Shoot Growth, Gamma-Terpinene and Essential Oil Content of *Satureja hortensis* L. in Response to Foliar Application of $FeSO_4$ and Citric Acid

Radmanesh E (M.Sc.)[1], Naghdi Badi H (Ph.D.)[2], Hadavi E (Ph.D.)[1], Mehrafarin A (Ph.D.)[2]*

1- Department of Horticultural Science, College of Agriculture and Natural Resources, Islamic Azad University, Karaj Branch, Iran
2- Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
* Corresponding author: Medicinal Plants Research Centre, Institute of Medicinal Plants, ACECR
P.O.Box: 33651/66591, Karaj, Iran
Tel: +98-26-34764010-9, Fax: +98-26-34764021
E-mail: A.Mehrafarin@gmail.com

Received: 28 Sep. 2014 **Accepted:** 21 Feb. 2015

Abstract

Background: Summer savory (*Satureja hortensis* L.) is one of the most important medicinal and spice plants cultivated in many parts of the world.

Objective: A greenhouse experiment was conducted to study the effects of $FeSO_4$ and citric acid application on the morphological traits and essential oil content and its component, gamma-terpinene in *S. hortensis*.

Methods: $FeSO_4$ in four levels (0, 3, 4 and 5 g L^{-1}) and citric acid in four levels (0, 4, 6 and 8 mM) through foliar application were considered as two studied factors in this factorial experiment.

Results: The results showed that the treatments of $FeSO_4$, and interaction of $FeSO_4$ and citric acid had a significant effect ($p<0.01$) on the all studied traits, whereas citric acid treatments only had significant effect on the essential oil content ($p<0.01$) and plant height ($p<0.05$). $FeSO_4$ and its interaction with citric acid significantly increased these traits. The optimum level of $FeSO_4$ fertilizer on this plant was 3 and 4 g L^{-1} and for citric acid was 6 and 8 mM which increased the essential oil content and its component, gamma-terpinene compared to control plants.

Conclusion: Foliar application of citric acid plus $FeSO_4$ had the capability to alter the relative percentages of the essential oil content. In general, foliar application of $FeSO_4$ with citric acid had synergistic effect on essential oil content, its component gamma-terpinene and agro-morphological traits of *S. hortensis*.

Keywords: ***Satureja hortensis* L., Essential oil, $FeSO_4$, Citric acid, Foliar application**

Introduction

The genus *Satureja* L. (Labiatae, Mentheae) comprises over 30 species with wide distribution in the Mediterranean region [1]. Among them, many are used as valuable medicinal and spice plants worldwide. Summer savory (*S. hortensis* L.) is an annual aromatic plant with linear to linear-oblanceolate leaves and white to pale red flowers, which are born in erect stems [2]. In folk medicine, *S. hortensis*is used as stomachic, stimulant, carminative, expectorant, aphrodisiac, antispasmodic and antidiarrhoeal [3, 4]. In addition, summer savory has wide application in the world food, drink and perfume industries [5, 6]. The essential oil of *S. hortensis* possesses many activities such as antioxidant, antibacterial and antifungal [7, 8]. Main essential oil constituents are phenolic compounds, carvacrol and thymol, as well as γ-terpinene, *p*-cymene, β-caryophyllene, linalool and other terpenoids [8]. Production of essential oil and its composition in plants is mainly dictated by the combined influences of both genetic factors and cultivation conditions such as climate, plant density, the use of fertilizers, etc. [9, 10].

In comparison with other crops, the concentration of main minerals such as Ca^{2+}, Mg^{2+} and Zn^{2+} in the leaves of *S. hortensis*is very high which makes it as a potential source of dietary minerals [11]. Beside, adaptability to harsh environmental conditions, high yield and short growing period make *S. hortensis*as a valuable alternative crop in agriculture [1]. Micronutrients, applied either as complex or single fertilizers, have been proved to be effective in improvement of biomass, yield of secondary metabolites, and, in some cases, quality of those metabolites [12, 13].

Iron is a trace element, required in higher amounts by plants than other micronutrients. The role of iron in biological redox systems (electron transfer chain in photosynthesis and respiration), enzyme activity, N_2 fixation, chloroplast development, heme proteins (cytochromes, catalase, peroxidase), iron-sulphur proteins (ferredoxin, isoenzymes of superoxide dismutase, aconitase), is well known and documented [14]. Less is known about the effect of iron on the secondary metabolism, either indirectly, by affecting availability of photosynthates provided by primary metabolism [15], or directly, through some factors responsible for efficient utilization of precursors coming from primary synthesis [16]. Root iron uptake involves a reduction of iron from Fe^{3+} to Fe^{2+} at the cell membrane of epidermal root cells; this reduction is catalyzed by the enzyme [17]. After iron is reduced in the roots, it is re-oxidized back to Fe^{3+} in the apoplast where Fe^{3+} then binds with citric acid [18]. Iron is then transported in the xylem from the roots to the leaves as ferric-citrate and re-reduced in the leaf apoplast to the Fe^{2+} form and is actively transported across the plasma membrane into the symplast where it is metabolized by the plant [17, 19, 20, 21]. Often, in calcareous soils, a sufficient quantity of iron is translocated from the roots to the leaves, but the reduction of Fe^{3+} to Fe^{2+} in the leaves is hampered by the high pH environment of the apoplast [17].

Recently, it has been proposed that root exudation of citrate may play an important role in supplying Fe to dicotyledonous plants. Studies in which different varieties of soybeans, tomatoes and corn were tested for Fe uptake efficiency showed that citric acid accumulation is always more pronounced in

the Fe-efficient genotypes. Acid treatments could release Fe immobilized within the plant by changing apoplastic pH [17]. The participation of organic acid excretion in the mechanisms that allow cells to cope with a low iron supply has been reported in diverse organisms ranging from bacteria to plants. For instance in *Eschericia coli* and *Pseudomonas putida*, under iron-limiting conditions ferric citrate activates the transcription of genes encoding a ferric-citrate transport system [22].

In calcareous soils, the correction of Fe chlorosis in plants is normally achieved by the application of Fe (III)-chelates such as Fe-EDDHA to the soil [18]. This practice has to be repeated every year because Fe is rapidly immobilized in the soil. Foliar application of Fe instead of soil application can avoid inhibitory effects of soil bicarbonate on Fe uptake and transport to the shoot [23].

In the recent years, the interest of growing herbs such as savory as alternative crops is highly increased [4-8]. With regard to the importance of $FeSO_4$ and citric acid application on the growth and essential oil, we studied the impacts of $FeSO_4$ and citric acid rates on the aerial shoot growth, essential oil content of *S. hortensis* L. and its components cultivated in greenhouse.

Materials and methods

In order to study the effects of $FeSO_4$ and citric acid on the aerial shoot and essential oil content of *S. hortensis* L., a greenhouse experiment was carried out based on randomized complete block design with three replications. The experiment was conducted at the research greenhouse of Institute of Medicinal Plants (IMP), Iranian Academic Centre for Education, Culture & Research (ACECR), Iran, in 2013 (altitude 50˚ 53 E, longitude 1235 M, latitude 35˚54 N). The average day and night temperatures during experiment were 26.5 and 18.5°C, respectively. The seeds of *S. hortensis*, a bred cultivar with high quality provided from the seed bank of Institute of Medicinal Plants (IMP). On July, the seeds were sown in pots (24 cm diameter and 10 cm deep) containing field soil and well rotten animal manure under green house conditions.

After full establishment of plants and when they had 10 or 15 fully expanded leaves the treatments were done as foliar application of $FeSO_4$ at four concentration levels including 0, 3, 4 and 5g L^{-1} and citric acid at four concentration levels comprised of 0, 4, 6 and 8 mM. Foliar application was applied 4 times during growth stages and cultivation season with 15 days intervals on shoot part of *S. hortensis*. The first spray was applied 20 days after sowing and the others were treated 35, 50 and 65 days after sowing. Sprays were carried out in a way that all above ground parts of rosemary plants were covered. To increase the absorption of solutions by plants, foliar application of the employed treatments was done before sunrise when plant stomata are open. During the growing period, the plants were weekly irrigated and kept free of weeds by hand hoeing. After establishment of plants, they were monitored carefully and precisely.

Some parameters were measured in greenhouse and some other in laboratory over a period of 100 days. Following parameters were recorded for each sample: shoot dry weight (g), plant height (cm), number of leaves per plant (leaves $plant^{-1}$), number of flowers per plant (flowers $plant^{-1}$), number of branches per plant (branches $plant^{-1}$), and essential oil content (%).

For essential oil isolation, the aerial parts of

harvested plants were air-dried in the shade, and then flower heads and leaves were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopeia method [24]. The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4°C prior to analysis. The essential oil content (v/w) was determined.

GC analysis was carried out on a Younglin Instrument Acme 6000M gas chromatograph equipped with Flame Ionization Detector (FID) and a HP-5 capillary column (30 m×0.25 mm; 0.25 μm film thicknesses). The oven temperature was held at 50ºC for 5 minutes, and then programmed at 3ºC min^{-1} to 240ºC and after that programmed at 15ºC min^{-1} to 300ºC (held for 3 minutes). Other operating conditions were: carrier gas, He with a flow rate of 0.8 mL min-1; injector and detector temperatures was 290ºC, and split ratio, 1:10. GC/MS analysis was performed on a GC mentioned above coupled with an Agilent Technologies 5973 Mass system. The other operating conditions were the same conditions as described above, mass spectra were taken at 70 eV. Mass range was from m/z 35–375 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. The gamma-terpinene were identified by comparison of their mass spectra and retention indices with those published in the literature [25, 26] and presented in the MS computer library. Each analysis was performed in triplicate.

Data of all measured parameters were subjected to analysis of variance using SPSS statistical software (ver. 17). Also, Duncan's multiple range tests was used to compare treatment means at a probability level of 0.05.

Results

The results of the present study showed that $FeSO_4$, and interaction of $FeSO_4$with citric acid treatments had a significant effect ($p<0.01$) on aerial parts of the plant, whereas citric acid treatment had no significant effect on the examined traits except plant height and essential oil content (Table 1). 5 g L^{-1} $FeSO_4$ and 0 Mm citric acid treatment significantly increased the shoot dry weight (1.85 g compared to 1.26 g at 3 g L^{-1} $FeSO_4$ and 0 Mm citric acid) (Figure 1). Plant height was positively affected by $FeSO_4$ ($p<0.01$), citric acid ($p<0.05$), and interaction of $FeSO_4$ and citric acid treatments ($p<0.01$) (Table 1), and it was the greatest (37.76 cm) at 3 g $L^{-1}$$FeSO_4$ and 8 mM citric acid (Figure 2). $FeSO_4$ and interaction of $FeSO_4$ and citric acid fertilizers significantly affected the number of leaves per plant ($p<0.01$), whereas citric acid treatment had no significant effect on the number of leaves (Table 1). The number of leaves was greater in the plants fertilized with 4 g L^{-1} $FeSO_4$ and 6 mM citric acid(189.33 leaves $plant^{-1}$) than those of 3 g L^{-1} $FeSO_4$ and 0 mM citric acid (153 leaves $plant^{-1}$) (Figure 3). The Effect of different levels of $FeSO_4$ on flower number per plant was significant ($p<0.01$), but citric acid had no significant effect on the flower number (Table 1). the maximal number of flowers was achieved from 5 g L^{-1} $FeSO_4$and 6 mM citric acid (77.71 flowers $plant^{-1}$) (Figure 4). Number of branches per plant increased significantly with $FeSO_4$, and $FeSO_4$ and citric acid fertilization ($p<0.01$) as the highest value (26.33) was observed with application of 3 g L^{-1} $FeSO_4$and 8 mM citric acid (Figure 5), whereas citric acid treatments had no significant effect on the number of

branches (Table 1). The effects of $FeSO_4$ and citric acid treatments on the essential oil content of *S. hortensis* L. and its components are presented in Table 1. Both $FeSO_4$ and citric acid applications had significant effect ($p<0.01$) on the essential oil content as it was the highest at the 0 g L^{-1} $FeSO_4$ and 8 mM citric acid (Figure 6). Application of $FeSO_4$, citric acid and their interaction had significant ($p<0.01$) effect on component of gamma-terpinene and the maximum value of this component was observed in 4 g L^{-1} $FeSO_4$ and acid citric 8 mM (Table 1 and Figure 7).

Table 1- Effects of $FeSO_4$ and citric acid application on shoot growth traits and essential content of *Satureja hortensis* L. under greenhouse condition

Source of Variation	df.	Means Squares (MS)						
		Shoot dry weight	Plant height	Number of leaves	Number of flowers	Number of branches	Essential oil content	Gamma-terpinene
Block	2	0.004^{ns}	0.624^{ns}	3.57^{ns}	2.895^{ns}	3.645^{ns}	0.0033^{ns}	9.12^{ns}
$FeSO_4$	3	0.25**	261.16**	91056.07**	25188.08**	306.44**	0.0183**	46.69**
Citric Acid	3	00.18^{ns}	4.003*	22.35^{ns}	226.58^{ns}	5.27^{ns}	0.0193**	5.87**
$FeSO_4$ × Citric Acid	9	0.016**	54.48**	18252.86**	5318.52**	67.31**	0.0109**	16.44**
Error	30	0.0045	1.36	24.82	109.65	3.57	0.002	6.50
Coefficicient of Variance (%)		14.75	3.67	2.98	12.73	10.26	4.781	1.05

*, ** and, ^{ns} significant at $p<0.05$, $p<0.01$, and non-significant, respectively; df., degree of freedom

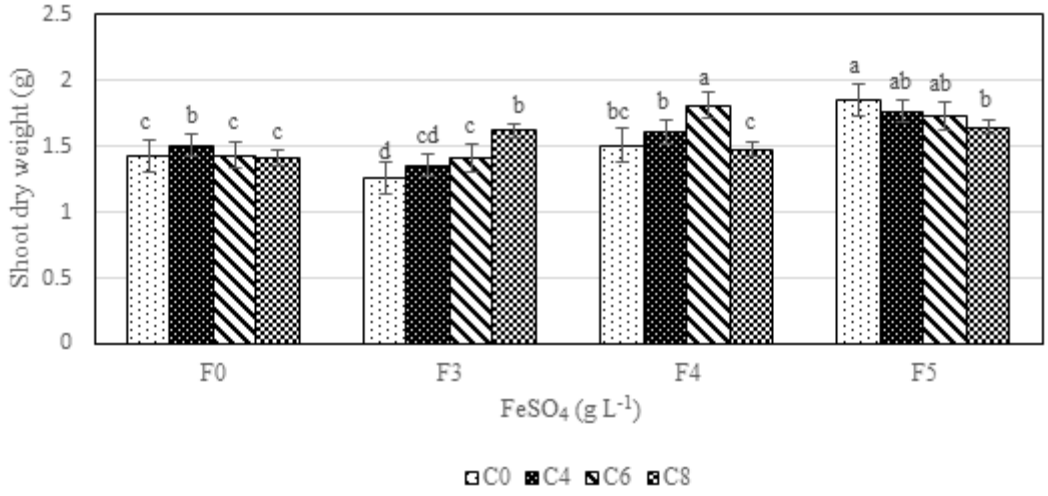

Figure 1- Effect of $FeSO_4$ and citric acid on shoot dry weight of *Satureja hortensis* L. Abbreviations: F0, F3, F4, F5 were 0, 3, 4 and 5 g L^{-1} $FeSO_4$, and also, C0, C4, C6, and C8 were 0, 4, 6 and 8 mM citric acid, respectively. The vertical bars represent standard errors of the means. Bars with the same letter(s) aren't significantly different at $p\leq0.05$

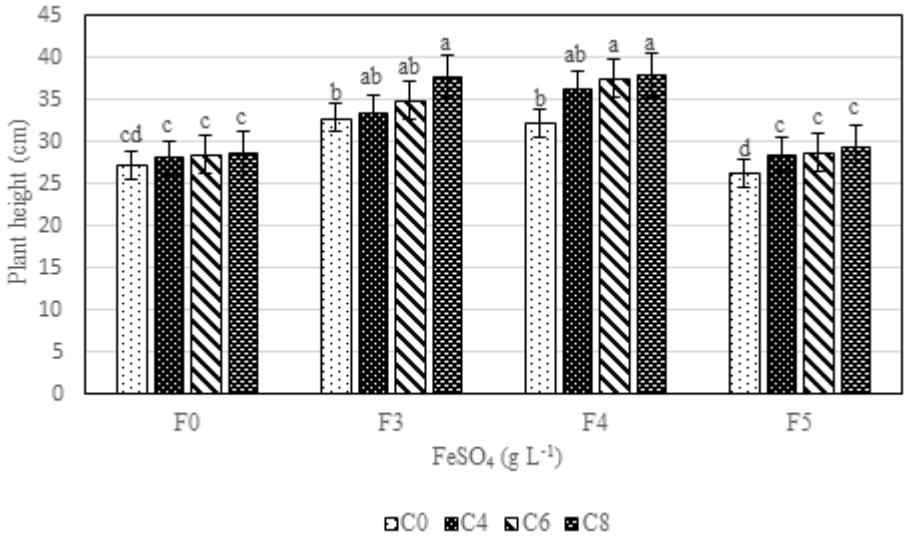

Figure 2- Effect of $FeSO_4$ and citric acid on plant height *Satureja hortensis* L. Abbreviations: F0, F3, F4, F5 were 0, 3, 4 and 5 g L^{-1} $FeSO_4$, and also, C0, C4, C6, and C8 were 0, 4, 6 and 8 mM citric acid, respectively. The vertical bars represent standard errors of the means. Bars with the same letter(s) aren't significantly different at p≤0.05

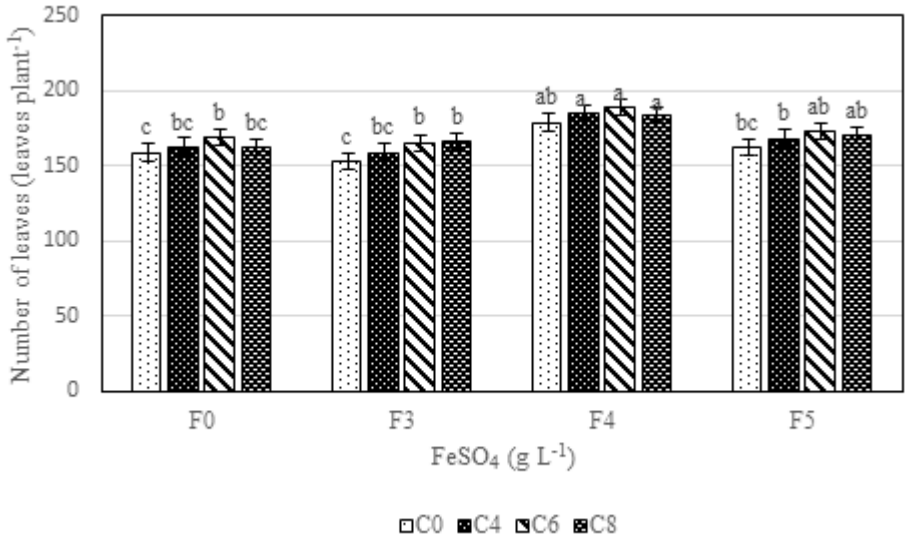

Figure 3- Effect of $FeSO_4$ and citric acidon number of leaves per plant of *Satureja hortensis* L. Abbreviations: F0, F3, F4, F5 were 0, 3, 4 and 5 g L^{-1} $FeSO_4$, and also, C0, C4, C6, and C8 were 0, 4, 6 and 8 mM citric acid, respectively. The vertical bars represent standard errors of the means. Bars with the same letter (s) aren't significantly different at p≤0.05

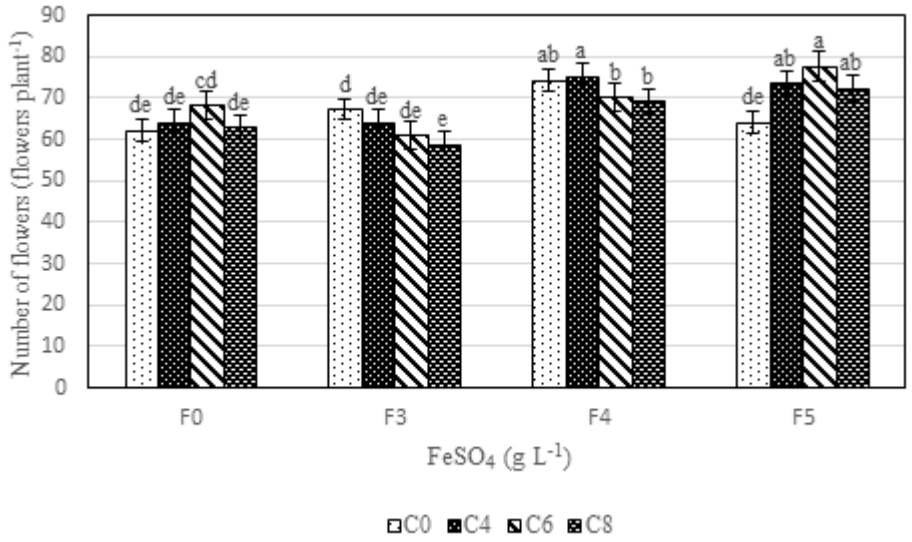

Figure 4- Effect of $FeSO_4$ and citric acid on number of flowers per plant of *Satureja hortensis* L. Abbreviations: F0, F3, F4, F5 were 0, 3, 4 and 5 g L^{-1} $FeSO_4$, and also, C0, C4, C6, and C8 were 0, 4, 6 and 8 mM citric acid, respectively. The vertical bars represent standard errors of the means. Bars with the same letter(s) aren't significantly different at p≤0.05

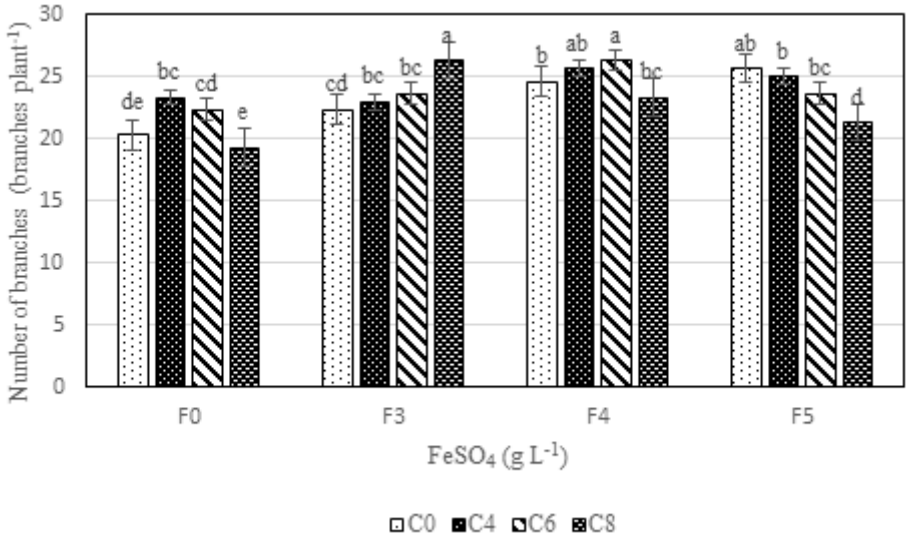

Figure 5- Effect of $FeSO_4$ and citric acid on number of branches per plant of *Satureja hortensis* L. Abbreviations: F0, F3, F4, F5 were 0, 3, 4 and 5 g L^{-1} $FeSO_4$, and also, C0, C4, C6, and C8 were 0, 4, 6 and 8 mM citric acid, respectively. The vertical bars represent standard errors of the means. Bars with the same letter (s) aren't significantly different at p≤0.05

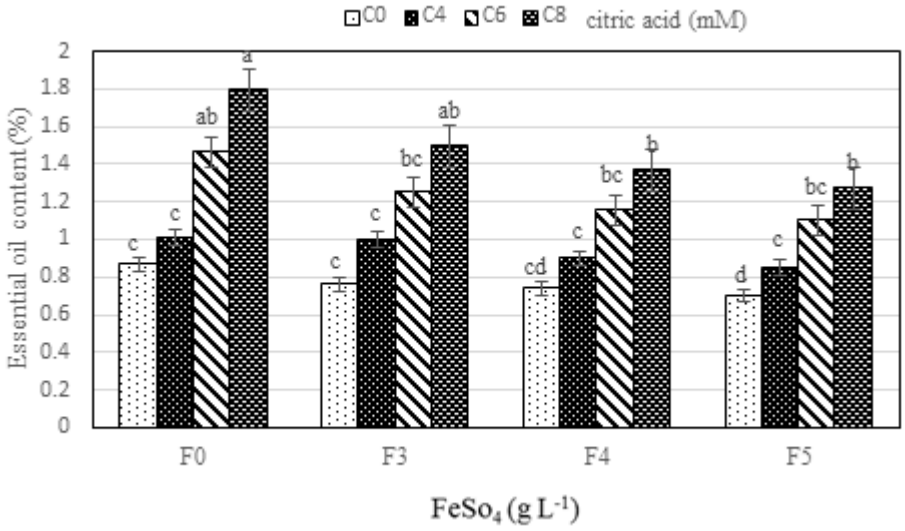

Figure 6- Effect of $FeSO_4$ and citric acid on essential oil content of *Satureja hortensis* L. Abbreviations: F0, F3, F4, F5 were 0, 3, 4 and 5 g L^{-1} $FeSO_4$, and also, C0, C4, C6, and C8 were 0, 4, 6 and 8 mM citric acid, respectively. The vertical bars represent standard errors of the means. Bars with the same letter(s) aren't significantly different at p≤0.05

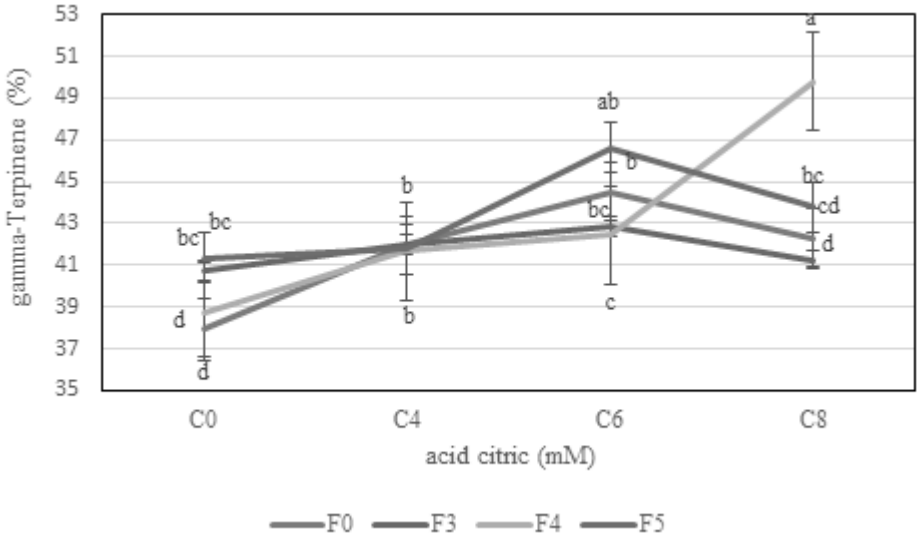

Figure 7- Effect of $FeSO_4$ and citric acid on gamma-terpinene content of *Satureja hortensis* L. Abbreviations: F0, F3, F4, F5 were 0, 3, 4 and 5 g L^{-1} $FeSO_4$, and also, C0, C4, C6, and C8 were 0, 4, 6 and 8 mM citric acid, respectively. The vertical bars represent standard errors of the means. Bars with the same letter(s) aren't significantly different at p≤0.05

Discussion

The present study showed that $FeSO_4$ fertilization significantly ($p<0.05$) influenced the growth of *S. hortensis* L. which is in agreement with the result of Nasiri *et al*. [27]. Also results indicated that treated plants with $FeSO_4$ were much superior in shoot dry weight, plant height, number of leaves,

number of flowers and number of branches compared to control plants whereas, the essential oil content of plants significantly decreased by all applied $FeSO_4$ rates.

Nutrients play a key role in the growth and development of all crops. In the case of medicinal plants containing essential oils, nutrients can effectively increase essential oil content and its components [28-33]. For adequate plant growth and production, micronutrients are needed in small quantities in balance of macronutrients [34]. However their deficiencies cause a great disturbance in the physiological and metabolic processes in the plant [35]. Plants normally take up nutrients from soils through their roots although nutrients can be supplied to plants as fertilizers by foliar application [36, 37]. Throughout the world microelements as Fe is added to foliar fertilizers, in order to compensate their deficiency especially in arid and semi-arid regions [38]. Totally, Iron (Fe) is a micronutrient, the lack of which causes chlorosis and is responsible for significant decrease in yield and quality of plants [39]. Fe acts 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 [41]. Therefore, sufficient amount of these nutrients in the plant is necessary for normal growth. Iron is necessary for the biosynthesis of chlorophyll and cytochrome besides. The function of Fe in the metabolism of chloroplast RNA, leading to increase in the biosyntheses materials (produced and accumulated), consequently, the growth was enhanced [40], Basavarajappa *et al*. [41] reported increased yield for cottonseeds treated with $FeSO_4$. Similarly, Kuzhandaivel and Venkatesan [42] observed that application of $FeSO_4$ and iron complex significantly increased production and growth of sorghum seeds. Singh et al. [43] reported that using $FeSO_4$ and iron citrate increased pod formation. Lalit Bhatt *et al*. [44] in their experiment on the effect of micronutrients on growth and performance of tomatoes found that application of $FeSO_4$ through leaf organ led to the maximum number of branches per plant, leaf number, leaf area, plant fresh weight, and dry stem weight. The stimulatory effect of Fe was recorded by [45-48]. Fe deficiency inhibited leaf growth, cell number, size and cell division, as well as protein, starch and sugar content. Thus, the fresh and dry weights of herb could be decreased following the Fe deficiency. As earlier expressed, the essential of content of *S. hortensis* L. was decreased with application of $FeSO_4$ treatments, but it was increased by citric acid application. Yeritsyan and Economakis [49] reported that essential oil content of *Origanum vulgar* was reduced by the application Fe. Similarly, Misra and Srivastava [13] obtained lower values for the concentration of the essential oil of *Mentha arvensis* L., when the plants were grown in a nutrient solution having high concentrations of iron. It could be concluded that the highest Fe concentration in the nutrient solution resulted in high iron uptake and high tissue (leaves and roots) Fe content, retarding growth and essential oil production of plants either directly (toxicity) or through induced deficiency of other nutrients.

Citric acid appears to improve to some extent the availability of Fe from previously unavailable internal pools in the plant [23]. These results suggest that citric acid causes changes apoplastic pH, by which plant are able to use the Fe more efficiently. With application of micronutrients photosynthetic activity will be increased. Considering the

effect of Iron in plant growth, one of the reasons for improvement of photosynthetic activity may be the role of this element in chloroplast structure and its activity that leads to increase of the essential oil glands in leaves [50]. It seems that acid citric with increase of Fe availability and its absorption by plant can improve the yield of essential oil of *S. hortensis* and its components.

Conclusion

In this study it was found that the $FeSO_4$ and citric acid fertilization had positive effects on the plant growth traits. The optimum level of the foliar application of $FeSO_4$ fertilizer for this plant was 3 and 4 g L^{-1}. Based on the obtained results, citric acid plus $FeSO_4$ had the capability to alter the relative percentages of essential oil content in *S. hortensis*. Also, application of citric acid at 6 and 8 mM was identified as the best treatment for the production of essential oil content and its component, gamma-terpinene.

References

1. Hadian J, Tabatabaei SMF, Naghavi MR, Jamzad Z and Ramak-Masoumi T. Genetic diversity of Iranian accessions of *Satureja hortensis* L. based on horticultural traits and RAPD markers. *Scientia Horticulture* 2008; 115: 196 - 202.

2. Rechinger KH. *Satureja*. Flora Desiranischen Hoclandes and der Umrahmenden Gebirge, vol. 150. Akademische Druku Verlags Antalt Graz, Austria, 1982, pp: 495 - 504.

3. Zargari A. Medicinal Plants (In Persian), vol. 4. Tehran University, 1970, pp: 42 - 5.

4. Hajhashemi V, Sadraei H, Ghannadi AR and Mohseni S. Antispasmodic and antidiarrhoeal effect of *Satureja hortensis* L. essential oil. *J. Ethnopharmacol.* 2000; 71: 1 - 2.

5. Sefidkon F, Abbasi K and Khaniki GB. Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis* L. *Food Chemistry*, 2006; 99: 19-23.

6. Skocibusic M, Bezic N, Dunkic V. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata Vis*. growing in Croatia. *Food Chemistry*, 2006; 96: 20-28.

7. Gulluce M, Sokmen M, Daferera D, Agar G, Ozkan H, Kartal N, Polissiou M, Sokmen A, Sahin F. *In vitro* antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis*. *J. Agric. Food Chemistry*, 2003; 51:3958-3965.

8. Sahin F, Karaman I, Gulluce M, Oguteu H, Sengul M, Adıguzel A, Ozturk S, KotanR Evaluation of antimicrobial activities of *Satureja hortensis* L. *Journal of Ethnopharmacology*, 2003; 87: 61-65.

9. Supanjani Tawaha ARM, Yang MS, Han HS, Lee KD. Calcium effects on yield, mineral uptake and terpene components of hydroponic *Chrysanthemum coronarium* L. *Research Journal of Agriculture and Biological Sciences*, 2005b; 1:146-151.

10. Salmasi SZ, Heidari F and Alyari H. Effect of microelements and plant density on biomass and essential oil production of peppermint

(*Mentha piperita* L.). *J. Plant Sciences and Res*. 2008; 1: 24 - 6.

11. Ozcan M. Mineral contents of some plants used as condiments in Turkey. *Food Chem.* 2004; 84: 437 - 40.

12. Haykazyan V, Khudaverdyan GR, Barsamyan LG and Piloyan AA. The peculiarities of peppermint water culture with additional supply of trace elements, In: Proceedings of the Republican Conference "The Contemporary problems of Soilless Cultivation of Plants." Published by NASRA, Yerevan, 1994, pp: 17 - 8.

13. Misra A and Srivastava NK. Iron nutrition related to growth and physiology of Japanese mint (*Mentha arvensis* L.). In: Proceedings of the International Congress of Plant Physiology. Society for plant Physiology and Biochemistry, India, Neo-art press, New Delhi 1989, pp: 1156 - 60.

14. Welch R M. Micronutrient nutrition of plants. *Critical Reviews in Plant Sciences* 1995; 14 (1): 49 - 82.

15. Srivastava NK and Luthra R. The relation between primary and secondary metabolism in peppermint under Fe-stress. *J. Essential Oil Res.* 1993; 5: 525 - 34.

16. Srivastava RK, Shervani TA and Fahey L. Market-Based Assets and Shareholder Value: A Framework for Analysis. *J. Marketing* 1998; 62: 2 - 18.

17. Mengel K, Planker R, Hoffmann B. Relationship between leaf apoplast pH and Fe chlorosis of sunflowers (*Helianthus annuus* L.). *Journal of Plant Nutrition*. 1994; 17:1053-1064.

18. Schmidt W. Review: mechanisms and regulation of reduction-based iron uptake in plants. *New Phytologist*. 1999; 141: 1 - 26.

19. De la Guardia MD, Alcántara E. Ferric chelate reduction by sunflower (*Helianthus annuus* L.) leaves: influence of light, oxygen, iron-deficiency and leaf age. *J. Experimental Botany* 1996; 47: 669 - 75.

20. Kosgarten H, Hoffmann B and Mengel K. The paramount influence of nitrate in increasing apoplastic pH of young sunflower leaves to induce Fe deficiency chlorosis, and the re-greening effect brought about by acidic foliar sprays. *J. Plant Nutrition and Soil. Sci.* 2001; 164: 155 - 63.

21. Taiz L, Zeiger E. Mineral nutrition. In: Plant Physiology (2nd Edition). Sinauer Assoc., Inc., Publishers, Sunderland, MA. 1998, 103 - 24.

22. Braun V and Killmann H. Bacterial solutions to the iron-supply problem, TIBS, 1999, pp: 104-9.

23. Pestana M, Correia PJ and Miguel G. Foliar treatment as a strategy to control iron chlorosis in orange trees. *Acta Horticulture* 2002; 594: 223 - 9.

24. Pharmacopee Europeene. 3Ed, Maisonneuve SA, Conseil de l'Europe, Sante Ruffine. 1997, 229-31.

25. Adams RP. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing. Carol Stream, IL, USA 2001, pp: 469.

26. Swigar AA, Silverstein RM. Monoterpenes. Aldrich Chemical, Milwaukee. 1981.

27. Nasiri Y, Zehtabe-Salmasi S, Nasrollahzade S, Najafi N and Ghassemi-Golezani K. Effect of foliar application of micronutrient (Fe and Zn) on flower yield and

essential oil of chamomile (*Matricaria chamomile* L.). *J. Medicinal Plants Res.* 2010; 4 (17): 1733 - 7.

28. Aziz E, El-Danasoury ME and Craker LE. Impact of sulphur and ammonium sulphate on dragonhead plants grown in newly reclaimed soil. *J. Herbs Spices& Medicinal Plants* 2010; 16 (2): 126 - 35.

29. Sharafzadeh S, Esmaeilli M and Mohammadi AH. Interaction effects of nitrogen, phosphorus and potassium on growth, essential oil and total phenolic content of sweet basil. Adv. *Environmental Biol.* 2011; 5 (6): 1285 - 9.

30. Sharafzadeh S, Khosh-Khui M and Javidnia K. Effect of nutrients on essential oil components, pigments and total phenolic content of lemon balm (*Melissa officinalis* L.). *Advanced Environmental Biol.* 2011; 5 (4): 639 - 46.

31. Zheljazkov VD, Cantrell CL, Astatkie T and Ebelhar MW. Peppermint productivity and oil composition as a function of nitrogen, growth stage, and harvest time. *Agronomy J.* 2010; 102 (1): 124 - 8.

32. Zheljazkov VD, Cantrell CL, Astatkie T and Cannon JB. Lemongrass productivity, oil content, and composition as a function of nitrogen, sulphur, and harvest time. *Agronomy J.* 2011; 103 (3): 805 - 12.

33. Jabbari R, Dehaghi MA, Sanavi AM and Agahi K. Nitrogen and iron fertilization methods affecting essential oil and chemical composition of thyme (*Thymus vulgaris* L.) medical plant. *Advances in Environmental Biology* 2011; 5(2): 433 - 8.

34. Sawan ZM, Hafez SA and Basyony AE. Effect of phosphorus fertilization and foliar application of chelated zinc and calcium on seed, protein and oil yields and oil properties of cotton. *J. Agricultural Sciences* 2001; 136: 191 - 8.

35. Bacha MA, Sabbah AM and Hamady MA. Effect of foliar application of iron, zinc and manganese on yield berry quality and leaf mineral composition of Thompson seedless and roomy red grape cultivars. *J. King Saud University- Science* 1997; 1: 127 - 40.

36. Bernal M, Cases R, Picorel R and Yruela I. Foliar and root Cu supply affect differently Fe and Zn-uptake and photosynthetic activity in soybean plants. *Environmental and Experimental Botany* 2007; 60: 145 - 50.

37. Baloch OB, Chacar OI and Tareen MN. Effect of foliar application of macro and micronutrients on production of green chilies (*Capcicum annuum* L.). *J. Agricultural Science and Technol.* 2008; 4 (2): 177 - 84.

38. Kaya M, Atak M, Mahmood Khawar K, Çiftçi CY and Özcan S. Effect of pre-sowing seed treatment with zinc and foliar spray of humic acids on yield of common bean (*Phaseolus vulgaris* L.). *International Journal of Agriculture and Biol.* 2005; 6 (7): 875 - 8.

39. Lucena JJ. Effect of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis: a review, *J. Plant Nutrition* 2000; 23: 1591 - 606.

40. Marschner H. Mineral nutrient of higher plants. Second Ed., Academic Press Limited. 1995. Harcourt Brace and Company, Publishers, London 1995, pp: 347 - 64.

41. Basavarajappa R, Koraddi VR, Kamath KS and Doddamani MB. Response cotton cv. Abadhita (*Gossypium hirsutum* L.) to soil and foliar application of micronutrients under rain

fed condition. *Karnataka J. Agric. Sci*. 1997; 10 (2): 287 - 91.

42. Kuzhandaivel H and Venkatesan S. Impact of iron toxicity on certain enzymes and biochemical parameters of tea. *Asian Journal of Biochemistry* 2011; 6 (5): 223 - 33.

43. Singh AL, Joshi YC, Chaudhari V and Zala PV. Effect of different sources of iron and sulphur on leaf chlorosis, nutrient uptake and yield of groundnut. *Fertilizer Res.* 1995; 13: 28 - 35.

44. Lalit Bhatt B, Srivastava K and Singh MP. Studies on the effect of foliar application of micronutrients on growth, yield and economics of tomato (*Lycopersicon esculentum* Mill.). *Progressive Horticulture* 2004; 28 (1): 222 - 23.

45. Aziz E and El-Sherbeny SE. Effect of some macro and micro- nutrients on growth and chemical constituents of *Sideritis montana* L. as a new plant introduced into Egypt. Arab Uni. Ain Shams Uni. Cairo Egypt, *J. Agricultural Science* 2004; 12 (1): 391 - 403.

46. Pande P, Anwar M, Chand S, Yadav VK and Patra D. Optimal level of iron and zinc in relation to its influence on herb yield and production of essential oil in menthol mint. *Communications in Soil Science and Plant Analysis* 2007; 38, Issue (5 & 6): 561 - 78.

47. Said-Al Ahl HAH and Omer EA. Effect of spraying with zinc and/or iron on growth and chemical composition of coriander (*Coriandrum sativum* L.) harvested at three stages of development. *J. Medicinal Food Plants* 2009; 1 (2): 30 - 46.

48. Said-Al Ahl HAH, Hussein MS and Abd El-Kader AA. Effect of nitrogen fertilizer and/or some foliar application on growth, herb yield, essential oil and chemical composition of dragonhead. *J. Medicinal Food Plants* 2010; 2 (1): 12 - 28.

49. Yeritsyan N and Economakis C. Effect of nutrient solution's concentration on growth and essential oil content of oregano plants grown in solution culture. *Acta Horticulture* 2002; 576: 277 - 83.

50. Evans WC. Pharmacognosy. 14th Edition. Chapter 21. Volatile Oils and resins. John Wiley, New York. 1996, pp: 450.