comparison to the none-Se-treated condition (Fig III).

In the case of proline and MDA contents, results showed that the proline and MDA accumulate significantly in *M. officinalis* treated with 50-200 mM NaCl. It is also obvious that in plants treated with 50 mg L^{-1} Se the proline and MDA contents decreased under different saline

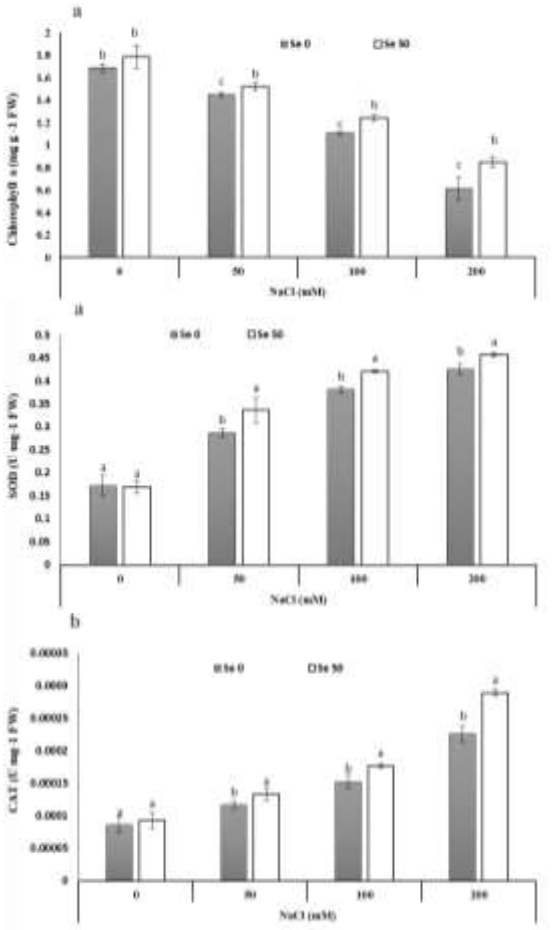

Fig. V. Effect of Se treatment on SOD and CAT activities; columns followed by the same letter are not significantly different according to LSD ($p<0.05$).

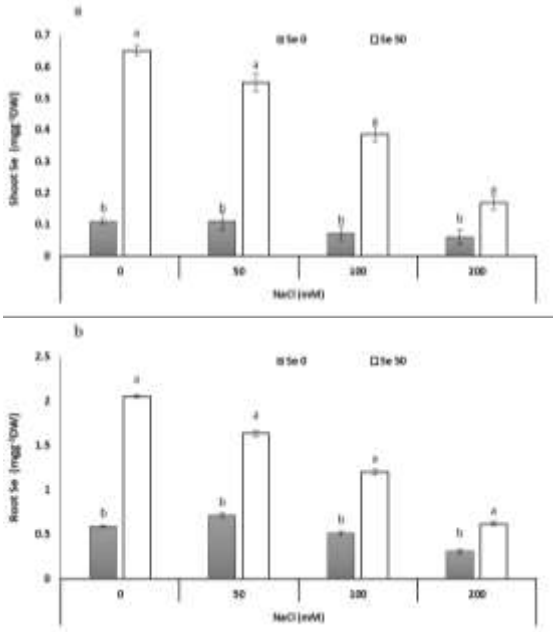

Fig. VI. Selenium accumulation in *M. officinalis* treated by Se and NaCl; columns followed by the same letter are not significantly different according to LSD ($p<0.05$).

Table 1
Shoot content of N, K, Ca, Mn, and Na

NaCl (mM)	Selenium (mg/L)	N (mg/g DW)	K (mg/g DW)	Ca (mg/g DW)	Mn (mg/g DW)	Na (mg/g DW)
0	0	461b	640.0e	541f	153b	145e
	50	484a	720.0d	590e	175a	144e
50	0	442c	843.0d	613e	98c	387c
	5	473ab	954.0c	664d	124bc	232cd
100	0	351e	922.0c	754cd	75e	450b
	5	383.0d	1053.0ab	798c	98c	390c
200	0	230.0f	1022b	904b	66e	587a
	5	263.0de	1143a	998a	85c	461b

Within each column, mean superscript with the same letter do not differ significantly (p≤0.05), LSD test

conditions (Fig. IV). Results also showed that salinity stress promoted a continuous increase in SOD and CAT activities. In addition, Se application in salt-stressed plants increased activities of SOD and CAT (Fig. V).

Compared with the control plants, Se accumulation in roots of Se-treated *M. officinalis* increased to 3.5-fold. In addition, in slat treated plants also foliar spray of Se increased Se concentration. The same results also were observed for Se accumulation in shoots but Se levels in shoot were lower than roots (Fig. VI).

Results clearly showed adding 50 mg L^{-1} Se could significantly enhance the RA production in 50 mM of salinity condition but when the concentration of salt increased up to 200 mM, the RA production experienced a dramatic decrease (Fig. VII).

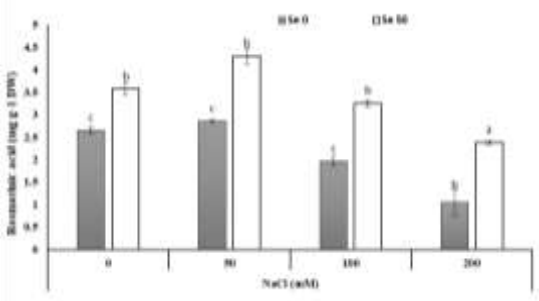

Fig. VII. Selenium effect on RA accumulation in *M. officinalis*; columns followed by the same letter are not significantly different according to LSD, p<0.05.

Discussion

Increasing salt concentration in plants may be problematic, but the decrease in growth was less seen in plants sprayed with 50 mg L^{-1} Se. Although the mechanism is still unknown, this study clearly demonstrated that the Se application can have a positive impact on growth parameters. Maneuvering on physiological and biochemical mechanisms demonstrated the beneficial role of Se in alleviating the negative effects of salinity on growth and fruit of strawberry plants studied recently which was in agreement with our study (Zahedi et al., 2019). Garlic is another plant whose physiological traits improved under NaCl stress using Se (Astaneh et al., 2018). In another study, salt-induced inhibitory effects were alleviated in maize plants after Se application resulting in growth and development (Jiang et al., 2017).

Hawrylak-Nowak (2009) investigated the salt alleviating properties of Se. Se treatments at 5 and 10 µM significantly improved the growth rate of cucumber (*Cucumis sativus* L.). The growth-promoting activity of Se at low concentrations of 5 and 10 µM could be a result of the antioxidative activity of Se (Hawrylak-Nowak, 2009). Bekhradi et al. (2015) reported that the physiological characteristics of the Iranian genotype of basil under salinity stress were examined. In the two Iranian cultivars of Green and Purple Iranian basil, the shoot length was significantly reduced (Bekhradi et al., 2015).

Astaneh et al. (2018) showed that applying 8 mg L^{-1} Se in garlic leaves treated with

30 mM NaCl and 4 mg L^{-1} under 60 mM salinity significantly improved chlorophyll contents (Astaneh et al., 2018). Evidence of another study suggests that the photosynthesis and antioxidative capacity of maize under salt-induced stress relieved by the application of Se (1 µM). Se application also enhanced the net photosynthetic rate and relieved the damage to chloroplast induced by NaCl. Ghazi (2018) reported that the salinity induced adverse effects on different plant growth parameters of the Coriander plant as well as total chlorophyll content. Under different salinity levels of irrigation wate,r Se-NP at 50 ppm showed the best effects on physiological characteristics and total chlorophyll contents of coriander plants (Ghazi, 2018).

Our results revealed accumulation of proline and MDA in arial parts of *M*. *officinalis* after treatment with salt, which was a sign of oxidative stress. The increment of antioxidative enzyme activities is a major mechanism for overcoming the oxidative conditions. like CAT and SOD enzymes activity. Hence, a remarkable increase in the activity of SOD and CAT by Se indicated that Se has useful impacts on salt tolerance of *M*. *officinalis* by elevating their antioxidant capacity (Feng, 2013).

Regarding the RA in our study, 50 mg L^{-1} of Se dramatically enhanced the amount of RA under salt which suggests an optimum condition for RA biosynthesis. Döring et al (2014) investigated the formation of the main phenolic ingredient of the pharmaceutically important plant *M. officinalis* under O3 stress. There was a quick up-regulation of both *RAS* and *PAL* genes from the beginning of the exposure. According to their result, there was also a close correlation between the specific activity of *RAS* and the decrease of RA concentration in *M. officinalis* leaves. According to our results, Se is an inducer for production of RA with or without salt stress. In the none-saline condition Se may induce the activity of genes and consequently the content of RA.

Salt stress increased Na contents since plants take up more Na when salt concentrations in the soil are high (Evelin et al., 2009). The increase in Na concentration disturbs the nutrient balance and osmotic regulation and cause ion toxicity (Munns and Tester, 2008). Also, salinity increased the K and Ca levels that was mostly because of the mechanism of abiotic stress resistance of *M. officinalis*. Moreover, Ca acts as a significant cellular messenger for plant growth signaling (El-Beltagi and Mohamed, 2013), which brings about raising the plant adjustment to salinity. Interestingly, the levels of N and Mn were decreased by salinity, which may be due to the antagonism between the essential nutrient and toxic ion, and furthermore to make them immobilized. The mechanism of selenium for enhancing mineral uptake in saline condition is not clear but there were many reports about antioxidative activity of Se. Interaction between Se and other elements have been established previously as they influence plant nutrient basis (Drahoňovský et al., 2016). The high contents of Mn, Ca, N, and K indicates that Se promoted their uptake. The reduced contents of Na in Se-treated plants proposed that Se prevented its uptake. There is some evidence of the positive effects of selenium on the growth and performance of tomato and canola (*Brassica napus* L.) (Hashem et al., 2013) at low concentrations. Sodium selenate has beneficial effects on the plants' growth and tolerance to the stresses through increasing their antioxidant capacity.

In general, it is concluded that Se can improve the negative effects of salinity. The interactive role of salinity and Se could promote the salt-induced inhibitory mechanisms in *M. officinalis*. The present study also suggests that Se foliar spray in *M. officinalis* is a useful strategy for improving *M. officinalis* tolerance to salinity. The beneficial effects of Se on *M. officinalis* growth performance under different salinity levels could be attributed to the protection of photosynthetic pigments for enhancing photosynthetic capacity, accumulation of minerals, and Rosmarinic Acid.

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