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Combined Effect of Soil Applied Iron and Sulfur Fertilisers on Monoterpene Content and Antioxidant Activity of *Satureja hortensis* L. Extract

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ABSTRACT

Chemical-composition/antioxidant-activity of *Satureja hortensis* L. extract is influenced by many factors including nutrient elements. A factorial completely randomized design greenhouse experiment was conducted to study the effect of three levels of soil-applied iron, Fe (0, 8 and 16mg kg^{-1} soil as ethylene-di-amine-die-hydroxyl-acetic-acid, Fe-EDDHA) and three levels of sulfur, S (0, 50 and 100mg kg^{-1} soil as elemental-S) on monoterpene production and antioxidant activity of *Satureja hortensis* L. The maximum (377.75 mg/L) and minimum (720.406 mg/L) antioxidant activity were obtained with 8mg Fe+100mg S treatment and control, respectively. The main oil constituents in control were γ-terpinene(67%), α-terpinene(11%), myrcene (4%), α-thujene (4%), p-cymene (4%), α-pinene (3%) and carvacrol (2%). The maximum content of α-thujene, α-pinene, myrcene and α-terpinene was obtained with 8mg Fe+50mg S application whereas the control was suitable for obtaining γ-terpinene. Carvacrol was mainly produced with addition of 16mg Fe+100mg S. Furthermore, the α-thujene, α-pinene, myrcene and α-terpinene contents increased with application of 8mg Fe+50mg S. The α-terpinene, myrcene, α-thujene and α-pinene increased by 13, 27, 21 and 43% compared to control, respectively when 8mg Fe+50mg S was applied. The entire component of monoterpenoid fraction with the major constituent of γ-terpinene, α-terpinene, myrcene, α-thujene, p-cymene, α-pinene and carvacrol that constitutes 99.9% of essential oil showed a same trend whereas identified sesquiterpenes and sesquiterpenoid components were relatively low (0.1%). The low molecular weight of γ-terpinene decreased as 8mg Fe+50mg S was applied. In general, it could be concluded that application of 8mg Fe+50mg S kg^{-1} was the most suitable treatment for obtaining

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higher amounts of α-terpinene, myrcene, α-thujene and α-pinene whereas addition of 16mg Fe+100mg S kg^{-1} was preferable for obtaining carvacrol.

Keywords: Fertiliser, monoterpene production, lamiaceae *Satureja hortensis*, savory, antioxidant activity

INTRODUCTION

Aromatic plants have been used as natural food additives and spices from a very long time ago due to their antiseptic and aromatic characteristics. Furthermore, these plants have medicinal and industrial applications. The genus *Satureja* L. (savory, saturei) with more than 30 species is one of the most famous aromatic plant species. These species produce essential oils and some secondary metabolites such as antimicrobial agents in their normal growth and development processes or in response to environmental stresses e.g. drought and temperature stresses, nutrient deficiency or toxicity, attack of pathogens etc. *Satureja hortensis* L. species is one of the most well-known species. In addition to having widespread application in cooking and the food industry, it is commonly used in the treatment of some diseases. Therefore, studying the chemical composition of essential oils and extracts of their above-ground parts is very important. The common application of *S. hortensis* L. as a natural compound in the treatment of inflammatory disorders, muscle troubles, spasms and many other disorders as well as in conservation of foods has been justified by some biological and pharmacological investigators due to the presence of anti-spasmodic (Leporatti & Ivancheva, 2003; Hajhashemi *et al*., 2000), anti-oxidant and anti-bacterial (Dorman & Hiltunen, 2003; Adiguzel *et al.,* 2007) and anti-fungal characteristics. Furthermore, high activity of its essential oil against clinical multi-resistant isolates from injuries has been reported by Mihajilov-Krstev *et al*. (2009). Stutte (2006) reported that environmental factors strongly affected the bio-synthesis of secondary metabolites in aromatic or medicinal plants. In this context, the use of organic and chemical fertilisers may increase the amount of essential oil or other major constituents produced by medicinal plants (Khalid *et al*., 2006).

Macro- and micronutrients are important and effective agents on plant yield and composition. High yielding crops need large and regular supply of macro- and micronutrients to develop high photosynthetic capacity and maintain the proper element concentration in the leaves (Lawlor, 1995). The lack of nutrients such as iron and sulfur reduces plant growth (Heidari *et al*., 2011). Sulfur (S) is needed for the optimal production of nutrients; it is also needed in the enzymatic and structural functions of the plant. Sulfur is required for synthesis of protein as a vital constituent of many essential amino acids. It is also needed in synthesis of chlorophyll. It has been reported that the biochemical structure of plant oils mainly depends on the amount of S as one of the major essential nutrients (Mengel & Kirkby, 1978). Sulfur deficiency

could decrease the uptake of nitrogen, yield of crop and plant quality (Marschner, 1995). Ahmad and Sharma (2008) reported that the uptake of S and its assimilation play a key role in determination of yield and quality of seeds in higher plants, and also in resistance to environmental stresses like pests. Higher plants require a continuous supply of sulfur from seed emergence to maturity due to its immobile nature within plants. Khan and Hussain (1999) reported the maximum yield of mustard (*Brassica juncea*) seed and oil was gained with application of 20kg S ha^{-1}. Alizadeh *et al*. (2010) stated that the fresh and dry weight of *S. hortensis* as well as its essential oil yield and efficiency increased in response to applied complete fertiliser. They recorded that 19 components in the essential oil of *S. hortensis* underwent different treatments that represented 97.58-99.24% of its oils, and of these, the main constituents were carvacrol (43.9-59.2%), γ-terpinene (30.7-40.2%), α-terpinene (2.8-4%) and *P*-cymene (1.8-2.2%). They reported that composition of essential oil did not affect significantly in response to different levels of applied fertiliser. They stated that the amount of specific constituents like γ-terpinene, α-terpinene and carvacrol were decreased significantly by fertiliser application while phenolic content and antioxidant activity increased. Zheljazkov *et al*. (2008) showed that application of N and S fertilisers had positive effects on biomass and oil yield of sweet basil as well as on the chemical composition of the plant's oil.

Among the micronutrients, iron plays an important role in the growth and development of plants. Marschner (1995) stated that micronutrients, especially Fe, act either as metal components of various enzymes or as functional, structural or regulatory cofactors; thus, it is associated with saccharide metabolism, photosynthesis and protein synthesis. Iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase as well as in photorespiration, the glycolate pathway and chlorophyll content (Marschner, 1995). Blakrishman (2000) showed that Fe caused an increase in activity of catalase, peroxidase and cytochrome oxidase enzymes. Nasiri *et al*. (2010) reported that foliar applied iron and zinc increased the flower yield of chamomile and the percentage of its essential oil significantly over the control. Yeritsyan and Economakis (2002) determined the growth parameter of oregano and the yield of its essential oil in a hydroponic culture in response to application of three levels of Fe-EDTA (2.5, 5 and 11mg/l). They showed that the content of essential oil decreased when the highest level of Fe (11 mg/L) was applied. Furthermore, they stated that the amount of biomass and essential oil decreased in response to a high level of Fe concentration. Abd El- Wahab (2008) reported that micronutrients such as Fe, Mn and Zn have important roles in plant growth and yield of aromatic and medicinal plants. Furthermore, the role of Fe in biological redox systems (electron transfer chain in photosynthesis and respiration), nitrogen fixation, chloroplast development, enzyme activation, heme proteins (cytochromes,

catalase, peroxidase), Fe-S proteins (e.g. ferredoxin, isoenzymes of superoxide dismutase, aconitase), is well known and documented (Welch, 1995).

Due to the demand for medicinal plants and herbal remedies in the world, the cultivation of these plants has significantly increased in recent years. Meanwhile, the use of chemical fertilisers and nutrients to increase the yield has become very popular (Yazdani *et al*., 2004). However, there has been little research into the effect of different amounts of chemical fertilisers on bioactive components and secondary metabolites in aromatic and medicinal plants. Therefore, this study aimed to evaluate the combined effect of soil applied iron (Fe) and sulfur (S) on essential-oil composition and antioxidant activity of *Satureja hortensis* grown on calcareous soil.

MATERIAL AND METHODS

Soil Analysis

The experiment was carried out on loamy calcareous soil [Typic Calcixerepts] with EC of 0.39mmho cm^{-1}, CCE of 44.8%; organic carbon (OC) of 0.87%; pH of 7.76; available P of 4.47mg kg^{-1}(Olsen *et al*., 1954), DTPA extractable (Lindsay & Norvell, 1978) copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe) content of 1.03, 0.957, 3.73 and 2.34mg kg^{-1} soil, respectively. The mentioned attributes of the studied soil were measured using standard methods.

Statistical Design of Experiment

A completely randomised design experiment with six replications was conducted in greenhouse conditions. Treatments consisted of control (without any Fe or S application), soil application of 8mg Fe and 50mg S kg^{-1} soil (8Fe+50S), 8mg Fe and 100mg S kg^{-1} soil (8Fe+100S), 16mg Fe and 50mg S kg^{-1} soil (16Fe+50S), and 16mg Fe and 100mg S kg^{-1} soil (16Fe+100S). Iron and S were applied as iron-ethylenediamine di-o-hydroxyphenylacetic acid (Fe-EDDHA) and elemental sulfur, respectively.

Soil Preparation and Satureja hortensis planting

Each pot contained 3kg soil. Aforementioned Fe and S treatments were applied before planting. For preventing any probable nutrient deficiency other than Fe and S, 60mg P as $Ca(H_2PO_4)_2.H_2O$, 30, 30 and 15mg Mn, Zn and Cu as in solutions of their sulfates were added uniformly to each pot. Nitrogen (450 mg) was added as $CO(NH_2)_2$ to each pot as well (one half of N was added before planting and the remainder was added as dressed application three weeks after emergence). Twenty seeds of *Satureja hortensis* were planted at a depth of about 10-mm and were reduced to 10 uniform plants two weeks after emergence. Plants were irrigated with distilled water to near FC (field capacity) and maintained at this level of moisture with addition of water to a constant weight. At the 12^{th} week after emergence, the plants were harvested. The plant samples were dried at shade and prepared for analysis.

Extraction of Plant Samples

For extraction of the metabolites in the

plant samples the following steps were carried out: 20g of dry matter was soaked in 0.2L of methanol/water (90/10) solution for 48h (the solvent was changed after 24h). The filtered extract was then concentrated using a rotary evaporator for <10 minutes. The yield was determined by weighting the obtained powder. The powder was preserved at -20°C until used. Just before each analysis, the desired amounts of powder dissolved in methanol and its antioxidant activity and the total content of phenol were determined.

Antioxidant Activity Measurement

The extract of the plant was tested to determine the antioxidant activity and the standard antioxidants on the basis of the radical-scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. gallic acid was used as a standard solution. An improved assay method proposed by of Bruits *et al*. (2001) wherein 0 . 2 ml of a 0.1M solution of DPPH radical in methanol was mixed with 0.02ml of 0.0125 and 3.200mg ml^{-1} of extracts and gallic acid, respectively was used. The solutions were kept at lab temperature for 0.5h. A micro-plate reader of Biotek ELx 808 model at 515nm was used to measure the DPPH-radical-inhibition. The half maximal inhibitory concentration (IC_{50}) of each extract (concentration in µg/ml required to inhibit DPPH radical formation by 50%) was calculated by MATLAB software package. The methanolic extract solution without DPPH was considered as a blank. Antioxidant activity (AOA) was calculated using the following equation:

$$AOA = 1- [A_{sample}-A_{blank} /A_{control}] \quad [1]$$

where A_{sample} is the absorbance of the samples. $A_{control}$ and A_{blank} correspond to absorbance of DPPH (without plant extract) and methanol, respectively.

The value of IC_{50} (the concentration of the test sample leading to 50-% reduction of the initial DPPH concentration) was determined for each extract sample from the nonlinear regression of the mean value (%) of activity for radical scavenging vs. the log-concentration of the test extract (µg/ml). The IC_{50} value is an appropriate way to measure the oxidation progress in oils and is therefore considered a good indicator for the effectiveness of antioxidants.

Extraction of Headspace Volatiles

Up to 0.003kg of each air-dried *Satureja hortensis* sample was crushed, placed in a 0.02L headspace vial and sealed with silicone-rubber septa and aluminum caps immediately. The vials were then transported to the headspace tray. The headspace continued on the ombiPAL system that consisted of a headspace auto-sampler, agitator and heater. The vial was heated up to 80°C and reserved for 20min while being agitated; the temperature of the sampling needle and transmission lines was 85°C.

Determining of the Oil Components by GC/MS

The GC-MS analysis was conducted using Agilent 7890 operating equipped with a HP-5 MS capillary column (phenyl-methyl-

siloxane, 30mx0.25mm i.dx 25pm) with split ratio of 1:50 and carrier gas of He at 70eV ionisation energy. The retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions was used to determine the retention indices. The N-alkanes were used as a standard in the determination of retention indices for all the constituents. The identified compounds from the retention indices (RRI, HP-5) were compared with those reported in the literature and by comparison of their mass spectra with the Adams Library, Wiley GC/MS Library, MassFinder 2.1 Library data published mass spectra data (Joulain *et al*., 2001; Adams, 2007; Adams & Yanke, 2007).

Statistical Analysis

The data were statistically analysed using MSTATC (Michigan State University, East Lansing, MI, USA) and Excel (Microsoft, Redmond, WA, USA) software packages and the mean values of the plant responses were compared statistically using Duncan's multiple range test at the probability level of 0.05.

RESULTS AND DISCUSSION

Antioxidant Activity

The combined effect of Fe and S application on the inhibitory effect of *Satureja hortensis* extract is shown in Fig.1. All the treatments possessed antioxidant potential, but variations were observed among them. All the extracts showed significant amounts of inhibitory effects (IC_{50}) from 377.75mg/l in treatment of 8Fe+100S (application of 8mg Fe and 100mg S per kg soil) to 720.406mg/l in control (Fig.1) and decreased in the following order:

8Fe+100S > 8Fe+50S > 16Fe+50S > 16Fe+100S > 0Fe+0S

The results indicated that the best plant was obtained with application of 8mg Fe + 100mg S kg^{-1} soil (IC_{50}=377.75mg/l), whereas the control plants showed the lowest antioxidant activity. Erdemoglu *et al*. (2006) reported that among 60 studied plants of Iran, *S.macrosiphon* (IC_{50} = 2.96 µg) and *S. hypoleuca* (IC_{50}=5.27 µg) failed to show significant antioxidant activity. Another research reported that the *S.macrosiphon* with 404.12mmol of $FeSO_4$ $100g^{-1}$ showed significant antioxidant activity using ferric ion reducing antioxidant power (FRAP) assay (Gohari *et al*., 2011). In the present study all the extracts showed significant amounts of inhibitory effects from 720.4mg/l in control to 377.75mg/l with application of 8 mg Fe+100mg S kg^{-1} by DPPH (Fig.1). Extensive research has been conducted into the antioxidant activity of some species of the Lamiaceae family (Shan *et al*., 2005). Shan *et al*. indicated that this plant family is apowerful antioxidant producer. Some investigators have reported that rosemary had the highest antioxidant effect whereas sage, oregano and basil had the lowest antioxidant activity. Similar to our findings, a linear relationship between the content of total phenolic compounds and their antioxidant

capacity has been demonstrated by some investigators (Djeridane *et al*., 2006; Katsube *et al*., 2004). The maximum antioxidant effect was obtained with application of 8mg Fe + 100mg S kg^{-1} soil. It could be ascribed to an increase in main phenolic content (carvacrol) by about of 1.6 fold because of positive correlation between phenolic contents and antioxidant activity. In other words, our findings revealed that aforementioned levels of applied fertilisers could improve the antioxidant activity of *S. hortensis*. As mentioned (Halliwell & Gutteridge, 1999; Miguel, 2010), plant phenols exhibit in-vitro antioxidant activity, inhibiting lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers. Phenols with two adjacent hydroxyl groups can bind transition metal ions e.g. Fe and Cu. In addition, phenols directly scavenge reactive oxygen species (hydroxyl radicals, peroxynitrite and hypochlorous acid). Sometimes phenols can act as pro-oxidants by reducing transition metal ions.

In contrast, Azaizeh *et al*.(2005) reported that application of chemical fertilisers decreased the antioxidant activity of some other medicinal plants e.g. *E. creticum*. They believed that leaf senescence of studied cultivated plants may account for differences in antioxidant activity in response to different fertilisation regimes.

Chemical Composition of Essential Oil

The identified constituents with their respective RIs and percentages are summarised in Table 1. The main constituents of the oils in control were γ-terpinene (67.4%), α-terpinene (10.8%), myrcene (4.4%), α-thujene (4.2%), p-cymene (3.8%), α-pinene (2.8%) and carvacrol (2.1%). The results showed

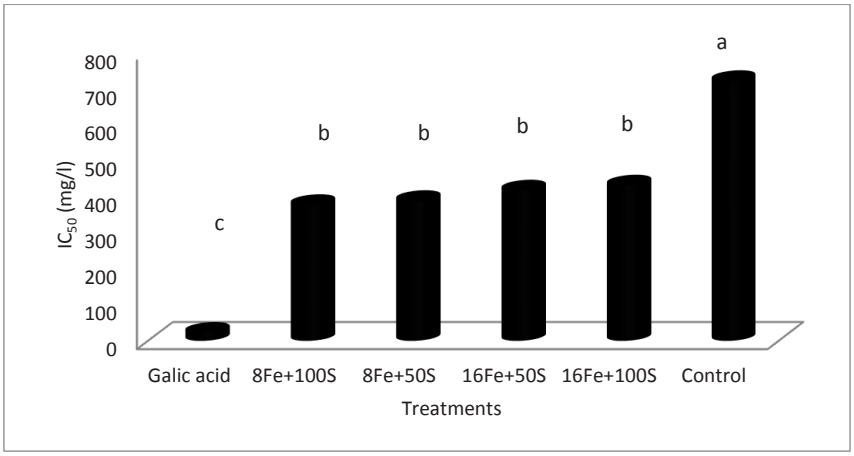

Fig.1: Comparison of antioxidant activity between treatments and gallic acid by DPPH assay. Treatments consisted of 8mg Fe kg^{-1} soil+50mg S kg^{-1} soil, 8mg Fe kg^{-1} soil+100mg S kg^{-1} soil, 16mg Fe kg^{-1} soil+50mg S kg^{-1} soil and 16mg Fe kg^{-1} soil+50mg S kg^{-1} soil (columns with the same statistical letter are not significantly ($P < 0.05$) different by Duncan's multiple range test).

that the maximum α-thujene, α-pinene, myrcene and α-terpinene percentage was obtained with application of 8mg Fe+50mg S kg^{-1} whereas the control was suitable for obtaining special components such as γ-terpinene. On the other hand, carvacrol was mainly produced with the application of 16mg Fe+100mg S kg^{-1}. The most important results of this study were the increasing trend in the quantities of α-thujene, α-pinene, myrcene and α-terpinene with the application of 8mg Fe+50mg S kg^{-1}. The α-terpinene was 10.8% in the control and reached to 12.2% with an application of 8mg Fe+50mg S kg^{-1}, an enhancement equal to 13.0% (Fig.2 and Table 1). The higher percentage of aforementioned constituents in essential oil composition of Fe and S treated plants may correspond to participation of these two studied elements in essential oil constituents or may be due to the improved growth conditions for plants when these two elements were applied. Other researchers like Mengel and Kirkby (1978) also stated that S is one of the major essential nutrients that plays a key role in the biochemical structure of plant oils. Besides, Wierdak (2013) stated that growing conditions, irrigation, cultivation method, fertilisation and date of harvest of plant material can considerably modify both the quantity and quality (composition) of essential oil.

The myrcene also represented a trend similar to that of α-terpinene in response to applied 8mg Fe and 50mg S kg^{-1} soil. This compound showed an increase of about 27.3%. Another important constituent that showed an interesting alteration in trend was α-thujene. As shown in Table 1, the quantity of α-thujene drastically increased by 21.4%. The amount of α-pinene in control harvested plant materials increased over time so that

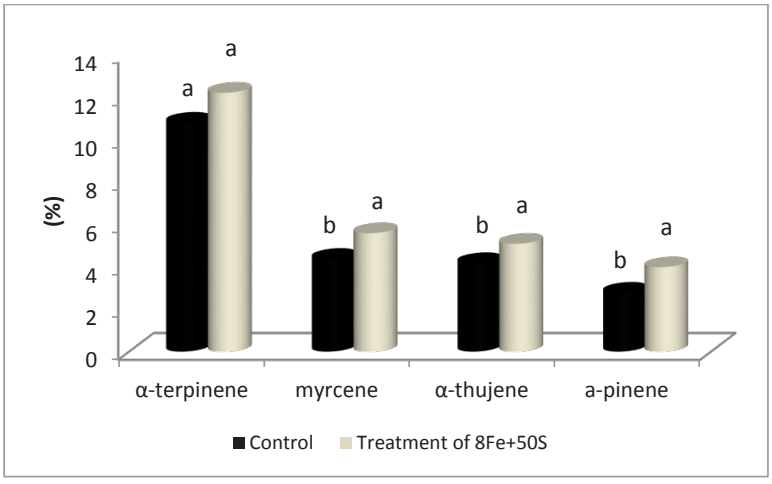

Fig.2: Changes of *S. hortensis* essential oil composition with application of 8mg Fe kg^{-1} and 50mg S kg^{-1} (columns with the same statistical letter are not significantly ($P < 0.05$) different by Duncan's multiple range test).

the amount was 2.8% immediately after oil extraction, gradually increasing to 4.0% and then to 42.85% at the end of the experiment period.

In total, 17 constituents were identified and quantified in the *S. hortensis* essential oil (EO) samples subjected to application of 8mg Fe and 50mg S kg^{-1} soil. The monoterpenoid fraction constituted 99.9% of the oil with the main components γ-terpinene, α-terpinene, myrcene, α-thujene, p-cymene, α-pinene and carvacrol. The percentage of the identified sesquiterpenes and sesquiterpenoid components was relatively low (0.1%). It has been reported that monoterpenes are the primary constituents of plant essential

TABLE 1
Combined Effect of Soil Applied S and Fe on the Chemical Composition (%) of *S. hortensis* Essential Oil

				Applied Fe and S fertilisers (mg kg^{-1} soil)			
No	Compound	RI[a]	Control	8Fe+50S	8Fe+100S	16 Fe+50 S	16Fe+100S
1	α-Thujene	923	4.2[b] b	5.1 a	4.4 b	4.1 b	3.9 b
2	α-Pinene	930	2.8 b	4.0 a	3.3 b	2.9 b	2.8 b
3	Camphene	945	0.2 a	0.3 a	0.2 a	0.2 a	0.2 a
4	Sabinene	969	0.3 bc	0.5 a	0.4 ab	0.3 bc	0.2 c
5	β -pinene	973	1.1 b	2.0 a	1.6 ab	1.4 b	1.3 b
6	Myrcene	987	4.4 b	5.6 a	5.0 a	5.1 a	4.9 ab
7	α-Phellandrene	1003	0.9 a	1.2 a	1.1 a	1.0 a	1.0 a
8	δ-3-Carene	1008	0.1 b	0.2 a	0.2 a	0.2 a	0.2 a
9	α-Terpinene	1014	10.8 a	12.2 a	11.5 a	11.2 a	10.9 b
10	p-Cymene	1021	3.8 b	3.9 b	4.1 b	5.1 a	5.1 a
11	Limonene	1025	0.9 a	0.6 c	0.8 ab	0.7 bc	0.6 c
12	β -Phellandrene	1026	0.2 b	0.8 a	0.5 a	0.6 a	0.7 a
13	(E)- β -Ocimene	1043	0.1 b	0.2 a	0.2 a	0.2 a	0.2 a
14	γ-Terpinene	1059	67.4 a	60.4 b	60.8 b	58.4 b	59.0 b
15	cis-Sabinene hydrate	1064	-	-	-	0.1	0.1
16	Terpinolene	1085	-	-	-	0.1	0.1
17	Thymol methyl ether	1239	-	-	0.1	0.1	0.2
18	p-Cymen-9-ol	1211	0.3	-	-	-	-
19	(Z)-Ocimenone	1224	0.1	-	-	-	-
20	Carvacrol	1299	2.1 b	2.8 b	5.5 ab	7.7 a	8.1 a
21	caryophyllene	1415	-	-	-	-	0.1
22	β -Bisabolene	1504	0.1 b	-	0.2 b	0.4 a	0.5 a
	Total		100%	99.8	99.8	99.8	99.9

[a] RI, retention indices. Treatments consisted of 8mg Fe kg^{-1} soil+50mg S kg^{-1} soil, 8mg Fe kg^{-1} soil+100mg S kg^{-1} soil, 16mg Fe kg^{-1} soil+50mg S kg^{-1} soil and 16mg Fe kg^{-1} soil+50mg S kg^{-1} soil.
[b] Means in each row followed by the same letter are not significantly ($P<0.05$) different by Duncan's multiple range test.

oils and the effects of many medicinal herbs are attributed to the presence of these compounds (Gherlardini *et al*., 2001). In this regard, the findings of our study showed that the concentration of γ-terpinene with a lower molecular weight decreased in response to application of 8mg Fe+50 mg S kg^{-1} (Table 1). This phenomenon could be due to evaporation, oxidation and other unwanted changes in essential-oil components with application of 8mg Fe + 50mg S kg^{-1}. It has been reported that essential-oil biosynthesis depends on a number of factors e.g. the presence of different input substances and enzymes, depending on the metabolic pathway in which a given group of compounds is formed (Woronuk *et al*., 2011). For example, Novak *et al*. (2002) reported that terpenes are produced from a small number of substrates whereas terpene synthases are capable of forming numerous terpene skeletons. Wierdak (2013) stated that the biochemical pathways for synthesis of some volatile compounds, which are essential-oil components, have not yet been fully described. However, some investigators like Lewinsohn *et al*. (2000) showed the combination of the effect of ontogenesis and chemotype on the activity of O-methyltransferase, the enzyme catalysing the transfer of the methyl group from methionine to the acceptor. In addition, these researchers demonstrated the presence of two types of activity of this enzyme in two basil chemotypes; one of them was highly specific for chavicol while the other could accept eugenol as a substrate. In general, as Wierdak (2013) stated, fertilisation and feeding of herbal plants seem to be important factors modifying their aromatic profile and the quantity and quality of their essential oils.

The genus *Satureja* presents great variability in the concentration of the major components of its essential-oil composition due to the presence of different species and subspecies, as well as because of various factors, mostly the environmental and climatic circumstances (Gulluce *et al*., 2003). With reference to previous studies, carvacrol and thymol, in particular, were found to be main components of the oils isolated from numerous Croatian *Satureja* species (Skoibuix & Bezix, 2004). It was interesting to note that different isolates of winter savory from Croatia and Bosnia and Herzegovina had carvacrol (up to 84.19%) as a major component (Kustrak *et al*., 1996). Cazin *et al*. (1985) revealed that the oil composition of winter savory showed large differences in the relative concentration of main constituents: carvacrol (5-69%), linalool (1-62%), γ-terpinene (1-31%) and p-cymene (3-27%), arising from the presence of different chemotypes. The main components of the *S. hortensis* (summer savory) essential oil were the phenols, thymol (29.0%), carvacrol (26.5%), *r*-terpinene (22.6%), p-cymene (9.3%) and other terpenoids (Gulluce *et al*., 2003). Finally, it could be concluded that application of 8mg Fe+50mg S kg^{-1} was the most suitable treatment for obtaining a higher percentage of α-terpinene, myrcene, α-thujene and α-pinene whereas addition of 16mg Fe+100 mg S kg^{-1} was preferable

for obtaining special components such as carvacrol. On the other hand, γ-terpinene was mainly produced in the control. Essential-oil composition in *Satureja* species showed it to be rich in phenolic components like carvacrol, γ-terpinene, thymol, p-cymene, β-aryophyllene, linalool and other terpenoids, but chemical composition and the amount of components varied between the different *Satureja* species oils (Baser *et al*., 2004; Novak *et al*., 2006; Sefidkon and Jamzad, 2006). Some researchers showed that the essential oil and extract of *Satureja* species showed a variety of activities including anti-bacterial and anti-fungal properties and they strongly inhibited the activity of a wide variety of bacteria and fungi in human, food and plant pathogens (Baydar *et al*., 2004; Gulluce *et al*., 2003; Hajhashemi *et al*., 2000). Recent studies showed that some plants from the lamiaceae families were very rich in phenolic compounds such as phenolic-acids, flavonoids and phenolic-diterpenes, and possessed high antioxidant activities (Aaby *et al*., 2004; Wong *et al*., 2006). Flavonoids and phenolic compounds exert multiple biological effects such as anti-oxidant activities, free radical scavenging and anti-inflammatory properties (Miliauskas *et al*., 2004; Shahidi, 2000). Oxidative damage in the human body plays a vital causative role in disease initiation and progression (Jacob & Burri, 1996).

CONCLUSION

S. hortensis L. is a medicinal and aromatic plant of the Lamiaceae family used in Iranian folk medicine for various purposes. Our results showed that chemical fertilisers increased the essential oil constituents. The amount of some components such as α-terpinene, myrcene, α-thujene, p-cymene, α-pinene and carvacrol could be changed in response to S and Fe fertiliser application. Our findings indicated that savoury oils, in addition to other properties, had potential in topical antioxidant activity and its antioxidant activity increased when plants received S and Fe fertilisers.

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