Foliar application of micronutrients on essential oils of borago, thyme and marigold

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Abstract

To study the effect of micronutrients foliar applications in medicinal plants contain of *Borago*, *Calendula* and *Thyme* 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^{2+} , Cu^{2+} , Zn^{2+} and Mn^{2+} applications on flavonoids, phenols, carotenoids, essence percentage, fresh/dry flower matter, number of flowers, and root/shoot dry/fresh matter in *Borago officinalis* and *Calendula officinalis* L.; carvacrol and thymol percentage, essence percentage, number of stem, height, and root/shoot dry/fresh matter in *Thymus vulgaris* L., the combinations of 400ppm of Fe, Cu, Zn and Mn ($\text{Fe}_3\text{Cu}_3\text{Zn}_3\text{Mn}_3$) produced the greatest amounts in most of measured characters. The most of phenols, carotenoids and flavonoids in *Calendula* and *Borago*; thymol and carvacrol in *Thyme*; weight of dry flower and number of flower per plant in two seasons were produced by $\text{Fe}_3\text{Cu}_3\text{Zn}_3\text{Mn}_3$ treatment.

Keywords: Essence, medicinal plants, nutrition, phytochemical

1. Introduction

Borago officinalis Linn. (Family; Boraginaceae) is a hairy annual herb 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 and is also considered useful to treat asthma, bronchitis, cramps, palpitations and kidney ailments. Optimum, minimum, and maximum temperatures for borage germination are 23, 9, and 37 °C, respectively (Gilbertson *et al.* 2014). Borage oil has been reported to lower serum cholesterol, phospholipids and triglyceride levels 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 and cytotoxic and free radical scavenging activities. Flavonoids and phenolic compounds were the important essential oils in this plant and do not have tannin (Lin *et al.* 2002; Wafa *et al.* 2011; RatzLyko *et al.* 2014). *Calendula* also known marigold is annual herb (Danielski *et al.* 2007). *Calendula* seeds contain high concentrations of calendic acid (C18:3) which can be used as tung and linseed oil substitutes. Optimum germination temperature is at

16–17 °C (Eberle *et al.* 2014). The vegetative parts of the plant are mid green while the stems are angular and covered in fine hairs. Marigold is cultivated for its flowers with receptacle or flowers without receptacle (Borghei *et al.* 2011) which are used as the medical raw material. Flavonoids, carotenoids and polyphenols are the active ingredient contents reported previously for *C.officinalis* flowers (Bunghez and Ion, 2011; Lim, 2014). The most essence in this plant is formed at full blooming (0.97%) and the least of essence (0.13%) before flowering (Sedghi *et al.* 2011). *Thymus vulgaris* L. (thyme), a member of the family Lamiaceae, is widely used in Iran folk medicine. *Thymus* species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants. The best time for harvesting of this plant is the beginning of flowering period (FriekeKuper *et al.* 2014). The major components are phenols (mainly thymol and carvacrol), monoterpen hydrocarbon and alcohol that have insecticide activity (Mendes *et al.* 2014; Deletre *et al.* 2015). Among these, thymol and carvacrol are the main compounds (Yadegari, 2012; Jamali *et al.* 2014). Although many studies performed about of macronutrients effects on plants (Quezada *et al.* 2007; Wyllyam Do Vale *et al.* 2008; Khan *et al.* 2014; Raza *et al.* 2014; Sandana and Pinochet, 2014; Yañez-Mansilla *et al.* 2015), few studies examining Iron, Copper, Zinc and Manganese fertility in *Borago*, *Calendula* and *Thyme* 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. 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 (Datta *et al.* 2011). Zinc is an important micronutrient associated with several enzymatic activities in all photosynthetic plants. Zn is necessary

in vital enzymes and growth regulators (Babaeian *et al.* 2012; Samia and Mohmoud, 2009).

Orioli Junior *et al.* (2008) showed that zinc application methods did not influence the initial growth of the wheat plants. The zinc concentration in leaves was influenced by the different application methods, being the leaf pulverization the one that was caused the largest zinc accumulation on aerial part dry matter but Mello Prado *et al.* (2007) reported that zinc application in the wheat seeds influenced the nutritional stage and plant dry matter production.

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 spray application of micro nutrient solve this problem and reduce soil fertilizer loss. 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 weak or damaged crops, speed growth and grow better (Said-Al Ahl and Mahmoud, 2010). Objectives of this study were to determine the effects of Iron, Zinc, Copper and Manganese foliar applications on vegetative, reproductive and phytochemical characters of three medicinal plants.

2. Materials and Methods

2.1. Plant material and fertilizers

Seeds of *Borago officinalis* Linn, *C.officinalis* var Qazvin and *T.vulgaris* L. obtained from the PakanBazr

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. During the vegetation, used practical measures were standard (3 earth up and pest protection). 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 (Fe_1 , Fe_2 , Fe_3 , Fe_4 were 0, 200, 400 and 600 ppm of Fe, respectively and similar in other micronutrients).

2.2. Experimental conditions

Field trials were established in 2012 and 2013 at Shahrekord (50°56' E, 32°18' N) South-Western Iran. The soil (Typiccalcixerocrepts) 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. Top soil 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 beginning of the blooming stage, shoots of thyme plants and in the end of the blooming stage, shoots of calendula and borago plants were harvested.

Table 1. Physical and chemical properties of soil for experiment

Year	Clay	Silt	Sand	N _{total}	K _{available}	P _{available}	O.C	pH	E.C	Depth
	(%)	(%)	(%)	(%)	(mg/kg)	(mg/kg)	(%)		(ds.m ⁻¹)	(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

2.3. Preparation of extracts from thyme, calendula and borago samples

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, phenols, thymol and carvacrol) 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. Harvested flowers were dried at room temperature for 1 week. 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* and *C.officinalis* methanolic extract was determined using the Folin–Ciocalteu (F–C) reagent. Dried samples were hydrolyzed, 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 multiwave length 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. 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 (Adams, 2001).

2.4. Data analysis

All data were subjected to ANOVA using the statistical computer package SAS and treatment means separated using Duncan's multiple range test at $P < 0.05$ level.

3. Results

Results showed the significant differences between treatments. Although in some of single treatments no significant differences, but in combinations and by Duncan's comparison, there were differences in most characters that result the Fe₃Cu₃Mn₃Zn₃ 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, calendula and thyme 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, phenols, thymol and carvacrol) 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 also the number of stem per plant in Fe₄Cu₄Mn₄Zn₄ 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 and calendula; thymol and carvacrol in thyme too. However, combination of $\text{Fe}_3\text{Cu}_3\text{Mn}_3\text{Zn}_3$ treatment was more effective than other treatments. Most of amount of measured characters (for example 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 $\text{Fe}_3\text{Cu}_3\text{Mn}_3\text{B}_3$, $\text{Fe}_3\text{Cu}_3\text{Mn}_2\text{Zn}_2$ and $\text{Fe}_3\text{Cu}_3\text{Mn}_1\text{Zn}_1$ made the maximum amount of characters, but $\text{Fe}_3\text{Cu}_3\text{Mn}_3\text{B}_3$ was the best combination (Table 4). In most times, control plants made alike of $\text{Fe}_4\text{Cu}_4\text{Mn}_2\text{Zn}_2$, $\text{Fe}_4\text{Cu}_4\text{Mn}_3\text{Zn}_3$ and $\text{Fe}_4\text{Cu}_4\text{Mn}_4\text{Zn}_4$. 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 $\text{Fe}_3\text{Cu}_3\text{Mn}_3\text{Zn}_3$. 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 were determined of medicinal plants were positive correlated and in best combinations of treatments (Table 3) the $\text{Fe}_3\text{Cu}_3\text{Mn}_3\text{Zn}_3$ was the best. Essence percentage positively correlated with the weight of shoot dry and fresh matter, number and weight of flowers. Also weight of shoot dry and fresh matter positively correlated with number and weight of flowers and essence percentage of medicinal plants (Table 3).

Table 2. Complex analysis of variance of variation of, flavonoids, phenols, carotenoids, essence percentage, fresh flower matter, dry flower matter, number of flowers, and root/shoot dry/fresh matter in plants of *Borago officinalis* L.

Source of Variation	Degree of freedom	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
Mean of Squares												
Year	1	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	0.05	12	0.008	0.0032	6.6	4.7	1.2	3.3	2.3	5.5	3.3
Fe	3	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	0.006*	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	0.005*	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	0.015**	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	0.004*	1421.2**	0.09**	0.00008*	2.7*	3.7*	1.2 ^{ns}	3.3*	1.8*	8.6**	3.4*
Cu×Fe	9	0.32*	18.4 ^{ns}	0.07**	0.00067**	6.6**	3.9*	1.3*	3.7*	3.4**	4.1*	8.8**
Mn×Fe	9	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	0.44**	14.1 ^{ns}	0.002*	0.012**	7.8**	5.8**	1.4*	7.3**	3.3**	11.1**	8.9**
Mn×Zn	9	0.1**	24.1*	0.0021*	0.000078*	2.7*	7.6**	4.7**	3.4*	4.4**	10.5**	10.3**
Cu×Mn	9	0.32**	55.4**	0.2**	0.000081*	9.9**	3.6*	3.3**	3.3*	2.05*	11.5**	3.5*
Cu×Fe×Zn	27	0.003*	19.1*	0.0015*	0.00007*	2.1*	3.1*	1.1*	1.9*	7.6**	3.2*	1.87*
Fe×Zn×Mn	27	0.22**	57.6**	0.7**	0.069**	10.6**	9.7**	5.4**	2.1*	1.5*	15.5**	14.4**
Cu×Mn×Fe	27	0.0034*	20.22*	0.8**	0.000075*	2.2*	9.1**	1.1*	9.8**	7.4**	12.5**	14.1**
Cu×Mn×Zn	27	0.14**	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	0.0024*	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	0.0004*	3.1*	0.0002*	0.000009*	0.3*	0.45*	0.16*	0.28*	0.19*	0.5*	0.3*
E	1020	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		1.1	2.2	3.3	1.2	9.7	8.7	6.3	9.1	12.1	14.4	18.3

Table 2- continued Complex analysis of variance of variation of, flavonoids, phenols, carotenoids, essence percentage, fresh flower matter, dry flower matter, number of flowers, and root/shoot dry/fresh matter in plants of *Calendula officinalis* L.

Source of Variation	Degree of Freedom	flavonoid	phenols	carotenoids	essence percentage	flower fresh weight	flower dry weight	number of flowers	root dry weight	root fresh weight	shoot dry weight	shoot fresh weight
Mean of Squares												
Year(Y)	1	0.0013	0.00002	0.00043	0.002	6.7	4.4	1.1	2.2	1.2	7.6	5.2
R/Y	4	0.0035*	0.00009	0.00032	0.0032	6.6	4.7	1.2	3.3	2.3	5.5	3.3
Copper (Cu)	3	0.013**	0.00015*	0.0002*	0.00004 ^{ns}	2.4 ^{ns}	2.2 ^{ns}	1.8*	4.2*	1.5 ^{ns}	5.5 ^{ns}	4.1*
Manganese (Mn)	3	0.0033*	0.00001 ^{ns}	0.0003*	0.00003 ^{ns}	2.5 ^{ns}	1.2 ^{ns}	1.5 ^{ns}	2.4 ^{ns}	1.4 ^{ns}	4.1*	3.9*
Iron (Fe)	3	0.017**	0.00007 ^{ns}	0.000092 ^{ns}	0.00004 ^{ns}	2.6 ^{ns}	1.9 ^{ns}	1.6 ^{ns}	2.7 ^{ns}	1.5 ^{ns}	4.7*	2.5 ^{ns}
Boron (B)	3	0.0013 ^{ns}	0.00008 ^{ns}	0.000001 ^{ns}	0.00005 ^{ns}	2.4 ^{ns}	1.3 ^{ns}	1.1 ^{ns}	2.5 ^{ns}	1.3 ^{ns}	5.4 ^{ns}	2.7 ^{ns}
Cu×Mn	9	0.0001 ^{ns}	0.000083*	0.0002*	0.00008*	2.7*	3.7*	1.2 ^{ns}	3.3*	1.8*	8.6**	3.4*
Cu×Fe	9	0.0001 ^{ns}	0.000087*	0.00023*	0.0067**	6.6**	3.9*	1.3*	3.7*	3.4**	4.1 ^{ns}	8.8**
Cu×B	9	0.0002*	0.0054**	0.00027*	0.00009*	2.8*	3.85*	2.6**	7.5**	1.9*	4.2 ^{ns}	8.6**
Mn×Fe	9	0.024**	0.00009*	0.00032**	0.012**	7.8**	5.8**	1.4*	7.3**	3.3**	11.1**	8.9**
Mn×B	9	0.00021*	0.000082*	0.00029*	0.000078*	2.7*	7.6**	4.7**	3.4*	4.4**	10.5	10.3**
Fe×B	9	0.003**	0.000089*	0.0003*	0.000081*	9.9**	3.6*	3.3**	3.3*	2.05*	11.5**	3.5*
Cu×Mn×Fe	27	0.002*	0.000075*	0.00009*	0.00007*	2.1*	3.1*	1.1*	1.9*	7.6**	3.2*	1.87*
Cu×Mn×B	27	0.003**	0.043**	0.00043**	0.069**	10.6**	9.7**	5.4**	2.1*	1.5*	15.5**	14.4**
Cu×Fe×B	27	0.0025*	0.000077*	0.000095*	0.000075*	2.2*	9.1**	1.1*	9.8**	7.4**	12.5**	14.1**
Fe×B×Mn	27	0.045**	0.024**	0.0045**	0.056**	15.4**	3.2*	1.2*	1.9*	1.7*	3.3*	1.8*
Cu×B×Mn×Fe	81	0.002*	0.000065*	0.00007*	0.00006*	1.7*	2.4*	0.9*	1.6*	1.1*	2.5*	1.5*
T(Cu,B,Mn,Fe)×Y	255	0.0002*	0.0000093*	0.000019*	0.000009*	0.3*	0.45*	0.16*	0.28*	0.19*	0.5*	0.3*
E	1020	0.0009	0.00004	0.000054	0.00004	1.34	1.1	0.67	1.2	0.85	2.1	1.2
CV		1.1	2.4	3.3	1.2	9.7	8.7	6.8	9.2	16.9	14.2	19.4

Table 2. continued Complex analysis of variance of variation of, Carvacrol percentage, Thymol percentage, essence percentage, number of stem, height, and root/shoot dry/fresh matter in plants of *Thymus vulgaris* L.

Source of variation	Degree of Freedom	carvacrol percentage	thymol percentage	essence percentage	number of stem	height	root dry matter	root fresh matter	shoot dry matter	shoot fresh matter
Mean of Square										
Year(Y)	1	0.05*	0.05*	0.051*	0.73*	0.97*	0.0002*	0.0002*	5.3*	2.2*
R/Y	4	0.041*	0.04*	0.032*	0.46*	0.61*	0.00013*	0.00012*	3.28*	1.35*
Copper (Cu)	3	0.22**	0.2**	0.1**	1.1**	1.8**	0.00024**	0.00022**	6.4**	2.6**
Manganese(Mn)	3	0.22**	0.22**	0.2**	0.99**	3.2**	0.0004**	0.0004**	7.1**	3.1**
Iron (Fe)	3	0.32**	0.04*	0.3**	2.5**	4.5**	0.001**	0.003**	6.5**	2.91**
Zinc (Zn)	3	0.04*	0.092*	0.15**	1.6**	2.2**	0.002**	0.0003**	7.3**	2.62**
Cu×Mn	9	1.01**	1.11**	0.05**	0.7**	0.85**	0.0002**	0.00015**	4.7**	2.1**
Cu×Fe	9	2.21**	2.3**	0.2**	1.2**	1.4**	0.001**	0.12**	5.1**	3.2**
Cu×Zn	9	0.4**	5.6**	0.1**	1.14**	1.1**	0.004**	0.0012**	6.7**	2.2**
Mn×Fe	9	1.3**	3.3**	0.99**	0.88**	0.9**	0.0004**	0.002**	6.02**	7.2**
Mn×Zn	9	1.5**	4.5**	0.08**	0.94**	2.21**	0.002**	0.56**	5.5**	3.4**
Fe×Zn	9	2.9**	3.9**	1.4**	1.7**	0.97**	0.003**	0.0021**	5.6**	2.43**
Cu×Mn×Fe	27	0.14**	0.04*	0.06**	1.4**	1.2**	0.00015**	0.00014**	2.8*	1.5**
Cu×Mn×Zn	27	0.14**	0.02*	0.03*	0.7**	0.6*	0.004**	0.0012**	4.5**	1.1*
Cu×Fe×Zn	27	0.05**	0.06**	0.027*	1.4**	0.51*	0.00017**	0.00008*	2.81*	2.2**
Fe×Zn×Mn	27	0.05**	0.043**	0.04**	0.4*	1.1**	0.00011*	0.0023**	2.78*	2.6**
Cu×Zn×Mn×Fe	81	0.14**	0.044**	0.03**	0.34*	2.2**	0.00009*	0.00019**	2.4*	1.2**
T(Cu,Zn,Mn,Fe)×Y	255	0.03**	0.02*	0.018*	0.26*	0.35*	0.00007*	0.00005*	1.9*	0.78*
E	1020	0.012	0.013	0.013	0.19	0.25	0.00005	0.00004	1.36	0.56
Coefficient of variation		8.9	3.5	5.2	15.1	12.2	14.1	16.1	16.1	18.9

Table 3. Results of correlation between characters in borago plants that are affected by several micronutrients (2012).

Characters	shoot fresh weight (1)	shoot dry weight (2)	root fresh weight (3)	root dry weight (4)	number of flowers (5)	flower dry weight (6)	flower fresh weight (7)	Extraction percentage (8)	Carotenoid (9)	Phenol (10)	Flavonoid (11)
1	1										
2	0.52*	1									
3	0.26	0.1	1								
4	0.36	0.56**	0.66**	1							
5	0.5*	0.58**	0.64**	0.52*	1						
6	0.5*	0.76**	0.5*	0.44	0.58**	1					
7	0.6**	0.59**	0.35	0.36	0.55**	0.58**	1				
8	0.48	0.56**	0.46	0.33	0.7**	0.65**	0.46	1			
9	0.1	0.68**	0.11	0.4	0.4	0.7**	0.26	0.69**	1		
10	0.11	0.76**	0.1	0.3	0.42	0.4	0.24	0.66**	0.48	1	
11	0.1	0.56**	0.3	0.55*	0.31	0.42	0.33	0.68**	0.26	0.36	1

Table 3. continued Results of correlation between characters in borago plants that are affected by several micronutrients (2013).

Characters	shoot fresh weight (1)	shoot dry weight (2)	root fresh weight (3)	root dry weight (4)	number of flowers (5)	flower dry weight (6)	flower fresh weight (7)	essence percentage (8)	Carotenoid (9)	Phenol (10)	Flavonoid (11)
1	1										
2	0.56**	1									
3	0.36	0.2	1								
4	0.36	0.66**	0.73**	1							
5	0.5*	0.68**	0.65**	0.7**	1						
6	0.48	0.8**	0.6**	0.4	0.8**	1					
7	0.78**	0.7**	0.3	0.3	0.87**	0.87**	1				
8	0.58*	0.6**	0.4	0.3	0.7**	0.7**	0.6**	1			
9	0.2	0.6**	0.14	0.4	0.3	0.8**	0.3	0.8**	1		
10	0.22	0.8**	0.2	0.3	0.4	0.3	0.4	0.5*	0.3	1	
11	0.3	0.7**	0.2	0.5*	0.3	0.4	0.2	0.48	0.3	0.4	1

Table 3 – continued Results of correlation between characters in *Calendula* plants that are affected by several micronutrients (2012)

Characters	shoot fresh weight (1)	shoot dry weight (2)	root fresh weight (3)	root dry weight (4)	number of flowers (5)	flower dry weight (6)	flower fresh weight (7)	essence percentage (8)	Carotenoid (9)	Phenol (10)	Flavonoid (11)
1	1										
2	0.9**	1									
3	0.89**	0.91**	1								
4	0.85**	0.9**	0.34	1							
5	0.76**	0.87**	0.9**	0.69**	1						
6	0.73**	0.78**	0.9**	0.59**	0.9**	1					
7	0.95**	0.96**	0.9**	0.54**	0.77**	0.79**	1				
8	0.94**	0.95**	0.44	0.42	0.8**	0.7**	0.68**	1			
9	0.82**	0.94**	0.42	0.37	0.8**	0.68**	0.78**	0.5	1		
10	0.92**	0.94**	0.37	0.36	0.6**	0.8**	0.76**	0.46	0.5	1	
11	0.87**	0.92**	0.6**	0.55**	0.65**	0.56**	0.6**	0.69**	0.69**	0.65**	1

Table 3 . continued Results of correlation between characters in *Calendula* plants that are affected by several micronutrients (2013).

Characters	shoot fresh weight (1)	shoot dry weight (2)	root fresh weight (3)	root dry weight (4)	number of flowers (5)	flower dry weight (6)	flower fresh weight (7)	essence percentage (8)	Carotenoid (9)	Phenol (10)	Flavonoid (11)
1	1										
2	0.91**	1									
3	0.99**	0.87**	1								
4	0.8**	0.82**	0.34	1							
5	0.7**	0.8**	0.89**	0.89**	1						
6	0.7**	0.88**	0.69**	0.59**	0.79**	1					
7	0.9**	0.86**	0.79**	0.74**	0.82**	0.89**	1				
8	0.92**	0.9**	0.34	0.42	0.68**	0.87**	0.88**	1			
9	0.8**	0.9**	0.22	0.27	0.78**	0.88**	0.88**	0.5	1		
10	0.9**	0.9**	0.37	0.26	0.6**	0.78**	0.86**	0.55*	0.5	1	
11	0.8**	0.9**	0.76**	0.65**	0.65**	0.76**	0.96**	0.9**	0.59*	0.6**	1

Table 3. continued Results of correlation between characters in Thyme plants that are affected by several micronutrients (2012).

Characters	1	2	3	4	5	6	7	8	9
Shoot dry matter (1)	1								
Shoot fresh matter (2)	0.92**	1							
Root dry matter (3)	0.99**	0.9**	1						
Root fresh matter (4)	0.75**	0.65**	0.66**	1					
Height (5)	0.66**	0.6**	0.66**	0.6**	1				
Number of stems (6)	0.77**	0.57**	0.67**	0.57**	0.87**	1			
Essence percentage (7)	0.94**	0.74**	0.79**	0.7**	0.7**	0.7**	1		
Carvacrol percentage (8)	0.91**	0.81**	0.85**	0.8**	0.92**	0.94**	0.98**	1	
Thymol percentage (9)	0.84**	0.8**	0.84**	0.72**	0.82**	0.92**	0.97**	0.78**	1

Table 3. continued Results of correlation between characters in Thyme plants that are affected by several micronutrients (2013).

Characters	1	2	3	4	5	6	7	8	9
Shoot dry matter (1)	1								
Shoot fresh matter (2)	0.9**	1							
Root dry matter (3)	0.89**	0.78**	1						
Root fresh matter (4)	0.85**	0.62**	0.64**	1					
Height (5)	0.76**	0.57**	0.63**	0.76**	1				
Number of stems (6)	0.73**	0.55**	0.62**	0.67**	0.97**	1			
Essence percentage (7)	0.95**	0.73**	0.89**	0.67**	0.77**	0.77**	1		
Carvacrol percentage (8)	0.94**	0.8**	0.87**	0.88**	0.9**	0.9**	0.9**	1	
Thymol percentage (9)	0.82**	0.76**	0.85**	0.78**	0.87**	0.96**	0.95**	0.88**	1

ns: Not Significant, *and**: Significant at $P=0.05$ and $P=0.01$ levels of probability, respectively.

Table 4. Means of characters measured in borago plants that are affected by micronutrients (400 ppm) concentration and control plants (1st year).

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

Table 4b. Means of Characters measured in borago plants that are affected by micronutrients (400 ppm) concentration and control plants (2nd year).

Treatments	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	0.08c	14c	0.07de	0.23d	15c	7.5d	10cd	16.2cd	32.5d	15.7c	39.3d
Zn	0.05cd	12c	0.11d	0.21d	15c	8.5cd	11c	14.9cd	32.5d	12.7c	39.6cd
Cu	0.06c	11c	0.095d	0.5c	15c	7.7d	11.1c	14.7cd	35.7d	18.4bc	36.3d
Mn	0.03cd	11.1c	0.07de	0.24d	11d	8.5cd	8.7d	13d	30.1e	15.4c	30.2de
Fe×Zn	0.09c	11.5c	0.23bc	0.5c	13.6cd	9.4cd	11.2c	14.8d	30.1e	18.4bc	42.5cd
Cu×Fe	0.09c	21cd	0.22bc	0.5c	13.4cd	9.7cd	12.3b	20.5c	35.3c	18.5bc	42.5cd
Mn×Fe	0.25b	22cd	0.2c	0.5c	15c	10.8c	11.5c	20.5c	40.2c	18.9bc	45.2c
Cu×Zn	0.15bc	30c	0.2c	0.61bc	14cd	10.5cd	12.6b	20c	41.3c	21bc	45.2c
Mn×Zn	0.12bc	31c	0.2c	0.54c	15c	10.9c	12.6b	18.6cd	42.3c	15.6c	45.2c
Cu×Mn	0.11bc	35bc	0.35bc	0.5c	15c	10.2cd	12.7ab	18.4cd	44.4c	18.5bc	51.7bc
Cu×Fe×Zn	0.3b	50b	0.35bc	0.75b	17b	11.8bc	13.3a	22bc	52.3bc	24ab	51.8bc
Fe×Zn×Mn	0.4b	38bc	0.4b	0.72b	19ab	13b	12.5b	23bc	54.5bc	24.3ab	54.2b
Cu×Mn×Fe	0.48b	72a	0.4b	0.9ab	17b	12.8b	12.5b	28b	56.3b	24.7ab	54.5b
Cu×Mn×Zn	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	0.62a	75a	0.8a	1.1a	22a	16.8a	13.9a	34.7a	60.8a	35.8a	70.2a
Control	0.015d	12d	0.05e	0.1d	11d	7.5d	9d	14d	25.4f	15.5c	27.4e

Table 4c. Means of Characters measured in calendula plants that are affected by micronutrients (400 ppm) concentration and control plants (1st year).

Treatments	Shoot dry matter	Shoot fresh matter	Root dry matter	Root fresh matter	Number of flower	Weight of flower	Essence percentage	phenols	Flavonoid
Copper (Cu)	6.6 c	12.2 d	7.1 cd	14.1 de	21.1 de	7 cd	0.24 d	28.4 d	14.1 c
Manganese(Mn)	5.6 c	12.5 cd	6.8 cd	13.1 de	22.1 d	7.5 cd	0.22 d	28.2 d	14.2 c
Iron (Fe)	7.3 bc	12.7 cd	6.6 cd	12.1 de	22.2 d	8 c	0.25 d	32.9 d	14.4 c
Boron (B)	6.1 c	12.2 d	6.6 cd	11.9 e	20.1 de	6.6 d	0.24 d	30.1 d	15.1 c
Cu×Mn	7.2 bc	13.2 cd	7.7 cd	14.6 d	22.4 cd	9.1 b	0.56 c	39.1 bc	15.5 c
Cu×Fe	7.4 bc	13.7 cd	8.1 cd	16.1 cd	23.1 cd	9.3 b	0.52 c	38.4 bc	16.2 c
Cu×B	7.8 bc	14.1 cd	9.1 c	17.9 c	24.4 cd	9.4 b	0.55 c	37 c	16.7 bc
Mn×Fe	8.1 bc	15.1 c	8.8 cd	17.1 cd	25.7 c	9.5 b	0.59 bc	38.8 bc	19.5 b
Mn×B	6.5 c	14.1 cd	8.5 cd	18.1 c	23.1 cd	9.5 b	0.54 c	38.1 c	18.5 b
Fe×B	7.3 bc	16.6 bc	8.3 cd	17.2 cd	25.5 c	9.6 ab	0.62 bc	40.2 bc	18 bc
Cu×Mn×Fe	8.9 ab	16.7 bc	10.1 bc	21.1 bc	27.2 b	10.2 a	0.71 b	44.1 b	20.1 b
Cu×Mn×B	9.2 ab	17.1 bc	10.2 bc	21.3 bc	27.4 b	10.4 a	0.7 b	42.2 b	22 b
Cu×Fe×B	9.6 ab	17.4 bc	11.1 b	23.1 b	27.6 b	10.4 a	0.8 ab	44 b	24 ab
Fe×B×Mn	9.1 ab	18.1 b	11.4 b	23.7 b	27.1 b	10.1 a	0.8 ab	43 b	23 b
Cu×B×Mn×Fe	10.8 a	22.1 a	15.2 a	32.6 a	33.1 a	10.8 a	0.9 a	55.3 a	30 a
Control	5.1 c	10.3 e	6.1 d	12.2 de	18.1 e	6.9 d	0.21 d	25.1 d	12.5 c

Table 4. continued Means of Characters measured in calendula plants that are affected by micronutrients (400 ppm) concentration and control plants (2nd year).

Treatments	Shoot dry matter	Shoot fresh matter	Root dry matter	Root fresh matter	Number of flower	Weight of flower	Essence percentage	phenols	Flavonoid
Copper (Cu)	6.8 c	12.4 cd	7.3 d	15.2 cd	22.1 cd	8 d	0.3 de	28.6 d	15.2 d
Manganese(Mn)	5.8 c	12.6 cd	7 d	14.2 d	23.1 cd	8.1 d	0.31 de	28.4 d	15.3 d
Iron (Fe)	7.8 ab	12.8 cd	6.8 d	12.4 d	23.2 cd	9 c	0.34 de	33.1 cd	14.7 d
Boron (B)	6.7 c	12.4 cd	6.4 d	12.5 d	21.1 d	7 d	0.27 e	31.1 d	15.5 d
Cu×Mn	7.7 ab	13.6 cd	7.9 cd	15.6 cd	23.4 cd	10.1 b	0.57 c	39.5 bc	15.6 d
Cu×Fe	7.5 ab	13.9 cd	8.6 cd	16.5 cd	24.1 c	10.2 b	0.55 c	38.6 c	16.5 d
Cu×B	8.1 ab	12.1 d	8.8 c	18.3 c	24.2 c	10 b	0.56 c	37.5 c	16.9 cd
Mn×Fe	8.5 ab	16.1 c	9.1 c	18.4 c	26.4 bc	10.2 b	0.6 c	39.8 c	20.1 c
Mn×B	6.7 c	14.2 c	8.7 cd	18.7 c	25.1 bc	10.1 b	0.57 c	38.5 c	19.9 c
Fe×B	7.9 ab	16.7 bc	8.4 cd	18.5 c	26.5 bc	10.2 b	0.64 c	41.2 bc	18.8 c
Cu×Mn×Fe	9.3 b	16.8 bc	11.2 b	22.1 bc	28.2 b	11.7 a	0.74 b	45.1 b	21.5 bc
Cu×Mn×B	9.7 b	17.3 bc	11.6 b	22.4 bc	28.4 b	11.6 a	0.73 b	43.2 bc	22.3 bc
Cu×Fe×B	10.1 ab	17.6 bc	11.4 b	24.5 bc	28.6 b	11.1 ab	0.82 b	45.2 b	24.5 b
Fe×B×Mn	10.1 ab	18.2 b	11.8 b	25.5 b	28.1 b	11.7 a	0.9 ab	45.1 b	23.4 b
Cu×B×Mn×Fe	12.1 a	23.4 a	16.3 a	34.1 a	33.5 a	11.9 a	1 a	56.2 a	31.2 a
Control	6.1 c	10.5 d	6.7 d	14 d	18.3 d	7.1 d	0.22 e	26.1 d	14.2 d

Table 4. continued Means of Characters measured in thyme plants that are affected by micronutrients (400 ppm) concentration and control plants (1st year).

Treatments	Shoot dry matter	Shoot fresh matter	Root dry matter	Root fresh matter	Height	Number of stems	Essence percentage	Carvacrol percentage	Thymol percentage
Copper (Cu)	6.6 c	12.2 d	7.1 cd	14.1 de	21.1 de	7 cd	0.24 d	28.4 d	14.1 c
Manganese(Mn)	5.6 c	12.5 cd	6.8 cd	13.1 de	22.1 d	7.5 cd	0.22 d	28.2 d	14.2 c
Iron (Fe)	7.3 bc	12.7 cd	6.6 cd	12.1 de	22.2 d	8 c	0.25 d	32.9 d	14.4 c
Zinc (Zn)	6.1 c	12.2 d	6.6 cd	11.9 e	20.1 de	6.6 d	0.24 d	30.1 d	15.1 c
Cu×Mn	7.2 bc	13.2 cd	7.7 cd	14.6 d	22.4 cd	9.1 b	0.56 c	39.1 bc	15.5 c
Cu×Fe	7.4 bc	13.7 cd	8.1 cd	16.1 cd	23.1 cd	9.3 b	0.52 c	38.4 bc	16.2 c
Cu×Zn	7.8 bc	14.1 cd	9.1 c	17.9 c	24.4 cd	9.4 b	0.55 c	37 c	16.7 bc
Mn×Fe	8.1 bc	15.1 c	8.8 cd	17.1 cd	25.7 c	9.5 b	0.59 bc	38.8 bc	19.5 b
Mn×Zn	6.5 c	14.1 cd	8.5 cd	18.1 c	23.1 cd	9.5 b	0.54 c	38.1 c	18.5 b
Fe×Zn	7.3 bc	16.6 bc	8.3 cd	17.2 cd	25.5 c	9.6 ab	0.62 bc	40.2 bc	18 bc
Cu×Mn×Fe	8.9 ab	16.7 bc	10.1 bc	21.1 bc	27.2 b	10.2 a	0.71 b	44.1 b	20.1 b
Cu×Mn×Zn	9.2 ab	17.1 bc	10.2 bc	21.3 bc	27.4 b	10.4 a	0.7 b	42.2 b	22 b
Cu×Fe×Zn	9.6 ab	17.4 bc	11.1 b	23.1 b	27.6 b	10.4 a	0.8 ab	44 b	24 ab
Fe×Zn×Mn	9.1 ab	18.1 b	11.4 b	23.7 b	27.1 b	10.1 a	0.8 ab	43 b	23 b
Cu×Zn×Mn×Fe	10.8 a	22.1 a	15.2 a	32.6 a	33.1 a	10.8 a	0.9 a	55.3 a	30 a
Control	5.1 c	10.3 e	6.1 d	12.2 de	18.1 e	6.9 d	0.21 d	25.1 d	12.5 c

Table 4. continued Means of Characters measured in thyme plants that are affected by micronutrients (400 ppm) concentration and control plants (2nd year).

Treatments	Shoot dry matter	Shoot fresh matter	Root dry matter	Root fresh matter	Height	Number of stems	Essence percentage	Carvacrol percentage	Thymol percentage
Copper (Cu)	6.8 c	12.4 cd	7.3 d	15.2 cd	22.1 cd	8 d	0.3 de	28.6 d	15.2 d
Manganese(Mn)	5.8 c	12.6 cd	7 d	14.2 d	23.1 cd	8.1 d	0.31 de	28.4 d	15.3 d
Iron (Fe)	7.8 ab	12.8 cd	6.8 d	12.4 d	23.2 cd	9 c	0.34 de	33.1 cd	14.7 d
Zinc (Zn)	6.7 c	12.4 cd	6.4 d	12.5 d	21.1 d	7 d	0.27 e	31.1 d	15.5 d
Cu×Mn	7.7 ab	13.6 cd	7.9 cd	15.6 cd	23.4 cd	10.1 b	0.57 c	39.5 bc	15.6 d
Cu×Fe	7.5 ab	13.9 cd	8.6 cd	16.5 cd	24.1 c	10.2 b	0.55 c	38.6 c	16.5 d
Cu×Zn	8.1 ab	12.1 d	8.8 c	18.3 c	24.2 c	10 b	0.56 c	37.5 c	16.9 cd
Mn×Fe	8.5 ab	16.1 c	9.1 c	18.4 c	26.4 bc	10.2 b	0.6 c	39.8 c	20.1 c
Mn×Zn	6.7 c	14.2 c	8.7 cd	18.7 c	25.1 bc	10.1 b	0.57 c	38.5 c	19.9 c
Fe×Zn	7.9 ab	16.7 bc	8.4 cd	18.5 c	26.5 bc	10.2 b	0.64 c	41.2 bc	18.8 c
Cu×Mn×Fe	9.3 b	16.8 bc	11.2 b	22.1 bc	28.2 b	11.7 a	0.74 b	45.1 b	21.5 bc
Cu×Mn×Zn	9.7 b	17.3 bc	11.6 b	22.4 bc	28.4 b	11.6 a	0.73 b	43.2 bc	22.3 bc
Cu×Fe×Zn	10.1 ab	17.6 bc	11.4 b	24.5 bc	28.6 b	11.1 ab	0.82 b	45.2 b	24.5 b
Fe×Zn×Mn	10.1 ab	18.2 b	11.8 b	25.5 b	28.1 b	11.7 a	0.9 ab	45.1 b	23.4 b
Cu×Zn×Mn×Fe	12.1 a	23.4 a	16.3 a	34.1 a	33.5 a	11.9 a	1 a	56.2 a	31.2 a
Control	6.1 c	10.5 d	6.7 d	14 d	18.3 d	7.1 d	0.22 e	26.1 d	14.2 d

4. Discussion

Results showed beneficial micronutrient application on medicinal plants (Table 2). Increasing of micronutrients can obtain more yield, essence and essential oils from calendula (Naguib *et al.* 2005). The beneficial application of micronutrients was reported by other researchers (Yadegari *et al.* 2012; Galavi *et al.* 2012). Results of this research showed that foliar application of micronutrient made the more essential oil in flower of medicinal plants (Tables 2, 4). A similar effect of micronutrient supply on this parameter was also reported on *M.chamomilla* (Nasiri *et al.* 2010), *Coriandrum sativum* (Said-Al Ahland Omer, 2009), *Menthasp* (Rawia *et al.* 2010) and *Ocimum basilicum* (Said-Al Ahland Mahmoud, 2010). In the present study, the effect of micronutrients applications was determined on the growth and yield of medicinal plants in two consecutive years.

Dry matter flower increased in both years with micronutrients applications. The essential oil yield increased with Fe²⁺, Cu²⁺, Zn²⁺ and Mn²⁺ applications because there was a significant increase in dry matter and number of flower. This is the first report in which the effects of Fe²⁺, Cu²⁺, Zn²⁺ and Mn²⁺ applications on certain physiological characteristics were determined for medicinal plants 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. It seems the weight of shoot dry matter was the best character that correlated by most of characters a finding with which others agree (Mhamdi *et al.* 2009; Naghdi Badi and Sorooshzadeh, 2011; Babaieian *et al.* 2012; Samia and Mohmoud, 2009; Jamali *et al.* 2014). From the present data, Its concluded that applications of Fe²⁺, Cu²⁺, Zn²⁺ and Mn²⁺ are beneficial for medicinal 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 medicinal plants. 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. Micronutrients can increase the weight of shoot dry matter and weight of root dry matter (Younis *et al.* 2013; Yarnia *et al.* 2012). It seems, 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. These results reflect the role of applying the four foliar fertilizers in improving the total essential oils in medicinal 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. 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 $\text{Fe}_4\text{Cu}_4\text{Mn}_4\text{Zn}_4$ made the equivalent amount of control plants.

5. Conclusion

Plants treated with 400 ppm of Iron, Zinc, Manganese and Copper, had the more fresh and dry weight of flowers and essence percentage. It could be concluded from the results that Iron, Zinc, Manganese and Copper fertilization had significant effects on measured characters as well as the chemical component of the essence of medicinal plants. The most suitable Fe - Zn - Mn - Cu supply for production of medicinal plants

to obtain the highest number and weight of flowers in Shahrekord, Iran is 400ppm foliar application of micronutrients. Effects of combined application of micronutrients fertilizers is suggested in compare with the separately use of them.

Results obtained from this study showed that the essential oils composition of lemon balm was significantly affected by foliar application of micronutrients. The highest amount of flavonoids, phenols, carotenoids in *Borago officinalis* and *Calendula officinalis* L.; carvacrol and thymol percentage, in *Thymus vulgaris* L., obtained from 400 ppm of micronutrients foliar application in two cultivation seasons. This study provides some useful information about the efficacy of foliar application of micronutrients in soils with undesirable characteristics, and chemical properties in particular. Since in some cases, these methods are relatively new and need further refinement with regard to rates, timing, and technique.

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