

## Effect of Soil Applied Iron Chelate Fertilizer on the Essential Oil Composition and Antioxidant Activity of *Satureja hortensis* under Greenhouse Conditions

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### Abstract

Savory (*Satureja hortensis* L.) from the *Lamiaceae* family is one of the most famous medicinal/spice-plant cultivated in most regions of the world. A greenhouse experiment was conducted to study the effect of iron, Fe, (0, 8 and 16 mg Fe kg<sup>-1</sup> soil as iron-ethylenediamine di-hydroxyphenylacetic acid (Fe-EDDHA)) application on the oil composition and antioxidant activity of the *S. hortensis*. Results showed that the maximum percentage of  $\gamma$ -terpinene,  $\alpha$ -terpinene and carvacrol were observed in control; whereas, the minimum percentage of the aforementioned constituents was obtained with application of 8 mg Fe kg<sup>-1</sup> soil. Results showed that inhibitory effects (IC<sub>50</sub>) of extract decreased, significantly in response to Fe application (IC<sub>50</sub> of 408 and 324.7 mg L<sup>-1</sup> were obtained with application of 8 and 16 mg Fe kg<sup>-1</sup> soil, respectively in comparison to IC<sub>50</sub> of 720.4 mg L<sup>-1</sup> that obtained in control). In another word, antioxidant-activity was improved with Fe application as a significant (P<0.05) linear regression with determination coefficient of 0.89. Findings indicated that savory-oils, in addition to other useful properties have a potential to use as a topical antioxidant.

**Keywords:** inhibitory effects; savory; essential oil; micronutrient; DPPH assay

### Introduction

Savory (*Satureja hortensis* L.) from the *Lamiaceae* family is one of the most famous medicinal and spice plants of significant antioxidant effects cultivated in the most regions of the world such as Iran (Hadian et al. 2008). The most important compounds of *Satureja hortensis* L. essential oil are carvacrol, thymol, and some phenolic compounds. Essential oils are concentrated hydrophobic liquid containing volatile aroma compounds from plants. They have an important impact on human health. Antioxidants are a group of substances when present at low concentrations compared to oxidized substrates significantly inhibit or delay oxidative processes, while being oxidized themselves (Seidler-Lozykowska et al. 2007; Vaya and Aviram 2003; Zhang et al. 2010). It has been established that

oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppressant, neurodegenerative diseases and others (Yong and Woodside, 2001). Phenolic compounds found in plants have been well known for their ability of scavenging free radicals, which is referred to as antioxidant activity (Jia et al., 1999). Production of essential oil and its composition in plants is mainly dictated by the combined influences of genetic factors, cultivation conditions such as climate, plant density, and nutrient status in growth medium, etc. (Milos et al. 2001; Salmasi et al. 2008). Micronutrients and macronutrients (like Fe and S, respectively) plays important roles in improvement of growth and development of plants or the composition of their essential oil (Najafian and Zahedifar, 2015;

Zahedifar and Najafian, 2015). Yeritsyan and Economakis (2002) determined the effect of three levels of Fe-EDTA (2.5, 5 and 11 mg L<sup>-1</sup>) on the growth and essential oil content of oregano in the nutrient solution. They showed that the biomass and essential oil content of the shoots decreased in the highest level of applied Fe. Nasiri et al. (2010) showed that foliar application of Fe and Zn increased flower yield, essential oil percentage and essential oil yield of chamomile, significantly. Effects of Fe on yield and composition of *Rosmarinus officinalis* L. essential oil in irrigated and non-irrigated plants described by Moretti et al. (1998). They reported Fe absorption did not produce any significant improvement in oil yield but appeared to cause a marked rise in verbenone concentration in the oil of irrigated plants. Mumivand et al. (2011) showed that the chemical composition of *S. hortensis* essential oil did not change due to nitrogen fertilization but CaCO<sub>3</sub> application increased the relative percentages of carvacrol,  $\gamma$ -terpinene and  $\beta$ -bisabolene.

Several assays have been frequently used to estimate antioxidant capacities for clinical studies including 2,2-azinobis, 3-ethyl-benzothiazoline-6-sulfonic acid, ABTS, (Leong and Shui 2002; Miller and Rice-Evans 1997). 2,2-diphenyl-1-picrylhydrazyl, DPPH, (Gil et al. 2002), ferric reducing antioxidant power, FRAP, (Guo et al., 2003; Jimenez-Escrig et al., 2001), and the oxygen radical absorption capacity, ORAC, (Prior et al. 2003).

The antioxidant activity of various foods can be determined accurately, conveniently, and rapidly using DPPH testing. This method can be used successfully for solid samples without prior extraction and concentration, which saves time (Prakash et al. 2001).

The DPPH can only be dissolved inorganic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants. Both radicals show similar bi-phase kinetic reactions with many antioxidants. However, the ferric reducing antioxidant power (FRAP) method is based on the reduction of a ferrous analogue, the Fe<sup>3+</sup> complex of tripyridyltriazine, Fe(TPTZ)<sup>3+</sup> to the intensely blue-coloured, Fe(TPTZ)<sup>2+</sup> to the intensely blue-coloured Fe<sup>2+</sup> complex, Fe(TPTZ)<sup>2+</sup> by

antioxidants in acidic medium. However, the reducing capacity does not necessarily reflect antioxidant activity (Katalinic and Milos 2006). There are various studies emphasizing that free radicals contribute to the development of many diseases, including hemorrhagic shock, arthritis, ageing, atherosclerosis, ischemia, Alzheimer and Parkinson's disease, disorders, tumor promotion and carcinogenesis (Bagchi et al., 2000). Since the content of free radical and the other effective component of plants may influence by essential nutrient elements. Therefore, the aim of this research was to evaluate the effect of Fe-chelate fertilizer on the essential oil composition and antioxidant activity of Savory under greenhouse conditions.

## Materials and Methods

### Soil Analysis

A greenhouse experiment in Eram Botanical Garden, Shiraz, Iran, was carried out on a loamy calcareous soil (Fine-loamy, carbonatic, thermic, Typic Calcixerpts) with pH of 7.8; electrical conductivity (EC<sub>e</sub>) of 0.40 dS m<sup>-1</sup>, calcium carbonate equivalent (CCE) of 45%; organic matter (OM) of 1.5%; sodium bicarbonate extractable P (Olsen et al., 1954) of 4.5 mg kg<sup>-1</sup> soil; DTPA-extractable Fe, Mn, Zn and Cu (Lindsay and Norvell, 1978) of 2.3, 3.7, 0.96 and 1.0 mg kg<sup>-1</sup> soil, respectively. The mentioned attributes of the studied soil were measured using the common standard methods

### Experimental Design

The experiment was a randomized complete design with 6 replicates. Treatments consisted of soil application of three Fe levels of 0, 8 and 16 mg Fe kg<sup>-1</sup> soil as Fe-ethylenediamine di-o-hydroxyphenylacetic Acid (Fe-EDDHA).

### Soil Preparation and *Satureja Hortensis* Planting

Each pot contained 3 kg soil. Pots were watered with distilled water to a near field capacity and maintained at this moisture level by adding water to a constant weight. For preventing any probable nutrient deficiency other than Fe, all pots received uniform application of 20 mg P kg<sup>-1</sup> soil as Ca

$(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ , 150 mg nitrogen (N)  $\text{kg}^{-1}$  soil as  $\text{CO}(\text{NH}_2)_2$  (one half of N was added at planting and the other half was shoot dressed three weeks after emergence), 10, 10 and 5 mg Mn, Zn and Cu  $\text{kg}^{-1}$  soil as their sulfates and in aqueous forms. Twenty *Satureja hortensis* seeds were planted about 1-cm deep and were thinned to ten uniform stands two weeks after emergence. Eight and 16 mg Fe  $\text{kg}^{-1}$  were applied to Fe treated soils before planting from Fe-EDDHA source. Twelve weeks after planting, plants were harvested. Plant samples were dried at shade and prepared to analysis.

### Samples Extraction

Metabolic extracts of the plants were prepared as follows: 20 g dry plant was macerated in 200 mL methanol/water (90/10) for 2 days with one change of solvent after 1 day. The extract was filtered and then concentrated in a rotary evaporator in less than 10 min. Powders were weighed to calculate the yield, and kept at  $-20^\circ\text{C}$  until used. Shortly before each experiment, the powder was dissolved in methanol at the desired concentration and was tested for antioxidant activity.

### Determination of Antioxidant Activity Using DPPH

The antioxidant activity of plant extract and the standard antioxidants were assessed on the basis of radical scavenging effect of the stable DPPH free radical. Gallic acid was used to prepare a standard solution. In a modified assay (Bruits et al., 2001), 200  $\mu\text{L}$  of a 100 mM solution of DPPH radical in methanol was mixed with 20  $\mu\text{L}$  of 12.5-3200  $\mu\text{g mL}^{-1}$  extracts, Gallic acid, respectively and solutions were left at room temperature for 30 minutes. The DPPH radical inhibition was measured at 515 nm using a micro-plate reader of Biotek ELx 808 model. The  $\text{IC}_{50}$  of each sample (concentration in  $\mu\text{g mL}^{-1}$  required to inhibit DPPH radical formation by 50%) was calculated by MATLAB software. The extract methanolic solution without DPPH was used as a blank to be subtracted from all measurements. The antioxidant activity (AOA) is given by Equation 1.

$$\text{AOA} = 100 - \left[ \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right] \times 100 \quad (1)$$

The  $\text{IC}_{50}$  value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the test extract ( $\mu\text{g mL}^{-1}$ ) against the mean percentage of the radical scavenging activity.

### Headspace Volatiles Extraction

Up to 3 g of each *Satureja hortensis* dried sample was crushed and placed in 20 mL headspace vial, and immediately sealed with silicone rubber septa and aluminum caps. The vials were then transferred to the headspace tray. The headspace proceeded on the ombiPAL system which was provided with headspace auto-sampler, heater and agitator. The vial was heated up to  $80^\circ\text{C}$  and retained for 20 min while being agitated; the temperature of the sampling needle and transmission lines' temperature was  $85^\circ\text{C}$ .

### Identification of the Oil Components by GC/MS

GC-MS analysis was carried out using an Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30 m x 0.25 mm i.d., 25  $\mu\text{m}$ ) with He as the carrier gas and split ratio 1:50. Retention indices were determined using retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library data published mass spectra data (Adams, 2001).

### Statistical Analysis

Data were analyzed statistically using Excel and MSTATC software packages and means were compared using Duncan's Multiple Range Test at the probability level of 5%.

## Results and Discussion

### Chemical Composition of Essential Oil

Results indicated that eighteen compounds were identified in the essential oils of control plants; whereas, twenty and fifteen compounds were obtained in *Satureja hortensis* L. subjected to soil application of 8 and 16 mg Fe kg<sup>-1</sup> soil, respectively (Table 1).

The identified constituents with their respective percentages and retention indices (RIs) were summarized in Table 1. The main oil constituents in control plants were  $\gamma$ -terpinene (67.4%),  $\alpha$ -terpinene (10.8%), borneol (6.5%), myrcene (4.4%),  $\alpha$ -thujene (4.2%), p-cymene (3.8%),  $\alpha$ -pinene (2.8%), and carvacrol (2.0%). Essential oil composition of *Satureja hortensis* L. seeds were identified by Ghannadi (2002). He detected forty-two components such as carvacrol (59.7%),  $\gamma$ -terpinene (12.8%) and p-cymene (9.3%). Findings

indicated that application of 8 mg Fe kg<sup>-1</sup> soil resulted in existence of 61.4%  $\gamma$ -terpinene, 10.3%  $\alpha$ -terpinene, 7.6% carvacrol, 5.0% p-cymene, 4.7% myrcene, 3.8%  $\alpha$ -thujene and 2.4%  $\alpha$ -pinene. Whereas, the essential oil consisted of 60.7, 12.3, 2.5, 4.1, 5.5, 5.0 and 4.0% of the aforementioned constituents, respectively when 16 mg Fe kg<sup>-1</sup> soil was applied. Results showed that the maximum percentage of  $\gamma$ -terpinene,  $\alpha$ -terpinene and carvacrol were observed in control plants; whereas the minimum percentage of the aforementioned constituents was obtained with application of 8 mg Fe kg<sup>-1</sup> soil. Effect of foliar Fe spray on yield and composition of *Rosmarinus officinalis* L. in irrigated and non-irrigated plants was investigated by Moretti et al. (1998). Their results showed that Fe application did not affect the oil yield of plant; however, Fe application resulted in a significant increase in verbenone concentration in the oil of irrigated plants.

**Table 1** Effect of foliar Fe-chelate (Fe-EDDHA) application on the chemical composition (% of constituents) of *Saturejahortensis* L. essential oil.

Number	Compounds	RI <sup>1</sup>	Applied Fe (mg kg <sup>-1</sup> soil)		
			0	8	16
1	$\alpha$ -thujene	923	4.2 ± 0.32 b <sup>2</sup>	3.8 ± 0.3 b	5.1 ± 0.36 a
2	$\alpha$ -pinene	931	2.8 ± 0.1 b	2.4 ± 0.14 b	4.1 ± 0.09 a
3	camphene	945	0.2 ± 0.07 a	0.2 ± 0.01 a	0.3 ± 0.01 a
4	sabinene	969	0.3 ± 0.02 a	0.2 ± 0.01 a	0.4 ± 0.02 a
5	$\beta$ -pinene	974	1.1 ± 0.1 b	1.0 ± 0.1 b	2.0 ± 0.3 a
6	myrcene	987	4.4 ± 0.4 b	4.7 ± 0.35 b	5.5 ± 0.34 a
7	$\alpha$ -phellandrene	1003	0.9 ± 0.08 a	1.0 ± 0.1 a	1.1 ± 0.08 a
8	$\delta$ -3-carene	1008	0.1 ± 0.01 a	0.2 ± 0.03 a	0.2 ± 0.03 a
9	$\alpha$ -terpinene	1015	10.8 ± 1.24 b	10.3 ± 1.1 b	12.4 ± 1.2 a
10	p-cymene	1021	3.8 ± 0.52 b	4.9 ± 0.2 a	4.1 ± 0.3 b
11	limonene	1025	0.9 ± 0.08 a	0.6 ± 0.04 a	0.8 ± 0.04 a
12	$\beta$ -phellandrene	1026	0.2 ± 0.01 b	0.6 ± 0.01 a	0.6 ± 0.01 a
13	(E)- $\beta$ -ocimene	1043	0.1 ± 0.06 a	0.2 ± 0.05 a	0.2 ± 0.06 a
14	$\gamma$ -terpinene	1059	67.4 ± 4.5 a	61.4 ± 6.4 b	60.7 ± 5 b
15	cis-sabinene hydrate	1064	-	0.1 ± 0.08	-
16	terpinolene	1085	-	0.1 ± 0.08	-
17	p-cymen-9-ol	1211	0.3 ± 0.01	-	-
18	(Z)-ocimenone	1224	0.1 ± 0.02	-	-
19	thymol methyl ether	1239	-	0.1 ± 0.02	-
20	carvacrol	1299	2.1 ± 0.1 b	7.6 ± 0.85 a	2.5 ± 0.12 b
21	(E)-caryophyllene	1415	-	0.1 ± 0.02	-
22	$\beta$ -bisabolene	1504	0.1 ± 0.02 b	0.5 ± 0.01 a	-
	Total		100	100	100

<sup>1</sup>RI is retention index.

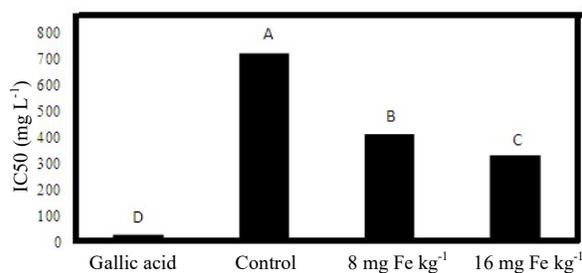
<sup>2</sup>Means in each row followed by the same letter are not significantly ( $P < 0.05$ ) different by Duncan's multiple range test.

Some investigators stated that the genus *Satureja* presents great variability in the concentration of the major components in its essential oil composition due to the existence of different species and subspecies, but also to a numerous of parameters, mainly the environmental and climatic conditions (Gulluce et al., 2003). With reference to previous studies, thymol and carvacrol, in particular, were found to be principal constituents of the oils isolated from several Croatian *Satureja* species (Milos et al., 2001; Skoibuix and Bezix, 2004). It is interesting that various isolates of winter savory from Croatia and Bosnia and Herzegovina have carvacrol up to 84.19% as main constituent (Kustrak et al., 1996). Cazin et al. (1985) revealed that the oil composition of winter savory shows large variations in the relative concentration of major components, for example 5 to 69% carvacrol, 1 to 62% linalool, 1 to 31%  $\gamma$ -terpinene, and 3 to 27% p-cymene arising from the existence of different chemo-types. The main constituents of the *S. hortensis* (summer savory) essential oil are phenols, thymol (about 29.0%), carvacrol (about 26.5%), r-terpinene (about 22.6%), p-cymene (about 9.3%) and other terpenoids (Gulluce et al., 2003). In general, it can be concluded that the control plants is the most suitable for obtaining the highest percentage of  $\gamma$ -terpinene; whereas, application of 8 mg Fe kg<sup>-1</sup> soil is preferable for attaining special components such as carvacrol. On the other hand,  $\alpha$ -terpinene is mainly produced in response to application of 16 mg Fe kg<sup>-1</sup> soil.

### Antioxidant Activity

As earlier mentioned, IC<sub>50</sub> is an appropriate way to measure the oxidation progress in oils and are therefore considered as good indicators for the effectiveness of antioxidants and Gallic acid is used as a standard to show antioxidant activity. The more an antioxidant is stronger, the more its IC<sub>50</sub> is closer to IC<sub>50</sub> of Gallic acid.

Results indicated that significant amounts of inhibitory effects (IC<sub>50</sub>) were obtained in response to Fe application. So that the IC<sub>50</sub> of extracts decreased significantly by about of 44 and 55% as compared to that of control when 8 and 16 mg Fe kg<sup>-1</sup> soil were applied, respectively (Figure 1). In



**Figure 1** Effect of Fe application on antioxidant activity (IC<sub>50</sub>) of *Saturejahortensis* L. extract as compared to that of Gallic acid (columns followed by the same capital letters are not significantly different ( $P < 0.05$ ) by Duncan's Multiple Range Test).

other words, the maximum IC<sub>50</sub> (720.4 mg L<sup>-1</sup>) corresponds to control; whereas, the minimum content of IC<sub>50</sub> (324.7 mg L<sup>-1</sup>) was obtained with application of 16 mg Fe kg<sup>-1</sup> soil that was more closer to that of Gallic acid (Figure 1). Findings revealed that antioxidant activity of *Satureja hortensis* L. extract was improved with iron application. In the other word, the IC<sub>50</sub> of extract decreased in response to applied Fe as the following significant ( $p < 0.01$ ) linear regression equation with determination coefficient ( $R^2$ ) of 0.89

Regression equation between IC<sub>50</sub> (Y) and Fe levels (X) is presented in Equation 2.

$$Y = -197.82X + 1077.9, R^2 = 0.89 (P < 0.01) \quad (2)$$

Where Y is IC<sub>50</sub> (mg L<sup>-1</sup>) of plant extract and X is the applied Fe (mg kg<sup>-1</sup> soil).

The influence of foliar and soil applications of Fe on variations in antioxidant activity of peanut was investigated by Panjtandoust et al. (2013). They showed that greater levels of Fe (4 g L<sup>-1</sup> and 20 kg Fe-EDDHA ha<sup>-1</sup> for sprayed and soil applications, respectively) had more positive effects on the activities of peroxidase and catalase than on superoxide dismutase. Zahedifar and Najafian (2015) also stated that the antioxidant activity of *Satureja hortensis* extract increased when plants received S and Fe fertilizers.

## Conclusions

Savory oils, in addition to other useful properties such as using for treating diarrhea, cramps, nausea and as muscle pain-reliever, or using as seasoning in the food-industry or antimicrobial agents due to their high phenolic contents, have a potential to use as a topical antioxidant. Results revealed that addition of Fe fertilizer could improve the antioxidant activity of plant extract, significantly. Therefore, application of Fe fertilizers can be recommended to improve both of agricultural products quality and their medicinal values.

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