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Effect of Foliar Application of Fe, Zn, Cu and Mn on Yield and Essential Oils of *Borago officinalis*

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ABSTRACT

Borago is one of the plants of Boraginaceae family. To study the effect of Fe, Zn, Cu and Mn foliar applications in Borago on yield and essence production, the investigations were performed in complete randomized block design with 3 replications at 2011 and 2012 in Shahrekord, Iran. Results showed that Fe, Zn, Cu and Mn had the significant effectiveness in percentage of essence, DPPH, carotenoids, flavonoids, phenols, weight of fresh and dry root matter, number of flower, and weight of dry and fresh of flower and weight of dry and fresh shoot matter. Combinations of 400ppm of Fe, Zn, Cu and Mn (Fe3Zn3Cu3Mn3) produced the greatest amounts in most of measured characters. The most of phenols, carotenoids and flavonoids was 75, 0.6, 0.6 mg/100 gr flower dry matter and 81, 0.8, 0.62 mg/100 gr flower dry matter in primary and secondary year respectively. The most weight of dry flower and number of flower per plant were 14.8, 16.8 gr and 10.9, 13.9 in 1st and 2nd year and made by Fe3Zn3Cu3Mn3 treatment.

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INTRODUCTION

Borago officinalis Linn. (family; Boraginaceae) is a hairy annual herb commonly known as 'Borage' and 'Gaozaban' locally in Iran. It has been known for its mood elevating properties as early as the first century A.D. (Tyler, 1993). The plant is reputed as antispasmodic, antihypertensive, antipyretic, aphrodisiac, demulcent, diuretic and is also considered useful to treat asthma, bronchitis, cramps, palpitations and kidney ailments (Duke *et al*., 2002; Yang, 2002). Borage oil has been reported to lower serum cholesterol, phospholipids and triglyceride levels (Gu *et al*., 1998; Hosseinpour, 2009) and increases the levels of 6 polyunsaturated fatty acids in the plasma, liver, aorta and renal artery tissues. Dietary use of borage oil exhibited immuno-modulatory (Harbige *et al*., 2000) and cytotoxic and free radical scavenging activities (Lin *et al*., 2002). Borage has gained importance, due to the occurrence of high levels of g-linolenic acid in its seed oil. Flavonoids and phenolic compounds were the important essential oils in this plant and no have tannin. In a recent publication, an extensive investigation on antioxidant properties of extracts of borage meal was reported (Wettasinghe and Shahidi, 1999; Wettasinghe *et al*., 2001; Makkizadeh tafti *et al*., 2006). Increasing of dry matter has negative correlation with seed moisture (Mhamdi *et al*., 2009). Borage extracts demonstrated excellent antioxidant properties and these effects were attributed to their phenolic constituents. Few studies examining Iron, Copper, Zinc and Manganese fertility in Borago have been conducted, but they are not specific to the medicinal use of this plant. Iron is one of the three micro essential nutrient elements required by plants. Fe is important in cytochrome structure. Iron occurs in concentrations of 7,000 to 500,000 mg kg-1 in soils, where it is present mainly in the insoluble Fe (III) (Fageria *et al*., 2002). The Fe (II) form is normally below the detection level in plants (Schönherr *et al*., 2005). Copper is an essential microelement in higher plants as it occurs as part of the prosthetic groups of several enzymes. It was shown to be associated with proteins or nuclear contaminants (Nabila *et al*., 2003; Doming and Bertling, 2004). Zinc is an important micronutrient associated with several enzymatic activities in all photosynthetic plants. Zn is necessary in vital enzymes and growth regulators (Swiader, 2000; Ved *et al*., 2002). Manganese is involved in many biochemical functions, primarily acting as an activator of enzymes such as dehydrogenases and decarboxylases involved in respiration, amino acid and lignin synthesis, and hormone concentrations (Younis *et al*., 2013). In alkaline soils nutrient concentration may be not enough and therefore micro nutrient in this soil immobilized quickly and roots of plants can't absorb from soil and some of nutrient no transition to leaves, in this places spry application of micro nutrient solve this problem (Dadhich and Somani, 2007; Foth and Ellis, 1996; Torun *et al*., 2001), and reduce soil fertilizer loss (Ahmad, 1998). Foliar fertilizer is particularly useful technique which can be designed to meet plants specific needs for one or more micro or macro nutrients especially trace minerals and enable to correct deficiencies, strengthen

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weak or damaged crops, speed growth and grow better and healthier plants (Khalifa *et al*., 2009; Malakoti and Davodi, 2002). Objectives of this study were to determine the effects of Iron, Zinc, Copper and Manganese foliar applications on percentage of essence, DPPH, carotenoids, flavonoids, phenols, weight of fresh and dry root matter, number of flower, and weight of dry and fresh of flower and weight of dry and fresh shoot matter of Borago.

Methodology:

Field trials were established in 2012 and 2013 at Shahrekord (50°56/ E 32°18/ N) South Western Iran on the parcel size 3000 m2. Used row spacing was 50 cm. Seeds of *Borago officinalis* Linn, obtained from the Pakan Bazr Company, Isfahan, Iran. Sowing was conducted manual, plant spacing in the each row was 5 cm, and two seeds were put in the soil. In the 3-5 leaves phase plants were thin out to final row distance (20 cm). During the vegetation, used practical measures were standard (3 earth up and pest protection). The soil (typic calci xerocrepts) physical and chemical properties are shown in Table 1. Experiments were arranged in a randomized complete block design with a factorial layout and three replications. All experiments were carried out in triplicate. Topsoil of the experimental plot area was kept moist throughout the growing season when necessary. After soil test, the required nutrients were added to soil. At the end of the blooming stage, shoots of plants were harvested. Four foliar fertilizers Librel Fe-Lo, Librel Cu, Librel Zn and Librel Mn were applied and all of them are mineral fertilizers. Librel Fe-Lo contains 13.2% chelated iron, Librel Zn is a foliar fertilizer which contains 14% Zinc in chelated form, Librel Cu has 14% copper in chelated form and Librel Mn inclusive of 13% Mn chelated with EDTA (obtained from The Chemical Company of England and Germany). Theses fertilizers were sprayed at four concentrations (Fe1, Fe2, Fe3, Fe4 were 0, 200, 400 and 600 ppm of Fe, respectively and similar in other micronutrients). In this research flowers harvested in 3 steps and before each step, in vegetation phase, treatments were done. At the end of the blooming stage, shoots of plants were harvested. Several parameters including dry and fresh weight of shoot and root, number of flower, dry and fresh flower matter and the amount of essence percentage as well as the chemical component of the essence (carotenoids, flavonoids, and phenols) and antioxidant were determined. Thermo Finnegan Trace 2000 GC/MS, made in the USA, was employed with a HP-5MS capillary column (30 m long and 0.25 mm wide, and a 0.25 µm of film thickness) at a 250°C of injector chamber. The initial column temperature was at 120°C for 5 min then raised to 280°C at the rate of 10°C/min. Helium was used as a carrier gas at a rate of 35 ml/min. MS parameters were as follows: ionization energy, 70eV; ion source temperature, 200°C; voltage, 3000 v; and mass range, 30 to 600. The compositions of the essential oil were identified by comparison of their retention indexes, retention times and mass spectra with those of authentic samples in Wiley library (Adams, 2001). Harvested flowers were dried at room temperature for 1week. Flower extracts were obtained by stirring 1 g of dry flower powder with 10 ml of pure methanol for 30 min. The extracts were then kept for 24 h at 4◦C, filtered through a Whatman no. 4 filter paper, and evaporated under vacuum to dryness and stored at 4◦C until analysed. The content of total phenolic of *B.officinalis* methanolic extract were determined using the Folin–Ciocalteu (F–C) reagent according to the method described by Dewanto *et al*. (2002) using Gallic acid as reference.

Table 1: Physical and chemical properties of soil for experiment.

Year	Clay (%)	Silt (%)	Sand (%)	N_{total} (%)	$K_{available}$ (mg/kg)	$P_{available}$ (mg/kg)	O.C (%)	pH	E.C ($ds.m^{-1}$)	Depth (Cm)
2012	39	33	28	0.158	741	15.9	1.13	8.05	0.76	0-30
2013	39	32	29	0.17	720	16.2	1.2	7.89	0.73	0-30

Dried samples from flowers were hydrolyzed according to the method of Proestos *et al*. (2006), slightly modified. 20 ml of methanol containing BHT (1 g l−1) were added to 0.5 g of a dried sample. Then 10 ml of 1M HCl were added. The mixture was stirred carefully and sonicated for 15min and refluxed in a water bath at 90◦C for 2 h. The obtained mixture was injected to HPLC. The phenolic compounds' analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC, Palo Alto, CA) coupled with an UV–vis multiwavelength detector. The separation was carried out on a 250×4.6-mm, 4µm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 ml min−1. The gradient programme was as follows: 15% A/85% B, 0–12min; 40% A/60% B, 12–14min; 60% A/40% B, 14–18min; 80% A/20% B, 18–20 min; 90% A/10% B, 20–24min; 100% A, 24–28 min (Bourgou *et al*., 2008). The injection volume was 20µl, and peaks were monitored at 280 nm. Samples were filtered through a 0.45µm membrane filter before injection. Peaks were identified by congruent retention times compared with standards. Analyses were performed by triplicate (Shibamoto, 1987).

The electron donation ability of the obtained methanol extracts was measured by bleaching of the purple-coloured solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al*. (1988). 2ml of methanolic extracts at different concentrations (borage flower extracts (10 and 50µg ml−1)) were added to 0.5 ml of a 0.2m mol l−1 DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity (three replicates per treatment) was expressed as IC50 (mg ml−1), the

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concentration required to cause a 50% DPPH inhibition. A lower IC50 value corresponds to a higher antioxidant activity of flower extract (Patro *et al*., 2005). The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH(\text{scavenging effect } \%) = [(A0 - A1) \div A0] \times 100$$

Where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min. Samples were analysed in triplicate. All data were subjected to ANOVA using the statistical computer package SAS and treatment means compared using Duncan's multiple range test at 5% level.

Results:

Results showed the significant differences between treatments. Although in many of single treatments no significant differences, but in combinations and by Duncan's comparison, there were differences in most characters that result the $Fe_3Cu_3Mn_3Zn_3$ treatment was the best (Tables 2-4). It seem that by synergist application of micro nutrient results to additive of effectiveness of measured characters but upper than 400 ppm, the action of fertilizer was toxicity. The components of borago oil were influenced by different treatments. The application treatment of Fe, Cu, Mn and Zn was significantly improved the growth characters including dry and fresh weight of shoot and root, number of flower, dry and fresh flower matter and the amount of essence percentage as well as the chemical component of the essence (carotenoids, flavonoids, and phenols) and antioxidant were determined showed significant response with increasing rates of foliar fertilizers (table 2). Meanwhile, the effect of combination of four micronutrients was surpassed that of single of them. Thus, the foliar application of 400 ppm Fe, Cu, Mn and Zn caused the highest increments compared with other treatments. Length of stem in control plants was the least but the number of stem per plant in $Fe_4Cu_4Mn_4Zn_4$ was the least (data no published). In character of number of flowers per plant, control plants and plants treated by treatments of 600 ppm of micronutrients were similar. The application of 400 ppm of Fe, Cu, Mn and Zn has increased flavonoids, phenols and carotenoids in the oil of borago. However, combination of $Fe_3Cu_3Mn_3Zn_3$ treatment was more effective than other treatments. Most of amount of measured characters (for example flavonoids, number and weight of flower) decreased by application upper than 400 ppm of Fe, Cu, Mn and Zn. Use of the upper range of optimum micro nutrient decreased the yield and essence production. Control treatment in many places made better yield components than 600 ppm concentration of Fe, Cu, Mn and Zn. In most treatments, combination of $Fe_3Cu_3Mn_3B_3$, $Fe_3Cu_3Mn_2Zn_2$ and $Fe_3Cu_3Mn_1Zn_1$ made the maximum amount of characters, but $Fe_3Cu_3Mn_3B_3$ was the best combination (tables 3, 4). In most times, control plants made alike of $Fe_4Cu_4Mn_2Zn_2$, $Fe_4Cu_4Mn_3Zn_3$ and $Fe_4Cu_4Mn_4Zn_4$. For toxicities of upper concentrations of Fe, Cu, Mn and Zn, the results obtained similar by control plants. It was clear from the presented data that the highest levels of the four foliar fertilizers were more effective than lower levels, and Librel Fe-Lo fertilizer was superior to other micronutrients. However, the highest essence percentage was found with $Fe_3Cu_3Mn_3Zn_3$ (0.9% and 1.1% in first and second year respectively). The most phenols made by $Fe_3Cu_3Mn_3Zn_3$ in two years (75% and 81 % in 1^{st} and 2^{nd} year). Concentration of micronutrients positively affected on characters measured and between the dry and fresh weight of shoot and root, number of flower, dry and fresh flower matter and the amount of essence percentage as well as the chemical component of the essence (carotenoids, flavonoids, and phenols) and antioxidant were determined of borago were positive correlated and in best combinations of treatments (tables 3, 4) the $Fe_3Cu_3Mn_3Zn_3$ was the best. Essence percentage positively correlated with the weight of shoot dry and fresh matter, number and weight of flowers (tables 3, 4). Weight of shoot dry and fresh matter positively correlated with number and weight of flowers and essence percentage of borago.

Table 2: Complex analysis of variance of shoot dry and fresh matter, root dry and fresh matter, sum of number of flower per plant, dry and fresh flower matter, essence percentage, carotenoids, flavonoids, phenols and antioxidant (mg/100 dry matter) plants that are affected by several micronutrients.

Source of variation	Degree of freedom	DPPH	flavonoids	phenols	carotenoids	essence percentage	fresh flower matter	Dry flower matter	number of flower	root dry matter	root fresh matter	shoot dry matter	shoot fresh matter
		M.S											
Year	1	0.14	0.067	11.1	0.009	0.002	6.7	4.4	1.1	2.2	1.2	7.6	5.2
R/Y	4	0.09	0.05	12	0.008	0.0032	6.6	4.7	1.2	3.3	2.3	5.5	3.3
Fe	3	1.77^{**}	0.036^{**}	95.1^{**}	0.09^{**}	0.00004^{ns}	2.4^{ns}	2.2^{ns}	1.8^{*}	4.2^{*}	1.5^{ns}	5.5^{*}	4.1^{*}
Zn	3	0.126^{**}	0.006^{*}	49.2^{**}	0.05^{**}	0.00003^{ns}	2.5^{ns}	1.2^{ns}	1.5^{ns}	2.4^{ns}	1.4^{ns}	4.1^{ns}	3.9^{*}
Cu	3	0.027^{*}	0.005^{*}	33.9^{*}	0.003^{*}	0.00004^{ns}	2.6^{ns}	1.9^{ns}	1.6^{ns}	2.7^{ns}	1.5^{ns}	4.7^{ns}	2.5^{ns}
Mn	3	0.002^{ns}	0.015^{**}	14.2^{ns}	0.0031^{*}	0.00005^{ns}	2.4^{ns}	1.3^{ns}	1.1^{ns}	2.5^{ns}	1.3^{ns}	5.4^{ns}	2.7^{ns}
Fe×Zn	9	0.019^{*}	0.004^{*}	1421.2^{**}	0.09^{**}	0.00008^{*}	2.7^{*}	3.7^{*}	1.2^{ns}	3.3^{*}	1.8^{*}	8.6^{**}	3.4^{*}
Cu×Fe	9	0.14^{**}	0.32^{*}	18.4^{ns}	0.07^{**}	0.0067^{**}	6.6^{**}	3.9^{*}	1.3^{*}	3.7^{*}	3.4^{**}	4.1^{*}	8.8^{**}
Mn×Fe	9	0.021^{*}	0.0038^{*}	23.86^{*}	0.0019^{**}	0.00009^{*}	2.8^{*}	3.85^{*}	2.6^{**}	7.5^{**}	1.9^{*}	4.2^{*}	8.6^{**}
Cu×Zn	9	0.23^{**}	0.44^{**}	14.1^{ns}	0.002^{*}	0.012^{**}	7.8^{**}	5.8^{**}	1.4^{*}	7.3^{**}	3.3^{**}	11.1^{**}	8.9^{**}
Mn×Zn	9	0.44^{**}	0.1^{**}	24.1^{*}	0.0021^{*}	0.000078^{*}	2.7^{*}	7.6^{**}	4.7^{**}	3.4^{*}	4.4^{**}	10.5^{**}	10.3^{**}
Cu×Mn	9	0.56^{**}	0.32^{**}	55.4^{**}	0.2^{**}	0.000081^{*}	9.9^{**}	3.6^{*}	3.3^{**}	3.3^{*}	2.05^{*}	11.5^{**}	3.5^{*}
Cu×Fe×Zn	27	0.016^{*}	0.003^{*}	19.1^{*}	0.0015^{*}	0.00007^{*}	2.1^{*}	3.1^{*}	1.1^{*}	1.9^{*}	7.6^{**}	3.2^{*}	1.87^{*}
Fe×Zn×Mn	27	0.33^{**}	0.22^{**}	57.6^{**}	0.7^{**}	0.069^{**}	10.6^{**}	9.7^{**}	5.4^{**}	2.1^{*}	1.5^{*}	15.5^{**}	14.4^{**}
Cu×Mn×Fe	27	0.24^{**}	0.0034^{*}	20.22^{*}	0.8^{**}	0.000075^{*}	2.2^{*}	9.1^{**}	1.1^{*}	9.8^{**}	7.4^{**}	12.5^{**}	14.1^{**}
Cu×Mn×Zn	27	0.017^{*}	0.14^{**}	96.67^{**}	0.9^{**}	0.056^{**}	15.4^{**}	3.2^{*}	1.2^{*}	1.9^{*}	1.7^{*}	3.3^{*}	1.8^{*}
Cu×Zn×Mn×Fe	81	0.011^{*}	0.0024^{*}	15.1^{*}	0.0011^{*}	0.00006^{*}	1.7^{*}	2.4^{*}	0.9^{*}	1.6^{*}	1.1^{*}	2.5^{*}	1.5^{*}
T(Cu,Zn,Mn,Fe)×Y	255	0.0022^{*}	0.0004^{*}	3.1^{*}	0.0002^{*}	0.000009^{*}	0.3^{*}	0.45^{*}	0.16^{*}	0.28^{*}	0.19^{*}	0.5^{*}	0.3^{*}
E	1020	0.01	0.002	12.56	0.001	0.00004	1.34	1.1	0.67	1.2	0.85	2.1	1.2
Coefficient of variation		4.4	1.1	12.2	3.3	1.2	9.7	8.7	6.3	9.1	8.9	14.4	12.3

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Table 3: Results of correlation between characters in borago plants that are affected by several micronutrients in first year.

Characters	Weight of shoot fresh matter	Weight of shoot dry matter	Weight of root fresh matter	Weight Of root dry matter	Number Of flowers	Weight of dry flower	Weight Of fresh flower	Essence percentage	carotenoids	phenols	flavonoids	DPPH
Weight of shoot fresh matter	1											
Weight of shoot dry matter	0.52*	1										
Weight of root fresh matter	0.26	0.1	1									
Weight of root dry matter	0.36	0.56**	0.66**	1								
Number of flowers	0.5*	0.58**	0.64**	0.52*	1							
Weight of dry flower	0.5*	0.76**	0.5*	0.44	0.58**	1						
Weight of fresh flower	0.6**	0.59**	0.35	0.36	0.55**	0.58**	1					
Essence percentage	0.48	0.56**	0.46	0.33	0.7**	0.65**	0.46	1				
carotenoids	0.1	0.68**	0.11	0.4	0.4	0.7**	0.26	0.69**	1			
phenols	0.11	0.76**	0.1	0.3	0.42	0.4	0.24	0.66**	0.48	1		
flavonoids	0.1	0.56**	0.3	0.55*	0.31	0.42	0.33	0.68**	0.26	0.36	1	
DPPH	0.11	0.56**	0.23	0.65**	0.22	0.5*	0.2	0.8**	0.1	0.33	0.22	1

Table 4: Results of correlation between characters in borago plants that are affected by several micronutrients in secondary year.

Characters	Weight of shoot fresh matter	Weight of shoot dry matter	Weight of root fresh matter	Weight Of root dry matter	Number Of flowers	Weight of dry flower	Weight Of fresh flower	Essence percentage	carotenoids	phenols	flavonoids	DPPH
Weight of shoot fresh matter	1											
Weight of shoot dry matter	0.56**	1										
Weight of root fresh matter	0.36	0.2	1									
Weight of root dry matter	0.36	0.66**	0.73**	1								
Number of flowers	0.5*	0.68**	0.65**	0.7**	1							
Weight of dry flower	0.48	0.8**	0.6**	0.4	0.8**	1						
Weight of fresh flower	0.78**	0.7**	0.3	0.3	0.87**	0.87**	1					
Essence percentage	0.58*	0.6**	0.4	0.3	0.7**	0.7**	0.6**	1				
Carotenoids	0.2	0.6**	0.14	0.4	0.3	0.8**	0.3	0.8**	1			
Phenols	0.22	0.8**	0.2	0.3	0.4	0.3	0.4	0.5*	0.3	1		
Flavonoids	0.3	0.7**	0.2	0.5*	0.3	0.4	0.2	0.48	0.3	0.4	1	
DPPH	0.18	0.6**	0.19	0.8**	0.1	0.7**	0.3	0.7**	0.2	0.2	0.1	1

Table 5: Means of Characters measured in borago plants that are affected by micronutrients (400 ppm) concentration and control plants (1st year).

Treatments	DPPH	flavonoids	phenols	carotenoids	essence percentage	fresh flower matter	Dry flower matter	number of flower	root dry matter	root fresh matter	shoot dry matter	shoot fresh matter
Fe	0.6c	0.03cd	12.1c	0.07de	0.24d	13c	6.8cd	7cd	15cd	21.3de	16.7c	32.3d
Zn	0.55cd	0.04cd	12.2c	0.11d	0.22d	10.7cd	6.5cd	8c	12.7cd	23.3de	15.7c	32.6cd
Cu	0.4cd	0.07c	12.4c	0.095d	0.25d	13c	6.3cd	8.1c	12.5cd	22.3de	17.4bc	32.8cd
Mn	0.5cd	0.09c	13.1c	0.1d	0.24d	10.7cd	6.5cd	6.7d	12.5cd	20.4de	16.2c	32.3d
Fe×Zn	0.6c	0.11c	13.5c	0.23bc	0.56c	11.6cd	7.4cd	9.2b	14.6cd	24.8d	17.3bc	33.3cd
Cu×Fe	0.7c	0.1c	19cd	0.22bc	0.52c	13c	7.7cd	9.3b	16cd	26.3cd	17.5bc	33.8cd
Mn×Fe	0.7c	0.28b	20cd	0.18bc	0.55c	13c	8.8c	9.5b	18c	28.1c	17.9bc	34.2cd
Cu×Zn	0.6c	0.29b	25c	0.17bc	0.59bc	12cd	8.5cd	9.6b	16.7cd	27.3cd	19.2bc	35.2c
Mn×Zn	0.6c	0.31b	22c	0.15c	0.54c	13c	8.1cd	9.6b	16.4cd	28.3c	16.6c	34.3cd
Cu×Mn	0.56cd	0.16bc	35bc	0.25bc	0.62bc	13c	8.2cd	9.7ab	16.2cd	27.4cd	17.5bc	36.7bc
Cu×Fe×Zn	0.8bc	0.15bc	49b	0.3b	0.71b	15b	9.8bc	10.3a	20bc	31.3bc	22ab	36.8bc
Fe×Zn×Mn	0.9bc	0.3b	50b	0.38b	0.7b	17ab	9.9bc	10.5a	20bc	32.5bc	21.3ab	37.2bc
Cu×Mn×Fe	1.1b	0.43ab	65ab	0.39b	0.8ab	15b	10.8b	10.5a	22b	34.3b	22.7ab	37.5bc
Cu×Mn×Zn	1b	0.45ab	55b	0.4b	0.8ab	15.3b	11.1b	10.2a	22.3b	34.9b	20.2b	38.2b
Cu×Zn×Mn×Fe	1.5a	0.6a	75a	0.6a	0.9a	19a	14.8a	10.9a	34.1a	44.8a	30.9a	42.2a
Control	0.3d	0.01d	8d	0.05e	0.2d	10d	5.5d	7d	12d	20.1e	10.3d	20.4e

Table 6: Means of Characters measured in borago plants that are affected by micronutrients (400 ppm) concentration and control plants (2nd year).

Treatments	DPPH	flavonoids	phenols	carotenoids	essence percentage	fresh flower matter	Dry flower matter	number of flower	root dry matter	root fresh matter	shoot dry matter	shoot fresh matter
Fe	0.7c	0.08c	14c	0.07de	0.23d	15c	7.5d	10cd	16.2cd	32.5d	15.7c	39.3d
Zn	0.59cd	0.05cd	12c	0.11d	0.21d	15c	8.5cd	11c	14.9cd	32.5d	12.7c	39.6cd
Cu	0.43cd	0.06c	11c	0.095d	0.5c	15c	7.7d	11.1c	14.7cd	35.7d	18.4bc	36.3d
Mn	0.55cd	0.03cd	11.1c	0.07de	0.24d	11d	8.5cd	8.7d	13d	30.1e	15.4c	30.2de
Fe×Zn	0.66c	0.09c	11.5c	0.23bc	0.5c	13.6cd	9.4cd	11.2c	14.8d	30.1e	18.4bc	42.5cd
Cu×Fe	0.7c	0.09c	21cd	0.22bc	0.5c	13.4cd	9.7cd	12.3b	20.5c	35.3c	18.5bc	42.5cd
Mn×Fe	0.77c	0.25b	22cd	0.2c	0.5c	15c	10.8c	11.5c	20.5c	40.2c	18.9bc	45.2c
Cu×Zn	0.6c	0.15bc	30c	0.2c	0.61bc	14cd	10.5cd	12.6b	20c	41.3c	21bc	45.2c
Mn×Zn	0.66c	0.12bc	31c	0.2c	0.54c	15c	10.9c	12.6b	18.6cd	42.3c	15.6c	45.2c
Cu×Mn	0.66c	0.11bc	35bc	0.35bc	0.5c	15c	10.2cd	12.7ab	18.4cd	44.4c	18.5bc	51.7bc
Cu×Fe×Zn	0.88bc	0.3b	50b	0.35bc	0.75b	17b	11.8bc	13.3a	22bc	52.3bc	24ab	51.8bc
Fe×Zn×Mn	0.99bc	0.4b	38bc	0.4b	0.72b	19ab	13b	12.5b	23bc	54.5bc	24.3ab	54.2b
Cu×Mn×Fe	1.2b	0.48b	72a	0.4b	0.9ab	17b	12.8b	12.5b	28b	56.3b	24.7ab	54.5b
Cu×Mn×Zn	1b	0.34b	68a	0.41b	0.72b	17.5b	13.1b	13.2a	24.5b	57.9b	30.2b	54.2b
Cu×Zn×Mn×Fe	1.6a	0.62a	75a	0.8a	1.1a	22a	16.8a	13.9a	34.7a	60.8a	35.8a	70.2a
Control	0.34d	0.015d	12d	0.05e	0.1d	11d	7.5d	9d	14d	25.4f	15.5c	27.4e

Discussion:

Our results showed beneficial micronutrient application on borago (table 1). The same results in *Calendula* (Naguib *et al*., 2005) and *Artemisia annua* L. (Glyn, 2002) were reported. Increasing of micronutrients can obtain more yield, essence and essential oils from borago (Naguib *et al*.,2005). The beneficial application of micronutrients was reported by other researchers (Asad and Rafique, 2000; Hussain *et al*., 2005; Ziaeian and Malakoti, 1998; Thalooth *et al*., 2006; Yadegari *et al*., 2012). Results of this research showed that foliar application of micronutrient made the more essential oil in flower of *Borago officinalis* (tables 5, 6). A similar effect of micronutrient supply on this parameter was also reported on *M.chamomilla* (Grejtovský *et al*., 2006; Nasiri *et al*., 2010), *S.farinacea* (Nahed and Balbaa, 2007), *Coriandrum sativum* (Said-Al Ahl and Omer, 2009), and *Ocimum basilicum* (Said-Al Ahl and Mahmoud, 2010). In the present study, the effect of Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} applications was determined on the growth and yield of borago in two consecutive years. According to our knowledge, this is the first report that shows that Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} can affect the yield and growth of borago and the same response can probably be found in other crop species that have requirements for Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} (Asad and Rafique, 2000; Aziz and El-Sherbeny, 2004; Doming and Bertling, 2004; Hussain *et al*., 2005; Said-Al Ahl and Omer, 2009; Said-Al Ahl and Mahmoud, 2010; Sharafi *et al*., 2002). Dry matter flower increased in both years with micronutrients applications. The percentage of essence and DPPH was affected by the Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} applications. The essential oil yield increased with Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} applications because there was a significant increase in dry matter and number of flower**.** This is the first report in which the effects of Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} applications on certain physiological characteristics were determined for borago and also their relationship with yield and essential oil content. It is obvious that weight and number of flowers can be used as a tool for selecting new cultivars with high yield. However, more research is needed to explore these tools for borago breeding and for better borago management. The DPPH level was found to correlate with essence percentage, flower and shoot dry matter. It seems the Weight of shoot dry matter was the best character that correlated by most of characters a finding with which others agree (Makkizadeh tafti *et al*., 2006; Mhamdi *et al*., 2009; Naghdi Badi and Sorooshzadeh, 2011). From the present data, we conclude that applications of Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} are beneficial for borago plants with concentrations of 400ppm or lower, and can result in an increase in dry matter yield of up to 40%. Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} have direct functions on growth and the development of plants. There are still many unanswered questions about how Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} act in increasing yield and its components (number of flower plant) for borago. One possibility is that the foliarly applied Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} can affect dry matter accumulation and increase dry matter flower. Fe^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} can increase the weight of shoot dry matter and weight of root dry matter. (Asad and Rafique, 2000; Aziz and El-Sherbeny, 2004; Dadhich and Somani, 2007; Doming and Bertling, 2004; Hussain *et al*., 2005; Said-Al Ahl and Mahmoud, 2010; Thalooth *et al*., 2006; Yadegari *et al*., 2012; Younis *et al*., 2013). It seem, control plants that no have foliar application, were better than plants that have upper concentration of micronutrients. Combinations of micronutrients upper concentration than 400 ppm had more reducer effect than single of them. Consumption of these fertilizers in *Mentha* sp can increase essence glands and therefore essence can increase (Evans, 1996). Increasing of auxin, chlorophyll and RUBP concentration, nitrogen use efficiency by Fe consumption was previously reported (Sharafi *et al*., 2002). Positive correlations were found between the content of antioxidant activity with flower dry weight and essence percentage in two seasons (tables 3, 4). Que *et al*. (2006) and Hee *et al*.(2006) showed also that essential oils in rice and wheat correlated with antioxidants activity. In conclusion by use the optimum amount of micro nutrient, the yield characters will increase. These results reflect the role of applying the four foliar fertilizers in improving the total essential oils in *Borago* species plants. It seems that Fe, Cu, Mn and Zn by the effect on absorption and transition of essential nutrients made the change of metabolism and growth and development and then increased upper phytochemicals (Malakoti and Davodi 2002; Ziaeian and Malakoti, 1998). The primitive effect of foliar fertilizers were in agreement with those obtained by El-Leithy (1998), Youssef (1998), Shirani (2011) and they reported that foliar spraying with micronutrients had stimulatory responses in growth characters. Similarly Khalil *et al* (2001), Refaat and Balbaa (2001), Aziz and El-Sherbeny (2004) showed that vegetative growth parameters and yield of different plants recorded significant increase with the application of applying various foliar fertilizer. Generally, the obtained results revealed that under Iranian conditions applying foliar fertilizer as Fe, Cu, Mn and Zn at 400 ppm resulted in highest improvement of growth character, yield and chemical constituents. Decreasing effect of $Fe_4Cu_4Mn_4Zn_4$ made the equivalent amount of control plants.

Conclusion:

Plants treated with 400 ppm of Iron, Zinc, Manganese and copper, had the more fresh and dry weight of flowers and essence percentage. Therefore, for maximum yield and quality of borago, foliar application of micronutrients by 400 ppm concentration is recommended. It could be concluded from the results that Iron, Zinc, Manganese and copper fertilization had significant effect on dry and fresh weight of shoot and root, number of flower, dry and fresh flower matter and the amount of essence percentage as well as the chemical component of the essence (carotenoids, flavonoids, and phenols) of borago. The highest number of flower per plant (13.9) and weight of flower per plant (16.8 g) and essence percentage (1.1%) was obtained from the plants were received 400 ppm of Iron, Zinc, Manganese and copper. Essence percentage significantly affected by micronutrients fertilizer. The highest DPPH (1.6) was measured from the flowers was received 400 ppm of Iron, Zinc, Manganese and copper. The most suitable Fe-Zn-Mn-Cu supply for production of borago to obtain the highest number and weight of flowers in Shahrekord, Iran is 400ppm foliar application of micronutrients. Effect of combined application of micronutrients fertilizers is suggested in compare with the separately use of them.

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