Investigation of the effect of selenium on growth, antioxidant capacity and secondary metabolites in *Melissa officinalis*

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Abstract

Melissa officinalis is a plant of Lamiaceae family with medicinal properties which is important for its aromatic, synthetic, and secondary metabolites. The aim of this research was to study the effect of selenium on secondary metabolites and antioxidant properties of *Melissa officinalis*. For this purpose, an experiment was done in a completely randomized design with four replications. The plants were treated with selenium (0, 0.2., and 5 µM). The experimental factors included root and shoot fresh and dry weights, protein contents, ascorbic acid, enzymes (catalase, ascorbate peroxidase, and superoxide dismutase), peroxide hydrogen, caryophyllene, and caryophyllene oxide. Results showed that application of selenium had positive effects on the wet weight of shoots and roots, dried weight of roots, and ascorbic acid, protein, enzyme (catalase, ascorbate peroxidase), peroxide hydrogen, caryophyllene, and caryophyllene oxide contents of the plants under study ($p \leq 0.05$). Also, high concentration of selenium (5 µM) lead to increased z-citral, citral, and geranyl acetate contents of *Melissa officinalis* essential oils while caryophyllene oxide content increased as a result of low concentration (0.2 µM). Therefore, application of selenium is concluded to play an effective role in increasing secondary metabolites in *Melissa officinalis*. In general, the study suggests that low concentration of selenium increases the growth of *Melissa officinalis* plants and improves their growth factors and morphology.

***Keywords*:** essential oils; *Melissa officinalis*; selenium

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Introduction

Melissa officinalis L. is a dicotyledon plant belonging to the Lamiales order of Lamiaceae family. It is an important medicinal plant that has found applications in medicinal, hygienic, and food industry for its special aromatic compounds and is widely used in the traditional medicine (Meftahizade, 2010). In Persian traditional medicine, *Melissa officinalis* L. has been used as a medicinal plant with a wide range of applications such as lowering the heart rate and as an anti-inflammation, antiviral, and antioxidant agent, local pain killer, neurotropic, sedative, cholinergic receptors' connectors, and also in intoxication with poisonous mushrooms (Akhondzadeh et al., 2003).

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Citral is a main compound in *Melissa officinalis* L. extract which has a strong lemon odor and is used in perfume industries. Caryophyllene, another important compound in *Melissa officinalis* L., is used as a flavoring in seasoning, and chewing gums (Chung et al., 2010).

Selenium is a metalloid in a diagonal area in the p-block region of the periodic table which because of its position near Sulphur, shares similar properties with it (Freeman et al., 2006). Although it is a necessary element for human, selenium is not necessary for plants and it is even poisonous at high concentrations. On the other hand, selenium has favorable effects on plant cell metabolism at low concentrations, regulating uptake of some ions (Rayman, 2008). Selenoproteins play an important role in several vital functions including formation of thyroid hormones, DNA synthesis, fertility and propagation, and antioxidant activities. Selenium can turn into various metabolites in the organisms. Some selenium compounds such as selenol have a remarkable role in prevention of cancer. This element also along with vitamin E plays a role in improving the muscle performance and recovery and slowing down the aging process (Yousef et al. 2013). Recent studies have shown that selenium not only improves the plant growth and development, but also increases the antioxidant capacity of the plants and their resistance against various stress conditions (Djanaguiraman et al., 2005).

As for accumulation of selenium, plants are divided into three groups including first grade selenium accumulators, second grade selenium accumulators, and non-selenium accumulators. Non-selenium accumulators contain less than 25 mg selenium per kg dry weight and most crops including cereals, grasses, fruit, vegetables, and weeds belong to this group. Second grade selenium accumulators usually grow normally in the media with low concentrations of selenium and can accumulate 25-100 mg Se/kg plant dry weight. First grade selenium accumulators can accumulate 100-100000 mg Se/kg plant dry weight (Terry et al., 2000).

Selenium is found in both organic and mineral form in the nature. The mineral form in the nature includes selenate (Seo^{-2}_4), selenite (Seo^{-2}_3), and selenide (Se^{-2}) while the main organic forms are selenocysteine (SeCy) and selenomethionine (SeMe) (Sors et al., 2005; Bodnar et al., 2012; Wu et al., 2015). Absorption, translocation, and distribution of selenium depends on the plant species, growth stages, form and concentration of selenium, physiological conditions (e.g., soil salinity and PH), and the mechanism of translocation in the plant (Zhao et al., 2005; Li et al., 2008; Renkema et al., 2012). The common form in agricultural soils is selenate which is more soluble in water compared with selenite (Sors et al., 2005; Missana et al., 2009). The aim of this study was investigating the effect of selenium on secondary metabolites and antioxidant properties of *Melissa officinalis*.

Materials and Methods

Seeds of *Melissa officinalis* L. were obtained from Pakan Bazr Co., Isfahan and the plants were grown using hydroponic method in the greenhouse of Payame Noor University, Najaf Abad Branch. The seeds were sterilized with sodium hypochlorite commercial 10% solution and were washed several times with distilled water to prepare for sowing. The sterilized seeds were spread on wet perlite and covered with a layer of perlite for germination. The seeds were irrigated with distilled water every two days and when necessary, every day. The seedlings were transferred to a hydroponic medium after one month. Two-litter containers were used for plants and the hydroponic cultivation medium was prepared based on Long Ashton nutrient solution (Hewitt, 1966) containing both micronutrients and macronutrients with the pH set within 6.5-7 using HCl and NaOH. The plants were grown under greenhouse condition with day and night temperatures set at 20±2 and 24±2° C, respectively and the relative humidity of 40% for two weeks.

Treatments included selenium (0, 0.2, and 5 µM) administered 21 days after transferring the plants to the hydroponic medium containing Long Ashton nutrient solution. Selenium was applied as selenite salt with five H_2O molecules ($Na_2SeO_3.5H_2O$) with the molecular weight 263.01 at three concentrations. The plants were removed from the hydroponic medium and fresh and dry

Table 1
Analysis of variance of the effects of selenium on morphologic features of *Melissa officinalis* L.

Feature	Source of Variability	Degree of Freedom	Mean Square	F	Significance Level
Shoot Fresh Weight	Selenium	2	60.983	12.841*	0.002
	Error	9	4.749		
Root Fresh Weight	Selenium	2	21.898	5.672*	0.025
	Error	9	3.861		
Shoot Dry Weight	Selenium	2	0.186	0.671$^{n.s.}$	0.535
	Error	9	0.277		
Root Dry Weight	Selenium	2	0.207	5.547*	0.027
	Error	9	0.037		

**, *, and n.s: significant at p≤0.01, p≤0.05, and non-significant, respectively.

weights were weighed at the end of the experiment.

The methods described by De Pinto et al. (1999), Bradford (1976), Jana and Choudhuri (1981), Aebi (1984), Gianopolitis and Ries (1997), and Nakano and Asada (1981) were used to assay ascorbic acid, protein, hydrogen peroxide, superoxide dismutase, and ascorbate peroxidase contents, respectively. The ground shoots of *Melissa officinalis* dried in shadow were used for extraction.

Statistical Analysis

The study was carried out in two separate experiments under room and greenhouse condition and the data were analyzed in a complete random design with three replications using SPSS and the T-test to compare the means based on Duncan's test at p≤0.05 and p≤0.01. Graphs were prepared using Microsoft Excel.

Results

Analysis of variance of the morphologic features showed that the effect of selenium on shoot and root fresh weight and root dry weight of *Melissa officinalis* were significantly different from those of the control plants. Selenium 5 µM reduced fresh weight compared with the control while it had no significant effect on the dry shoot weight (Table 1; Fig. I).

Analysis of variance of physiologic features of the plants under study showed that the effects of selenium treatment on ascorbic acid, catalase, protein, ascorbate peroxidase, and hydrogen peroxide contents of the plants were significant (Table 2). In fact, application of

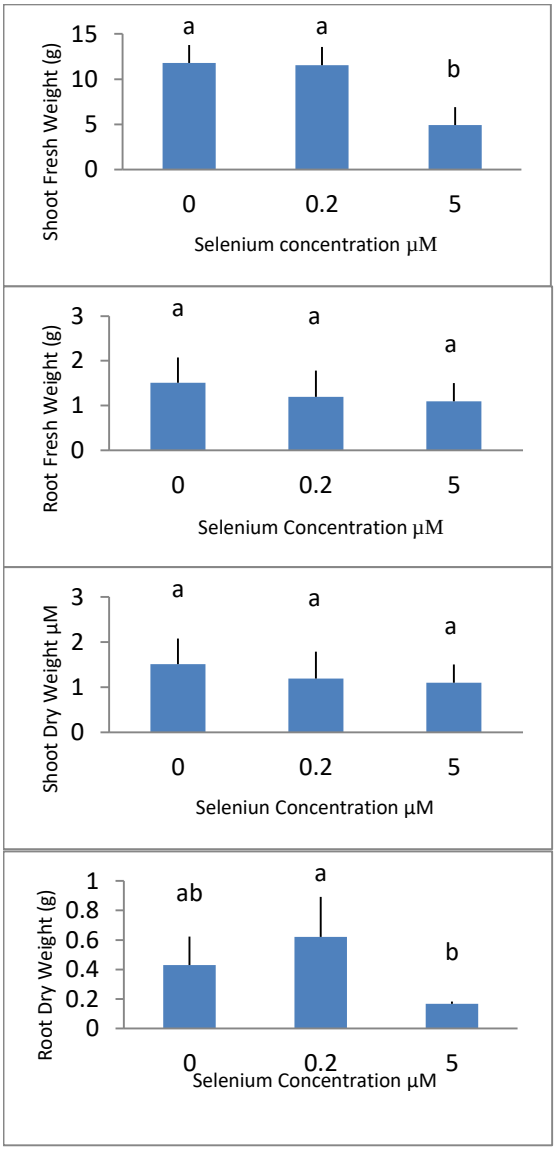

Fig. I. Effect of various concentrations of selenium on morphologic features of *Melissa officinalis*

selenium had a significant effect on ascorbic acid, catalase, and protein at p≤0.01 and p≤0.05

Table 2
Analysis of variance of the effects of selenium on physiologic features of *Melissa officinalis* L.

Feature	Source of Variability	Degree of Freedom	Mean Square	F	Significance Level
Ascorbic Acid	Selenium	2	0.179	9.421**	0.006
	Error	9	0.019		
Catalase	Selenium	2	7392489.30	8.714**	0.008
	Error	9	848311.01		
Protein	Selenium	2	0.945	7.98*·	0.010
	Error	9	0.118		
Superoxide Dismutase	Selenium	2	0.145	3.787$^{n.s.}$	0.064
	Error	9	0.038		
Hydrogen Peroxide	Selenium	2	33453.766	47.853**	0.00
	Error	9	699.123		

**, *, and n.s: significant at p≤0.01, p≤0.05, and non-significant, respectively

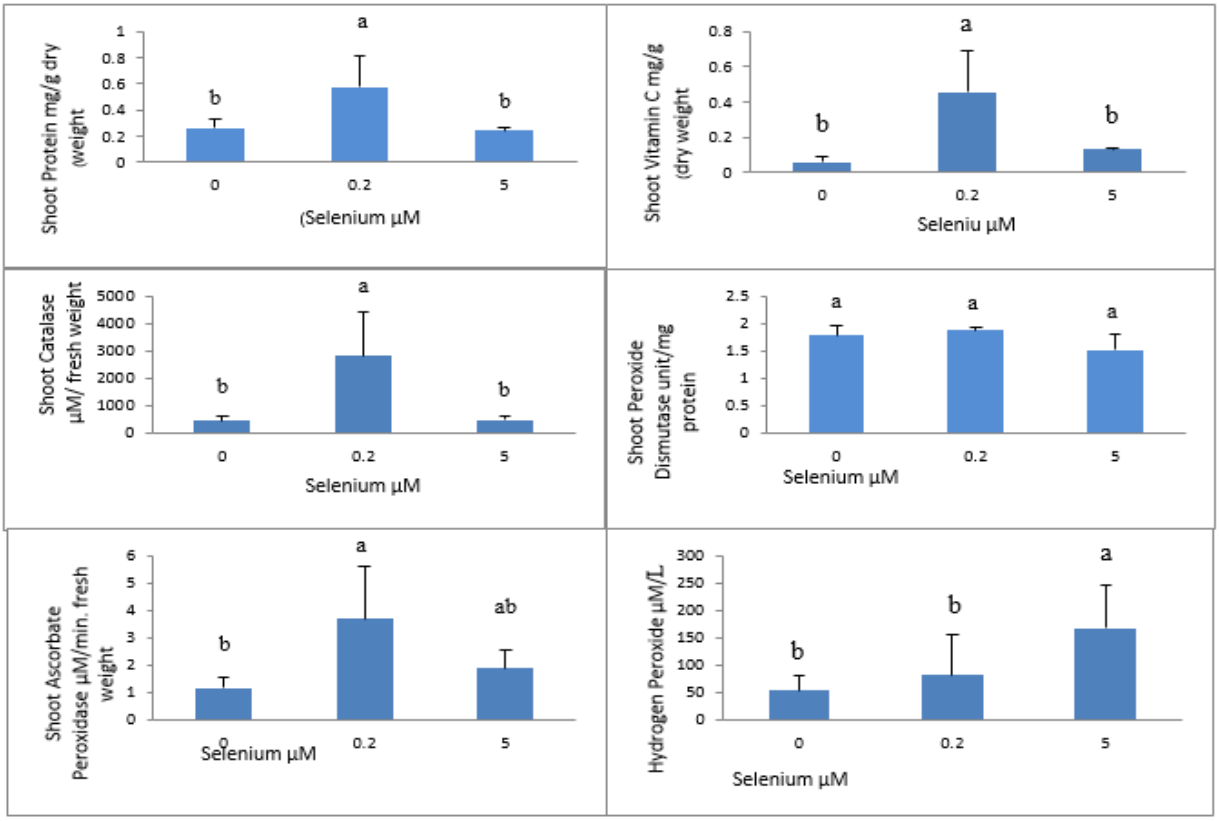

Fig. II. Effect of various concentrations of selenium on physiologic features of *Melissa officinalis*

showing a significant difference between 0.2 µM selenium treatment and control and also between the two concentrations of selenium in the study. The highest ascorbic acid, catalase, and protein contents were recorded with the treatment containing 0.2 µM selenium. Moreover, results of analysis of variance showed a significant difference between the effect of 0.2 µM selenium and control on the ascorbate peroxidase contents while the difference between control and 5 µM selenium was not statistically significant and the highest content of this enzyme was observed in the treatment containing 0.2 µM selenium. Also, a significant difference was observed in the hydrogen peroxide content of the plants at p≤0.05 and p≤0.01 and the treatment with 5 µM selenium significantly increased hydrogen peroxide content of the plants showing a significant difference with the control plants (Fig. II). On the other hand, no significant difference was observed between the

various selenium concentrations in the superoxide dismutase content of the plants under the study (Table 2; Fig. II).

Concentration of z-citral reduced in the plants with application of selenium and the highest level of z-citral (31.7%) was observed in the control plants. Application of 0.2 µM selenium reduced z-citral content by 8% and reached 26.75% (Fig. I). Similarly, treatment with 0.2 µM selenium reduced citral content of the plants in comparison with the control so that the content of citral in control (26.12) reduced by 7.41% in the plants treated with 0.2 µM selenium and reached 18.71%. However, application of 5 µM selenium resulted in a remarkable increase in the citral content of the treated plants which showed 11.72% increase compared with the control and reached 37.84% (Fig. II).

Caryophyllene content of the plants treated with 0.2 µM selenium increased to 50.75% in comparison with the control (42.19%) while increasing the dose of selenium to 5 µM led to a 22.96% reduction in caryophyllene content compared with the control, reaching 19.23% (Fig. III). Also, the treatment with 0.2 µM selenium increased caryophyllene in comparison with the caryophyllene oxide content of the plants and an optimum caryophyllene oxide content was obtained in the treatment with 0.2 µM selenium. Increase in the concentration of selenium beyond 0.2 µM led to a decrease in the caryophyllene oxide content of the plants under study from 6.79% to 2.87% while in comparison with the control, the caryophyllene oxide content of the plants treated with 5 µM selenium increased (Fig. III).

Geranyl acetate contents of *Melissa officinalis* essential oils showed no difference with control under 0.2 µM selenium treatment; however, increasing the concentration of selenium to 5 µM resulted in a sudden increase by 9.45% in geranyl acetate contents of the treated plants (Fig. III).

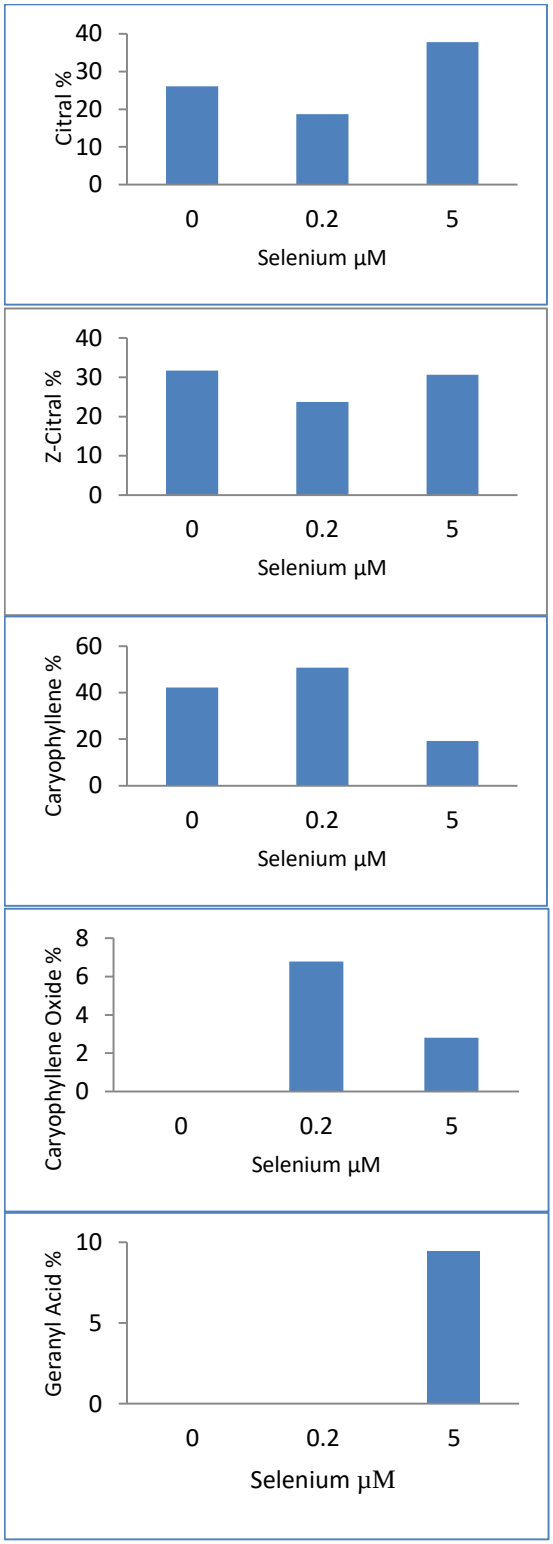

Fig. III. The effect of different concentrations of selenium on components of essential oils of *Melissa officinalis*

Discussion

High concentration of selenium reduced fresh root and shoot and dry root weights of *Melissa officinalis*. Selenium at high levels can cause toxicity and control growth in most plants that cannot accumulate high concentrations of selenium while at low concentrations, this metalloid can induce growth and improve plant resistance against various stresses (Feng et al.,

2013). Research has shown that low concentrations of selenium improves plant growth through increasing photosynthesis pigments, stabilization of carbon, and also synthesis and hydrolysis of starch and sucrose while high concentration of selenium reduces chlorophyll content and synthesis of carbohydrates which in turn reduces plant growth (Tailin et al., 2001; Han-Wens et al., 2010). Accordingly, application of 5 µM selenium reduces root and shoot fresh weights of *Melissa officinalis* which was also reported in previous studies (Mudgal and Madaan, 2011). Applying high concentration of selenium induced oxidative stress and resulted in decomposition of membranes in *Melissa officinalis* reducing their growth (Freeman et al., 2010).

Findings of the present study revealed that applying 0.2 µM selenium increased protein contents of shoots in *Melissa officinalis* plants showing a significant difference with the control plants ($p \leq 0.05$). Also, increasing the concentration of selenium in the treatment to 5 µM reduced protein contents of shoots. This reduction in plants' protein and biomass as a result of increase in selenium concentration reported in various plants can be attributed to the reduction in photosynthesis pigments and the probable effect of selenium on the whole plant (Chen et al., 2005). Yassen et al. (2011) showed that sprinkling potato plants with selenium had a significant positive effect on protein percentage of potatoes compared with the control. Similarly, Munshi et al. (1990) found that sprinkling potatoes with selenium solution significantly increased protein contents of their tubers.

Treatment of *Melissa officinalis* plants with 0.2 µM selenium in the present study had a significantly positive effect on their ascorbic acid contents ($p \leq 0.05$). Ascorbic acid is a substrate for many peroxides which is used as a scavenger for reactive oxygen species and protection of the cells against oxidative stresses (Jithesh et al., 2006; Abdul Jaleel et al., 2009). Through enzymatic method of removing free radicals, the plant uses free radical neutralizing enzymes such as superoxide dismutase, catalase, and peroxidase. Catalase and peroxidase play an important role in neutralizing reactive oxygen species in plants and depending on the plant species and intensity of the stress, the level of activity of these enzymes vary (Alscher et al., 2002). Low concentrations of selenium have a positive effect on growth of the crops and their resistance against abiotic stresses (Turakainen et al., 2004). Treatment of the plants with 5 µM selenium had a significant effect on catalase activity in this study. The highest level of activity was recorded in 0.2 µM selenium treatment and with an increase in the concentration of selenium, catalase activity reduced. Increased activity of catalase with 0.2 µM selenium treatment suggests that this concentration is toxic for *Melissa officinalis* and induces antioxidant defense system by increasing catalase activity. Applied at suitable concentrations, selenium improves plants, antioxidant system potential through activating antioxidant enzymes such as catalase while reducing malondealdehyde content produced as a result of damaging cell membranes and by this, improves their resistance against oxidative stresses (Kong et al., 2005). Rios et al. (2009) showed that high concentrations of selenium induces a reduction in shoot biomass of lettuce which is attributed to a reduction in enzymes such as catalase that detoxify hydrogen peroxide. Nowak et al. (2004) found that enzyme activities such as those of catalase were significantly increased in wheat in response to different concentrations of selenium. Also, Saidi et al. (2014) showed that treatment with suitable concentrations of selenium made a significant difference in catalase activity of sunflower. Unlike this study that found no significant effect of various concentrations of selenium on superoxide dismutase content of *Melissa officinalis* shoots, Hartikanan et al. (2000), Djanaguiraman et al. (2005), and Hu et al. (2013) showed increased activity of superoxide dismutase enzyme following the treatment with selenium in ryegrass, soybean, and melon, respectively. Also, Xue et al. (2013) found increased activity of superoxide dismutase in withered lettuce as a result of applying selenium. On the other hand, Saidi et al. (2014) reported a reduction in superoxide dismutase activity of sunflower plants whose seeds were primed with selenium. Our different findings may be due to variable responses of plants to selenium depending on the method of application, and the

plant organ under study e.g., leaves, fruit, or stems (Idrees et al., 2010). On the other hand, the plants under study have probably employed some other defense mechanisms to protect themselves against harmful effects of oxidative stress and therefore, there was no change in superoxide dismutase content.

Antioxidant enzymes such as ascorbate peroxidase (APX) are the main component of ROS detoxification system (Cao et al., 2004). A significant difference was observed in the study between APX activities of the plants treated with selenium and control ($p \leq 0.05$). It can be observed that under stress condition, selenium increased antioxidant activities of the plant as a stress mitigation factor. This is in line with the findings of Kong et al. (2005) who reported that the activities of enzymes effective on detoxification of hydrogen peroxide e.g., APX increased in the plants treated with selenate. The highest level of hydrogen peroxide in this study was recorded in treatment with 5 µM selenium. Reactive oxygen species such as hydrogen peroxide are among important factors in oxidative stress that remarkably affect cell growth and secondary metabolism. Since 5 µM selenium in the study induced production of peroxide hydrogen and increased its content, it is contended that the plant growth is controlled in this way. Accumulation of peroxide hydrogen and malondealdehyde at higher concentrations of selenium is indicative of oxidative stress and the reason for toxic effects of selenium (Rios et al., 2009). Increased level of hydrogen peroxide under 5 µM selenium treatment in this study can be a reason for a reduction in catalase activity at this concentration of selenium while appropriate concentration of selenium reduces hydrogen peroxide content and the resulting damages (Labanowska et al., 2012). The other studies have also shown that the activation of antioxidants through applying appropriate levels of selenium reduces hydrogen peroxide contents in plants (Kumar et al., 2012).

The content and chemical components of the essential oils of *Melissa officinalis* were significantly different which can be attributed to the growth condition and genetic variability of the plant. The essential oils of *Melissa officinalis* have antioxidant properties (Fratianni et al., 2010). The main components of the essential oils include caryophyllene oxide, citral, z-citral, beta caryophyllene, citronellal, and geranyl. Many studies have determined the components of essential oils in *Melissa officinalis* (Pino et al., 1999; Tinmaz et al., 2001). In fact, various compounds have been determined in different studies as the main components of the *Melissa officinalis* essential oils. Pino et al. (1999) determined neral and geranyl as the main components. These variabilities are because of different factors including genetic and climatic factors among others. There is no study on the effect of various concentrations of selenium on the components of *Melissa officinalis* essential oils. The findings of the present study on the components of *Melissa officinalis* essential oils suggest that applying 5 µM selenium increased z-citral, citral, and geranyl acetate contents of the essential oils while this increase in caryophyllene and caryophyllene oxide contents was observed in the treatments involving 0.2 µM selenium. Some studies consider z-citral, citral, and geranyl acetate as the main components of the essential oils. It is likely that when faced with high concentrations of selenium, *Melissa officinalis* experiences stress and these compounds, which have antioxidant properties, increase to control the effects of stress. Khosravi et al. (2012) found the highest neral, geranyl, and geranyl acetate biosynthesis in the *Melissa officinalis* plants sprinkled with methanol 53% solution. Generally, the findings of their study suggested that applying methanol and ethanol hydro-alcoholic treatments could change and improve biosynthesis of the components of *Melissa officinalis* essential oils.

Medicinal plants need suitable levels of micronutrients to grow and produce effective compounds (Sarmad Nia and Koochaki, 1992). Through foliar sprinkling of micronutrients it is possible to improve the plants' growth under stress conditions. For example, applying micronutrients increased the number of glands that secrete essential oils in spearmint and therefore improved essential oils performance (Evans, 1996). It was also show that foliar sprinkling of micronutrients increased dry matter and essential oils performance of spearmint (Heidari et al., 2008).

Conclusion

This study investigated the effect of using selenium on the medicinal plant *Melissa officinalis* L. in terms of the components of performance, antioxidant properties, and components of essential oils. Findings suggested that treatment with selenium had a significant positive effect on the fresh weight of roots and shoots, dry weight of roots, ascorbic acid, protein, catalase, ascorbate peroxidase, hydrogen peroxidase, caryophyllene, and caryophyllene oxide contents of the plants compared with the control while the highest levels of phenol and flavonol contents were observed in the control plants. In other words, selenium reduced these compounds. Based on the experiments carried out, lower concentrations of selenium increase biomass in plants and improve their growth through increasing chlorophyll, protein, carbohydrates, phenolic compounds, antioxidant enzymes activities (such as catalase, polyphenol oxidase, guaiacol peroxidase, and superoxide dismutase), and antioxidant compounds (e.g., ascorbate) and also through preventing peroxidation of lipids and increasing the absorption of nutrients. However, higher concentrations of this metalloid has reverse effects and unlike antioxidants has oxidation effects. High levels of selenium through damaging carbohydrates, protein, and chlorophyll synthesis system reduce the plant growth and even result in its death. Therefore, application of appropriate concentrations of selenium considering the plant species and its growth stage can be beneficial. Results of this study showed that low levels of selenium increased the growth in *Melissa officinalis*; however, with an increase in the concentration of selenium, the growth rate decreased. Generally, it was found that although selenium is not a necessary element for plants, lower concentrations of this metalloid (0.2 µM) improves plant growth indexes and morphology such as root and shoot weights.

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