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Foliar Application of Fe, Cu, Mn and B on growth, yield, and essential oil yield of marigold (*Calendula officinalis*)

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ABSTRACT

To study the effect of different concentrations of Iron, Copper, Manganese and Boron foliar application in marigold on yield and essence production, the investigation was performed in complete randomized block design with 3 replications. Each micronutrient applied in four concentrations (0, 200, 400, 600 ppm). The experiment was repeated at two growth periods (2012 and 2013) in southwestern Iran in a sandy clay soil. Objectives of this study were to determine the effects of foliar Fe^{2+}, Cu^{2+}, $Mn^{2+}$and H_2Bo^{3-} applications on length of stem, leaf and root, number of flower, fresh and dry matter of root and shoot, stem diameter, fresh and dry weight of flower, carotenoids, flavonoids, polyphenols, essence percentage of marigold. Results showed that Fe^{2+}, Cu^{2+}, $Mn^{2+}$and H_2Bo^{3-} applications significantly increased the measured characters. In two year, the greatest number of harvested flower (harvest1: 3500, harvest2: 6500, harvest3: 6000), length of stem (36 cm), shoot dry matter (0.39gr), root dry matter (0.38 gr) produced by combination of 400 ppm of Fe^{2+}, Cu^{2+}, $Mn^{2+}$and H_2Bo^{3-}. The most of poly phenols, carotenoids and flavonoids was 25%, 42%, 25.5% and 27%, 40%, 24.5% in primary and secondary year respectively. The most weight of dry flower, number of flower per plant was 14.8 gr, 16.8 gr and 10.9, 13.9 in 1st and 2nd years respectively, were made by combination of 400 ppm of all micronutrients ($Fe_3Cu_3Mn_3B_3$). These results showed that applications of micronutrients can affect the growth and yield of marigold, especially when the plant is grown in alkaline soils, and in this the 400 ppm concentrations of micronutrients were the best. Perhaps, the physiological basis of this effect is immobilization of micronutrients in this soil.

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INTRODUCTION

Calendula officinalis L., also known marigold is annual herb (Danielski *et al*., 2007). It grows wild in the southern, eastern and central Europe (Wyk and Wink, 2004; Marisol and Rosemarie, 2003; Martin, 2005). It is usually multi stemmed with a strong tap root. The vegetative parts of the plant are mid green while the stems are angular and covered in fine hairs. The composite flowers could be yellow or orange (Gilman and Howe, 1999), which blossom in the spring-summer seasons. Marigold is cultivated for its flowers with receptacle or flowers without receptacle (Varban *et al*., 2008) which are used as the medical raw material. The flower contains essential oils which are used for high blood-fat and treatment of inflammation intestine organs. Flavonoids, carotenoids and polyphenols are the active ingredient contents reported previously for C.officinalis flowers (Re *et al*., 2009; Mrda *et al*., 2007). The most essence this plant is formed at full blooming (0.97%) and the least of essence (0.13%) before flowering (Okoh *et al*., 2007). Few studies examining Iron, Copper, Manganese and Boron fertility in Marigold 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). 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

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concentrations (Younis *et al*., 2013). Boron made resistance of plasmalema and combination by other minerals and necessary for plants (Widom and Mihalkovic, 2008). 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 weak or damaged crops, speed growth and grow better and healthier plants. Micro and macro elements play an important role in promoting the growth and production of plants. Micro elements participate in most of the enzymatic reaction and they also play a role indirectly through the synthesis of several growth regulators (Khalifa *et al*., 2009; Malakoti and Tehrani, 1999). Objectives of this study were to determine the effects of Iron, Copper, Manganese and Boron foliar applications on length of stem and root, number of flower and stem, root and shoot fresh and dry matter, flower fresh and dry weight, carotenoids, flavonoids, polyphenols, essence percentage of marigold.

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 C.officinalis var Qazvin, 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 (10 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. Plants were sown in pots in the farm condition on 22 May 2012 and 20 May 2013. At the end of the blooming stage, shoots of plants were harvested.

Four foliar fertilizers Librel Fe-Lo, Librel Cu, Librel Mn and Unibor were applied and all of them are mineral fertilizers. Librel Fe-Lo contains 13.2% chelated iron. Librel Cu has 14% copper in chelated form. Librel Mn inclusive of 13% Mn chelated with EDTA and Unibor contains 15% B (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). Soil analysis result for this region is presented in Table 1. 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 plant, number of shoots and the amount of essence production as well as the chemical component of the essence (carotenoids, flavonoids, poly phenols) were determined. Essential oil composition was evaluated by capillary GC/MS (Shibamoto, 1987). The marigold petals and leaves were clean with distillated water, and then were dried 24 hours at 68°-72°C in oven. The product (approx 15 g) was extracted with 30 mL ethanol 95% by using Soxhlet method. The extract was filtered through a filter bed of cotton wool in a 100 mL volumetric flask. The filtrate was reintroduced in the recipient contained the plant residue and then was extracted again, for 10 minutes, with 30 mL with initial solvent; the process has been repeated for several times. Finally, the extract was concentrated by using a rotary evaporator; the final extract reached a 10 mL volume. This filtrate was cooled at -14°C, and was kept in the dark (Bunghez and Ion, 2011). After transit plants to laboratory and separate the petals and leaves, roots of them are cleaned and dried in oven similar to leaves and then root dry matter measured. 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. All parameters were calculated in Growing Degree Day (GDD). GDD was measured by the following formula. In this, basal temperature is the temperature that growth is beginning, in calendula is 5.5°C (Joly *et al*., 2013). $\sum((\frac{T\max + T\min}{2}) \_ Tb)$

Results:

Results showed the significant differences between treatments. However 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 $Fe_3Cu_3Mn_3B_3$ 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 Calendula oil were influenced by different treatments. The application treatment of Fe, Cu, Mn and B was significantly improved the growth characters including essence percentage, number of harvested flowers, length of leaf and root, flower dry and fresh weight, fresh and dry weight of leaves and roots, length of stem, leaf width, stem diameter 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 B 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 $Fe_4Cu_4Mn_4B_4$ was the least (data no published). In character of number of leaves 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 B has increased flavonoids, polyphenols and carotenoids in the oil of Calendula. However, combination of $Fe_3Cu_3Mn_3B_3$ treatment was more effective than other treatments. Most of amount of measured characters (for example flavonoids, width of leaves, length of stem) decreased by application upper than 400 ppm of Fe, Cu, Mn and B. 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 400 ppm concentration of Fe, Cu, Mn and B. In most treatments, combination of $Fe_3Cu_3Mn_3B_3$, $Fe_3Cu_3Mn_2B_2$ and $Fe_3Cu_3Mn_1B_1$ 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 $Fe_4Cu_4Mn_2B_2$, $Fe_4Cu_4Mn_3B_3$ and $Fe_4Cu_4Mn_4B_4$. For toxicities of upper concentrations of Fe, Cu, Mn and B, 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 essential oils were found with $Fe_3Cu_3Mn_3B_3$ (0.9% and 1% in first and second year respectively). The most polyphenols made by $Fe_3Cu_3Mn_3B_3$ in two years (55.3% and 56.2 % in 1st and 2nd year). Concentration of micronutrients positively affected on characters measured and between the length of stem and root, number of flower and stem, root and shoot fresh and dry matter, flower fresh and dry weight, carotenoids, flavonoids, polyphenols, essence percentage of marigold were positive correlated (Table 2) and in best combinations of treatments (tables 3, 4) the $Fe_3Cu_3Mn_3B_3$ was the best. Essence percentage positively correlated with the shoot dry and fresh matter, root dry and fresh matter, number of stems per plant, number and weight of flowers (Table 5, 6). Shoot dry and fresh matter positively correlated with root dry and fresh matter, length of stem and root of Calendula.

Table 1: Some physical and chemical properties of soil for experiment (0 -30) cm.

Year	Texture	E.C ($ds.m^{-1}$)	N_{total}	O.C	pH	K	P	B	Mn	Fe	Cu
			%			$mg.kg^{-1}$					
1	Loam	8.1	0.11	0.2	8.2	770	45	0.97	11.2	8.1	1.3
2	Loam	7.8	0.11	0.2	8.1	745	44	1	10.1	7.1	1.1

Table 2: Complex analysis of variance of variation of essence percentage, flavenoids, carotenoids, polyphenols, number of harvested flowers in harvest of 1, 2, 3, length of leaf and root, flower dry and fresh weight in plants of *Calendula officinalis* L.

Source of variation	Degree of Freedom	Mean of Squares									
		Essence percentage	Flavonoid	Carotenoids	Poly phenols	Number of flowers (3)	Number of flowers (2)	Number of flowers (1)	Leaf length	Leaf width	Flower dry weight
Year(Y)	1	0.002	0.0013	0.00043	0.00002	1.1	1.2	1.9	1.1	0.08	4.4
R/Y	4	0.0032	0.0035*	0.00032	0.00009	1.2	0.5	2.2	2.1	1.8	4.7
Copper (Cu)	3	0.00004^{ns}	0.013**	0.0002*	0.00015*	1.8*	1.6*	4.5**	2.2*	2.5*	2.2^{ns}
Manganese (Mn)	3	0.00003^{ns}	0.0033*	0.0003*	0.00001^{ns}	1.5^{ns}	1.1^{ns}	1.2^{ns}	1.03^{ns}	1.4^{ns}	1.2^{ns}
Iron (Fe)	3	0.00004^{ns}	0.017**	0.000092^{ns}	0.00007^{ns}	1.6^{ns}	0.99^{ns}	1.3^{ns}	1.1^{ns}	1.2^{ns}	1.9^{ns}
Boron (B)	3	0.00005^{ns}	0.0013^{ns}	0.000001^{ns}	0.00008^{ns}	1.1^{ns}	1.4^{ns}	1.6^{ns}	1.3^{ns}	1.1^{ns}	1.3^{ns}
Cu×Mn	9	0.00008*	0.0001^{ns}	0.0002*	0.000083*	1.2^{ns}	1.7^{ns}	1.5^{ns}	1.2^{ns}	2.2^{ns}	3.7*
Cu×Fe	9	0.0067**	0.0001^{ns}	0.00023*	0.000087*	1.3*	3.5**	5.4**	4.6**	5.7**	3.9*
Cu×B	9	0.00009*	0.0002*	0.00027*	0.0054**	2.6**	2.9**	2.5**	4.2**	4.8**	3.85*
Mn×Fe	9	0.012**	0.024**	0.00032**	0.00009*	1.4*	1.8*	1.7*	2.02*	2.4*	5.8**
Mn×B	9	0.000078*	0.00021*	0.00029*	0.000082*	4.7**	4.2**	4.5**	5.5**	6.5**	7.6**
Fe×B	9	0.000081*	0.003**	0.0003*	0.000089*	3.3**	3.9**	5.2**	4.8**	5.9**	3.6*
Cu×Mn×Fe	27	0.00007*	0.002*	0.00009*	0.000075*	1.1*	1.5*	1.1*	1.4*	6.4**	3.1*
Cu×Mn×B	27	0.069**	0.003**	0.00043**	0.043**	5.4**	5.7**	5.5**	7.7**	8.6**	9.7**
Cu×Fe×B	27	0.000075*	0.0025*	0.000095*	0.000077*	1.1*	1.5*	1.4*	1.9*	2.1*	9.1**
Fe×B×Mn	27	0.056**	0.045**	0.0045**	0.024**	1.2*	1.7*	1.6*	1.1*	1.9*	3.2*
Cu×B×Mn×Fe	81	0.00006*	0.002*	0.00007*	0.000065*	0.9*	1.1*	1.6*	3.9**	2.2*	2.4*
T(Cu,B,Mn,Fe)×Y	255	0.000009*	0.0002*	0.000019*	0.0000093*	0.16*	0.17*	0.55**	0.23*	0.33*	0.45*
E	1020	0.00004	0.0009	0.000054	0.00004	0.67	0.53	0.6	0.5	0.6	1.1
CV		1.2	1.1	3.3	2.4	6.8	7.7	8.3	12.2	14.4	8.7

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Tabei 2: continue

Source of variation	Degree of Freedom	Mean of Squares								
		Flower fresh weight	Shoot Fresh weight	Shoot dry weight	Root Fresh weight	Root dry weight	Stem length (2)	Stem length (1)	Root length	Stem diameter
Year(Y)	1	6.7	5.2	7.6	1.2	2.2	100.1	101.4	91.1	0.05
R/Y	4	6.6	3.3	5.5	2.3	3.3	120.5	133.2	122.5	1.6
Copper (Cu)	3	2.4^{ns}	4.1*	5.5^{ns}	1.5^{ns}	4.2*	200.1*	199.4*	181.1*	2.2*
Manganese (Mn)	3	2.5^{ns}	3.9*	4.1*	1.4^{ns}	2.4^{ns}	102^{ns}	110^{ns}	108.3.1	1.2
Iron (Fe)	3	2.6^{ns}	2.5^{ns}	4.7*	1.5^{ns}	2.7^{ns}	104^{ns}	99.3^{ns}	92.2	1.3
Boron (B)	3	2.4^{ns}	2.7^{ns}	5.4^{ns}	1.3^{ns}	2.5^{ns}	111^{ns}	121^{ns}	111.4	1.4
Cu×Mn	9	2.7*	3.4*	8.6**	1.8*	3.3*	133.2*	123.9*	112.6*	4.2
Cu×Fe	9	6.6**	8.8**	4.1^{ns}	3.4**	3.7*	121.1*	124.8*	122.2*	7.7**
Cu×B	9	2.8*	8.6**	4.2^{ns}	1.9*	7.5**	332.1**	321.8**	299.2**	10.8**
Mn×Fe	9	7.8**	8.9**	11.1**	3.3**	7.3**	280.3**	291.5**	289.1**	2.9*
Mn×B	9	2.7*	10.3**	10.5	4.4**	3.4*	123.2*	132.6*	137.2*	10.5**
Fe×B	9	9.9**	3.5*	11.5**	2.05*	3.3*	140.4*	154.7*	157.9*	11.9**
Cu×Mn×Fe	27	2.1*	1.87*	3.2*	7.6**	1.9*	78.4*	86.1*	99.4*	4.4
Cu×Mn×B	27	10.6**	14.4**	15.5**	1.5*	2.1*	111.2*	122.2*	132.2*	11.6**
Cu×Fe×B	27	2.2*	14.1**	12.5**	7.4**	9.8**	445.2**	443.3**	451.2**	4.1*
Fe×B×Mn	27	15.4**	1.8*	3.3*	1.7*	1.9*	121.6*	124.3*	132.3*	2.9*
Cu×B×Mn×Fe	81	1.7*	1.5*	2.5*	1.1*	1.6*	87.1*	99.9*	101.1*	2.5*
T(Cu,B,Mn,Fe)×Y	255	0.3*	0.3*	0.5*	0.19*	0.28*	15.7*	14.4*	15.1*	0.22*
E	1020	1.34	1.2	2.1	0.85	1.2	44.1	42.4	39.2	0.6
CV		9.7	12.4	14.2	8.9	9.2	15.7	16.4	14.4	14.4

Table 3: Means of Characters measured in Calendula plants that are affected by micronutrients (400ppm) concentration (1st year).

Treatments	Shoot dry matter	Shoot fresh matter	Root dry matter	Root fresh matter	Number of flower	Weight of flower	Essence percentage	Poly 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: Means of Characters measured in Calendula plants that are affected by micronutrients (400ppm) concentration (2nd year).

Treatments	Shoot dry matter	Shoot fresh matter	Root dry matter	Root fresh matter	Number of flower	Weight of flower	Essence percentage	Poly 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 5: Results of correlations between characters in Calendula plants that are affected by Fe, Cu, Mn and B micronutrients in 1st year.

Characters	Flower fresh weight	Flower dry weight	Root length	Leaf length	Number of flowers (1)	Number of flowers (2)	Number of flowers (3)	Poly phenols	Carotenoids	Flavonoid
Flower fresh weight	1									
Flower dry weight	0.9**	1								
Root length	0.89**	0.91**	1							
Leaf length	0.85**	0.9**	0.34	1						
Number of flowers (1)	0.76**	0.87**	0.9**	0.69**	1					
Number of flowers (2)	0.73**	0.78**	0.9**	0.59**	0.9**	1				
Number of flowers (3)	0.95**	0.96**	0.9**	0.54**	0.77**	0.79**	1			
Poly phenols	0.94**	0.95**	0.44	0.42	0.8**	0.7**	0.68**	1		
Carotenoids	0.82**	0.94**	0.42	0.37	0.8**	0.68**	0.78**	0.5	1	
Flavonoid	0.92**	0.94**	0.37	0.36	0.6**	0.8**	0.76**	0.46	0.5	1
Essence percentage	0.87**	0.92**	0.6**	0.55**	0.65**	0.56**	0.6**	0.69**	0.69**	0.65**
Stem diameter	0.82**	0.87**	0.55**	0.34	0.9**	0.65**	0.69**	0.49	0.46	0.4
Leaf width	0.65**	0.7**	0.34	0.37	0.94**	0.89**	0.9**	0.5	0.39	0.46
Stem length (1)	0.56**	0.6**	0.7**	0.47	0.9**	0.9**	0.69**	0.6**	0.45	0.39
Stem length (2)	0.55**	0.6**	0.7**	0.47	0.87**	0.79**	0.8**	0.6**	0.46	0.45
Root Fresh weight	0.74**	0.8**	0.7**	0.46	0.67**	0.7**	0.6**	0.53*	0.46	0.46
Root dry weight	0.62**	0.74**	0.6**	0.55**	0.6**	0.7**	0.66**	0.6**	0.5	0.46
Leaf dry weight	0.7**	0.75**	0.55**	0.54**	0.6**	0.66**	0.66**	0.6**	0.46	0.5
Leaf Fresh weight	0.6**	0.72**	0.54**	0.55**	0.68**	0.76**	0.6**	0.46	0.36	0.46

Table 5: Continue

Characters	Essence percentage	Stem diameter	Leaf width	Stem length (1)	Stem length (2)	Root Fresh weight	Root dry weight	Leaf dry weight	Leaf Fresh weight
Flower fresh weight									
Flower dry weight									
Root length									
Leaf length									
Number of flowers (1)									
Number of flowers (2)									
Number of flowers (3)									
Poly phenols									
Carotenoids									
Flavonoid									
Essence percentage	1								
Stem diameter	0.59**	1							
Leaf width	0.6**	0.37	1						
Stem length (1)	0.74**	0.56**	0.5	1					
Stem length (2)	0.72**	0.5	0.24	0.45	1				
Root Fresh weight	0.7**	0.34	0.3	0.34	0.3	1			
Root dry weight	0.67**	0.37	0.4	0.33	0.33	0.46	1		

Leaf dry weight	0.6**	0.47	0.3	0.24	0.4	0.39	0.6**	1	
Leaf Fresh weight	0.6**	0.37	0.4	0.14	0.3	0.45	0.6**	0.7**	1

Table 6: Results of correlation between characters in Calendula plants that are affected by Fe, Cu, Mn and B micronutrients (2nd year).

Characters	Flower fresh weight	Flower dry weight	Root length	Leaf length	Number of flowers (1)	Number of flowers (2)	Number of flowers (3)	Poly phenols	Carotenoids
Flower fresh weight	1								
Flower dry weight	0.91**	1							
Root length	0.99**	0.87**	1						
Leaf length	0.8**	0.82**	0.34	1					
Number of flowers (1)	0.7**	0.8**	0.89**	0.89**	1				
Number of flowers (2)	0.7**	0.88**	0.69**	0.59**	0.79**	1			
Number of flowers (3)	0.9**	0.86**	0.79**	0.74**	0.82**	0.89**	1		
Poly phenols	0.92**	0.9**	0.34	0.42	0.68**	0.87**	0.88**	1	
Carotenoids	0.8**	0.9**	0.22	0.27	0.78**	0.88**	0.88**	0.5	1
Flavonoid	0.9**	0.9**	0.37	0.26	0.6**	0.78**	0.86**	0.55*	0.5
Essence percentage	0.8**	0.9**	0.76**	0.65**	0.65**	0.76**	0.96**	0.9**	0.59*
Stem diameter	0.77**	0.81**	0.65**	0.3	0.77**	0.75**	0.9**	0.55*	0.51*
Leaf width	0.62**	0.72**	0.24	0.3	0.9**	0.8**	0.9**	0.53*	0.52*
Stem length (1)	0.56**	0.62**	0.57**	0.41	0.79**	0.79**	0.9**	0.76**	0.4
Stem length (2)	0.55**	0.63**	0.87**	0.37	0.8**	0.87**	0.87**	0.76**	0.4
Root Fresh weight	0.7**	0.84**	0.7**	0.46	0.6**	0.87**	0.76**	0.59*	0.4
Root dry weight	0.6**	0.64**	0.76**	0.65**	0.86**	0.77**	0.86**	0.76**	0.55*
Leaf dry weight	0.71**	0.65**	0.65**	0.64**	0.76**	0.86**	0.86**	0.66**	0.26
Leaf Fresh weight	0.62**	0.7**	0.75**	0.65**	0.88**	0.78**	0.76**	0.46	0.17

* and **: significant at the 5% and 1% levels of probability, respectively.

Table 6: Continue

Characters		Flavonoid	Essence percentage	Stem diameter	Leaf width	Stem length (1)	Stem length (2)	Root Fresh weight	Root dry weight	Leaf dry weight	Leaf Fresh weight
Flower fresh weight											
Flower dry weight											
Root length											
Leaf length											
Number of flowers (1)											
Number of flowers (2)											
Number of flowers (3)											
Poly phenols											
Carotenoids											
Flavonoid		1									
Essence percentage		0.6**	1								
Stem diameter		0.34	0.59*	1							
Leaf width		0.4	0.76**	0.3	1						
Stem length (1)		0.59*	0.7**	0.56*	0.4	1					
Stem length (2)		0.4	0.78**	0.55*	0.27	0.4	1				

Root Fresh weight		0.4	0.87^{**}	0.3	0.32	0.3	0.36	1			
Root dry weight		0.4	0.86^{**}	0.3	0.45	0.22	0.3	0.22	1		
Leaf dry weight		0.55^{*}	0.76^{**}	0.4	0.3	0.2	0.43	0.31	0.76^{**}	1	
Leaf Fresh weight		0.46	0.86^{**}	0.44	0.22	0.18	0.36	0.4	0.86^{**}	0.81^{**}	1

Discussion:

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 *Calendula* (Naguib *et al.*,2005). The beneficial application of micronutreints was reported by other researchers (Asad and Rafique, 2000; Hussain *et al.*, 2005; Ziaeian and Malakoti, 1998; Thalooth *et al*., 2006). Results of this research showed that foliar application of micronutrient made the more essential oil in flower of *Calendula officinalis.* 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). 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). 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 *Calendula* species plants. It seems that Fe, Cu, Mn and B by the effect on absorbtion 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 micronutriens 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 B at 400 ppm resulted in highest improvement of growth character, yield and chemical constituents. Decreasing effect of $Fe_4Cu_4Mn_4B_4$ made the equivalent amount of control plants.

Conclusion:

Plants treated with 400 ppm of Iron, copper, Manganese and Boron, had the more fresh and dry weight of flowers and essence percentage. Therefore, for maximum yield and quality of marigold, foliar application of micronutrients by 400 ppm concentration is recommended. It could be concluded from the results that Iron, copper, Manganese and Boron fertilization had significant effect on dry and fresh weight of flower, dry and fresh flower matter of flower and the amount of essence percentage as well as the chemical component of the essence (carotenoids, flavonoids, and polyphenols) of marigold. The highest number of flower per plant (33.5) and weight of dry flower per plant (11.9 g) and essence percentage (1%) was obtained from the plants were received 400 ppm of Iron, copper, Manganese and Boron. Essence percentage significantly affected by micronutrients fertilizer. The highest polyphenols (56.2) was measured from the flowers was received 400 ppm of Iron, copper, Manganese and Boron. The most suitable Fe-B-Mn-Cu supply for production of marigold 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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