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Effects of soil and foliar applications of iron and zinc on flowering and essential oil of chamomile at greenhouse conditions

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ABSTRACT

In order to study the effects of soil and foliar applications of iron (Fe) and zinc (Zn) on flowering, flower yield and essential oil production of German chamomile a pot experiment was conducted under greenhouse conditions at the Faculty of Agriculture, University of Tabriz, Iran in 2012. The experiment was arranged as completely randomized design with 12 treatments and three replications. Treatments were as follow: T₁: control – without Fe or Zn fertilizers, T₂: 30 mg FeSO₄.7H₂O kg⁻¹ dry soil, T₃: 22 mg ZnSO₄.7H₂O kg⁻¹ dry soil, T₄: 30 mg FeSO₄.7H₂O + 22 mg ZnSO₄.7H₂O kg⁻¹ dry soil, T₅: foliar spraying of FeSO₄.7H₂O (3.5 g L⁻¹), T₆: foliar spraying of FeSO₄.7H₂O (7.0 g L⁻¹), T₇: foliar spraying of ZnSO₄.7H₂O (2.5 g L⁻¹), T₈: foliar spraying of ZnSO₄.7H₂O (5.0 g L⁻¹), T₉: T₅+T₇, T₁₀: T₅+T₈, T₁₁: T₆+T₇, T₁₂: T₆+T₈. The foliar spraying was done two times during the growing period. The results revealed that the flower number, flower yield, essential oil content and essential oil yield were significantly increased by soil and foliar applications of Fe + Zn, compared with the control (untreated). The highest flower number (477 plant⁻¹), flower yield (11.6 g pot⁻¹), essential oil content (0.88 %) and essential oil yield (119 mg pot⁻¹) were recorded for the soil application of Fe + Zn (T₄) by 58, 68, 21.4 and 105 % increment compared to the control, respectively. Foliar application of Fe + Zn (T₁₂) was placed at the next rank; however this treatment had no significant difference with the soil application of Fe + Zn (T₄). Other treatments did not show significant differences with the control. Generally, the results showed that soil or foliar application of Fe + Zn can be effective on increase or improve of quantity and quality of chamomile yield. Moreover, use of foliar application as a low cost method especially in areas with alkaline or calcareous soils can be recommended.

Key words: Application methods, Essential oil, Iron, *Matricaria chamomilla*, Zinc

IZVLEČEK

UČINKI TALNEGA IN FOLIARNEGA DODAJANJA ŽELEZA IN CINKA NA CVETENJE IN VSEBNOST ETERIČNIH OLJ PRAVE KAMILICE (*Chamomilla recutita* (L.) Rauschert), GOJENE V RASTLINJAKU

Lončni poskus gojenja prave kamilice (*Chamomilla recutita* (L.) Rauschert) je bil izveden v rastlinjaku z namenom ugotavljanja talnega in foliarnega dodajanja železa (Fe) in cinka (Zn) na njeno cvetenje, pridelok cvetov in produkcijo eteričnih olj na Faculty of Agriculture, University of Tabriz, Iran, leta 2012. Poskus je bil izveden kot popoln naključni poskus z 12 obravnavanji in tremi ponovitvami. Obravnavanja so bila: T₁: kontrola – brez gnojenja s Fe ali Zn, T₂: 30 mg FeSO₄.7H₂O kg⁻¹ suhih tal, T₃: 22 mg ZnSO₄.7H₂O kg⁻¹ suhih tal, T₄: 30 mg FeSO₄.7H₂O + 22 mg ZnSO₄.7H₂O kg⁻¹ suhih tal, T₅: škropljenje listov z FeSO₄.7H₂O (3.5 g L⁻¹), T₆: škropljenje listov z FeSO₄.7H₂O (7.0 g L⁻¹), T₇: škropljenje listov s ZnSO₄.7H₂O (2.5 g L⁻¹), T₈: škropljenje listov z ZnSO₄.7H₂O (5.0 g L⁻¹), T₉: T₅+T₇, T₁₀: T₅+T₈, T₁₁: T₆+T₇, T₁₂: T₆+T₈. Škropljenje listov je bilo opravljeno dvakrat v rastni dobi. Rezultati so pokazali, da je talno in foliarno gnojenje z Fe + Zn značilno povečalo število cvetov, pridelok cvetov, vsebnost in pridelok eteričnih olj v primerjavi s kontrolo. Največje število cvetov (477 na rastlino), največji pridelok cvetov (11.6 g na lonec), največja vsebnost eteričnih olj (0.88 %) in največji pridelok eteričnih olj (119 mg na lonec) so bili izmerjeni pri talnem dodajanju Fe + Zn (T₄), povečanje je bilo za 58, 68, 21.4 in 105 % glede na kontrolno obravnavanje. Učinek foliarnega dodajanja Fe + Zn (T₁₂) je bil takoj za talnim dodajanjem Fe + Zn (T₄), vendar se od njega ni značilno razlikoval. Druga obravnavanja niso dala značilnih odstopanj od kontrole. V splošnem so rezultati pokazali, da lahko tako talno kot foliarno dodajanje Fe + Zn učinkovito poveča ali izboljša količino in kvaliteto pridelka prave kamilice. Uporabo foliarnega dodajanja bi kot poceni način gnojenja še posebej priporočali na območjih, kjer so tla bazična ali apnenčasta.

Ključne besede: metode gnojenja, železo, cink, *Matricaria chamomilla*, eterična olja

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1 INTRODUCTION

Today medicinal plants are one of the resources of drugs for treatment of many diseases. *Matricaria chamomilla* is an annual plant belonging to the Asteraceae family. It is widely used and well-documented medicinal plants in the world. It is included in the pharmacopoeia of 26 countries (Hendaway and Khalid, 2011). Chamomile has many pharmacological properties. It is a traditional treatment for numerous disorders, including sleep disorders, digestion/intestinal conditions, skin infections/inflammation (including eczema), wound healing, infantile colic, teething pains, and diaper rash. It has been also reported that chamomile has moderate antioxidant and antimicrobial activities (Simpson, 2001; McKay and Blumberg, 2006).

In order to obtain high quality and yield of crop, nutrients must be sufficient in growing environment of plant. Micronutrients as iron (Fe) and zinc (Zn) are the trace elements that play essential role in plant growth and increasing crop yields. Moreover, they improve plant nutrition and increase soil productivity (Marschner, 1995). Many crops respond to foliar and soil applications of micronutrients in terms of growth and crop yields. It is widely reported that foliar application of micronutrients at active growth stages will improve plant growth and consequently yield and quality in various crops (Kalidasu et al., 2008).

Iron is a cofactor for a large number of enzymes that catalyze several biochemical processes within the plant (Brittenham, 1994; Marschner, 1995). It plays a vital role in the chlorophyll formation, thylakoid synthesis and chloroplast development and also functions in the respiratory enzymes. Moreover, iron serves in the transportation of energy in the plant (Miller et al., 1995).

Zinc is known to have an important role either as a metal component of enzymes or as a functional, structural or regulatory cofactor of many enzymes. Zinc also has many essential roles in the plant growth and development including production of biomass, chlorophyll production, pollen function, fertilization, metabolism of RNA, proteins and the DNA formation (Marschner, 1995; Pandey et al., 2006; Cakmak, 2008). It is also, required for the synthesis of tryptophan, a precursor of IAA

(Indole-3-Acetic Acid) which acts as a growth promoting substance (Marschner, 1995; Miller et al., 1995).

Generally plants obtain their nutrients requirements from the soil, but they are capable to absorb nutrients through the leaves. Foliar plant nutrition is one of the techniques that farmers use for plant nutrition since 1950s, when they were learned that foliar fertilization was effective and economic (Ebrahimian et al., 2010). Foliar fertilization is extensively used as a practice to accurate the nutritional deficiencies in plants caused by inappropriate deliver of nutrients to roots (Silberbush and Ling, 2002). The most important use of foliar sprays has been in the application of micronutrients (Havlin et al., 2004).

Micronutrients are added to foliar fertilizers, in order to compensate their deficiencies especially in arid and semi-arid regions with calcareous soils (Nasiri et al., 2010). Many recent researches have shown that a small amount of nutrients as Zn, Fe and Mn, applied by foliar spraying increases significantly the yield of crops (Said-Al Ahl and Mahmoud, 2009 & 2010; Nasiri et al., 2010; Zehtab-Salmasi et al., 2008 & 2012; Saedh et al., 2009). Nasiri et al. (2010) reported that flower yield, essential oil percentage and essential oil yield of chamomile were increased by foliar application of Fe and Zn compared with the control at farm conditions. Also Said-Al Ahl and Mahmoud (2010) reported that the highest plant height, branches per plant, fresh and dry biomass and essential oil yield of basil plants were obtained by foliar application of Zn and/or Fe in normal soil. The highest seed yield, oil yield, oil percentage, thousand seed weight and protein percentage of sunflower were obtained from the soil and foliar applications of Fe + Zn (Ebrahimian et al., 2010). Foliar spraying of Zn (100 mg L^{-1}) in blue sage enhanced the length of peduncle and main inflorescence, number of inflorescence and florets, and fresh and dry weight of inflorescences/plant (Abd El-Aziz and Balbaa, 2007). Application of micronutrients increased fresh and dry mater, leaf area of plant, bush and leaf essential oil percentage and essential oil yield of peppermint (Zehtab-salmasi et al., 2008).

Although the importance of micronutrients (such as Fe and Zn) on the growth and production of herbs in many research presented; however, there is little information about effectiveness of application methods of Zn and Fe on the growth and development of chamomile. In our previous research we investigated the effects of only foliar

application of Fe and Zn on German chamomile at field conditions (Nasiri et al., 2010). Therefore, the purpose of present investigation was to study the effects of Fe and Zn application methods (Foliar spraying and Soil application) and different concentrations of them on flowering, yield and essential oil content of German chamomile.

2 MATERIALS AND METHODS

Chamomile plants (*Matricaria chamomilla*) were grown in a sandy loam alkaline soil in the greenhouse of the Faculty of Agriculture, University of Tabriz, Iran in 2012. The seeds obtained from Hungary were sown in plastic pots (30 cm diameter) filled with 6 kg of dry soil which according to Table 1 was deficient in Fe and Zn (Hazelton and Murphy, 2007). Physicochemical

characteristics of the soil used in the study were measured by methods of Gee and Bauder (1986) and Sparks et al. (1996). Each pot was supplied with 450 mg NH_4NO_3 , 44 mg $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, 150 mg K_2SO_4 , 8 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 85 mg $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and 20 mg $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ per kg of dry soil according to the soil testing.

Table 1. Physicochemical characteristics of the soil used as potting media.

pH	7.81
EC_e (dS m^{-1})	0.71
Organic carbon content (%)	0.11
Calcium carbonate equivalent (%)	Negligible
Sand (%)	70
Silt (%)	18
Clay (%)	12
Texture	Sandy loam
Total N (%)	0.08
Available-P (mg kg^{-1})	5.7
Available-K (mg kg^{-1})	250
Available-Mg (mg kg^{-1})	99.1
Available-Ca (mg kg^{-1})	1149
Available-Fe (mg kg^{-1})	1.8
Available-Mn (mg kg^{-1})	1.1
Available-Zn (mg kg^{-1})	0.42
Available-Cu (mg kg^{-1})	1.3

EC_e = Electrical conductivity of saturated soil paste extract

Before seed planting, the above mentioned nutrients were dissolved in enough water and then were mixed with the pot soil. After the emergence of the seedlings four plants were kept per pot.

Treatments in the experiment were as follow: T₁: control, T₂: 30 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ kg^{-1} dry soil, T₃: 22 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ kg^{-1} dry soil, T₄: 30 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 22 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ kg^{-1} dry soil, T₅: foliar spraying of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (3.5 g L^{-1}), T₆: foliar spraying of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (7.0 g L^{-1}), T₇: foliar spraying of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (2.5 g L^{-1}), T₈:

foliar spraying of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (5.0 g L^{-1}), T₉: T₅+T₇, T₁₀: T₅+T₈, T₁₁: T₆+T₇, T₁₂: T₆+T₈. These 12 treatments were arranged in a completely randomized design (CRD) with three replicates. The volume of the spraying solution was maintained just to cover completely the plant foliage till drip. The plants were sprayed twice at stem elongation and flowering stages.

The plant flowers were harvested eight times in 4-5 days intervals. After each harvest flowers were

dried in a shady place and were kept in a convenient location for essential oil extraction. The data recorded were: number of flowers in plant, dry weights of flowers in each pot as flower yield, essential oil percentage, and essential oil yield. Five grams dry flowers were hydro-distilled in a modified Clevenger apparatus in 1000 mL round bottomed flask with 500 mL distilled water for 4 h (Hoelz and Demuth, 1975; Letchamo, 1993).

Essential oil yield was determined by multiplying essential oil percentage \times average of dry weights of flowers per pot.

The results were statistically analyzed using MSTATC software. The graphs were plotted using Excel software and the Duncan's Multiple Range Test at 5 % level was used to compare the means of treatments.

3 RESULTS AND DISCUSSION

3.1 Flower number

The results in Table 2 show that flower number was significantly ($p < 0.05$) affected by different micronutrient fertilizer treatments. Means comparison indicated that the highest number of flowers (average 462 per plant) was noticed with the soil application of Fe + Zn (30 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 22 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ kg^{-1} dry soil) and foliar

spraying of Fe + Zn (7.0 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ L^{-1} and 5.0 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ L^{-1}). These treatments increased flower number by 58 and 48 % compared with the control, respectively. Although soil application of Fe and Zn increased flower number 6 % compared to the foliar spraying, but there was no significant difference between these two methods of fertilizer application.

Table 2: Effects of iron and zinc on flower, flower yield and essential oil of chamomile

Treatments	Flower number/plant*	Flower yield (g/pot)**	Essential oil content (%)**	Essential oil yield (mg/pot)***
T ₁ : Control– without Fe or Zn fertilizer	301 \pm 9.81 ^c	6.9 \pm 0.28 ^c	0.84 \pm 0.024 ^b	58 \pm 2.43 ^c
T ₂ : Fe (30 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ kg^{-1} dry soil)	349 \pm 42.2 ^{bc}	8.19 \pm 1.25 ^c	0.87 \pm 0.034 ^b	72 \pm 13.51 ^{bc}
T ₃ : Zn (22 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ kg^{-1} dry soil)	354 \pm 14.16 ^{bc}	8.14 \pm 0.52 ^c	0.88 \pm 0.031 ^b	71 \pm 6.2 ^a
T ₄ : Fe + Zn (T ₂ + T ₃)	477 \pm 38.4 ^a	11.6 \pm 0.56 ^a	1.02 \pm 0.026 ^a	119 \pm 8.5 ^{bc}
T ₅ : Fe foliar spraying (3.5 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ L^{-1})	371 \pm 20.42 ^{abc}	8.17 \pm 0.44 ^c	0.86 \pm 0.029 ^b	70 \pm 4.02 ^{bc}
T ₆ : Fe foliar spraying (7.0 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ L^{-1})	382 \pm 23.58 ^{abc}	8.44 \pm 0.54 ^{bc}	0.85 \pm 0.027 ^b	72 \pm 6.61 ^{bc}
T ₇ : Zn foliar spraying (2.5 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ L^{-1})	329 \pm 25.35 ^c	7.87 \pm 0.45 ^c	0.86 \pm 0.033 ^b	68 \pm 5.95 ^{bc}
T ₈ : Zn foliar spraying (5.0 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ L^{-1})	339 \pm 6.62 ^{bc}	7.3 \pm 0.13 ^c	0.85 \pm 0.025 ^b	62 \pm 2.74 ^{bc}
T ₉ : T ₅ + T ₇	363 \pm 14.61 ^{bc}	8.32 \pm 0.20 ^{bc}	0.92 \pm 0.026 ^{ab}	76 \pm 0.41 ^{bc}
T ₁₀ : T ₅ + T ₈	385 \pm 32.06 ^{abc}	8.01 \pm 0.40 ^c	0.93 \pm 0.024 ^{ab}	74 \pm 4.04 ^{bc}
T ₁₁ : T ₆ + T ₇	333 \pm 42.89 ^c	8.46 \pm 0.85 ^{bc}	0.93 \pm 0.026 ^{ab}	78 \pm 6.1 ^b
T ₁₂ : T ₆ + T ₈	447 \pm 28.91 ^{ab}	10.53 \pm 0.47 ^{ab}	1.01 \pm 0.019 ^a	106 \pm 3.86 ^a
F Test	*	**	**	***

Value represents mean \pm standard error of three replicates.

F Test: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Means followed by the same letter in each column are not significantly different according to Duncan's Multiple Range Test at 5 % level.

3.2 Flower yield

Data presented in Table 2 show that soil application of 30 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 22 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ per kg of dry soil) or foliar application of 7.0 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 5.0 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ L^{-1} significantly ($p < 0.01$) increased flower yield in pot. The increments on flower yield were by 68.1 and 52.6 % respectively for the Fe + Zn soil application (T₄) and Fe + Zn foliar

application (T₁₂) compared to the control. The lowest dry flower yield (6.9 g pot^{-1}) was recorded in control treatment. Increment of flower yield might be due to the increased number of flowers per plant as a result of positive effects of iron and zinc application that mentioned in the previous section. Increment of flowers number is directly responsible for higher flower yield in chamomile.

3.3 Essential oil content

The response of essential oil (EO) content (%) of chamomile to soil or foliar application with Fe and Zn is available in Table 2. EO % was significantly ($p < 0.01$) increased as a result of soil and foliar applications of Fe + Zn (T_4 and T_{12} treatments). The increments were 21.4 and 20.2 %, respectively

compared to the control plant. Although other treatments also increased this parameter compared to the control in chamomile plants, but these increments were not significant. Chamomile essential oil changes affected by the different treatments of this study are shown in Figure 1.

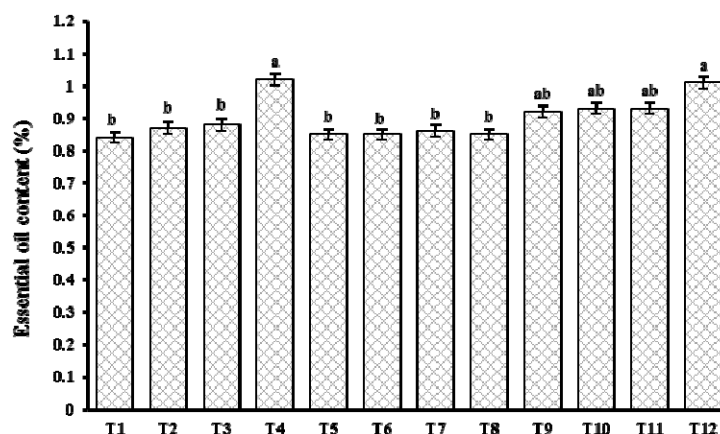


Figure 1: Mean comparison of essential oil content of chamomile in different treatments of iron and zinc application. The same letters in columns indicate no significant difference according to Duncan's Multiple Range Test at 5 % level. Error bars represent standard errors ($n=3$).

T₁: Control, T₂: 30 mg FeSO₄.7H₂O kg⁻¹ dry soil, T₃: 22 mg ZnSO₄.7H₂O kg⁻¹ dry soil, T₄: 30 mg FeSO₄.7H₂O + 22 mg ZnSO₄.7H₂O kg⁻¹ dry soil, T₅: foliar spraying of FeSO₄.7H₂O (3.5 g L⁻¹), T₆: foliar spraying of FeSO₄.7H₂O (7.0 g L⁻¹), T₇: foliar spraying of ZnSO₄.7H₂O (2.5 g L⁻¹), T₈: foliar spraying of ZnSO₄.7H₂O (5.0 g L⁻¹), T₉: T₅+T₇, T₁₀: T₅+T₈, T₁₁: T₆+T₇ and T₁₂: T₆+T₈.

3.4 Essential oil yield

The obtained results in Table 2 show significant differences ($p < 0.001$) were manifested in the plant essential oil yield (EOY) of chamomile due to Fe and Zn application treatments. The highest values of this parameter were obtained from the Fe + Zn soil application (T_4) (119 mg pot⁻¹), Fe + Zn

foliar application (T_{12}) (106 mg pot⁻¹), and T_{11} (78 mg pot⁻¹) that were 105, 85 and 34.4 % greater than the control (58 mg pot⁻¹), respectively. Although EO yield was increased by other treatments but these increments were not significant compared to the control (Figure 2).

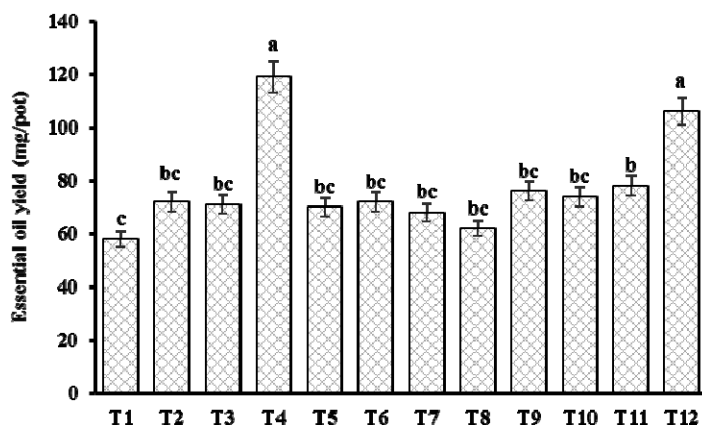


Figure 2: Mean comparison of essential oil yield of chamomile in different treatments of iron and zinc application. The same letters in columns indicate no significant difference according to Duncan's Multiple Range Test at 5 % level. Error bars represent standard errors (n=3).

T₁: Control, T₂: 30 mg FeSO₄.7H₂O kg⁻¹ dry soil, T₃: 22 mg ZnSO₄.7H₂O kg⁻¹ dry soil, T₄: 30 mg FeSO₄.7H₂O + 22 mg ZnSO₄.7H₂O kg⁻¹ dry soil, T₅: foliar spraying of FeSO₄.7H₂O (3.5 g L⁻¹), T₆: foliar spraying of FeSO₄.7H₂O (7.0 g L⁻¹), T₇: foliar spraying of ZnSO₄.7H₂O (2.5 g L⁻¹), T₈: foliar spraying of ZnSO₄.7H₂O (5.0 g L⁻¹), T₉: T₅+T₇, T₁₀: T₅+T₈, T₁₁: T₆+T₇ and T₁₂: T₆+T₈.

The results of this experiment show that flower number and flower yield affected by soil or foliar application of Fe and Zn treatments. These results were in consonance with the findings of Abd El-Aziz and Balbaa (2007) on blue sage, Kaldiasu et al. (2008) on coriander and Ravi et al. (2008) on safflower. They reported the beneficial effects of iron and zinc on flower production of different plants. This beneficial effect of Zn and Fe can be attributed to the role of Zn in the synthesis of IAA, photosynthesis and nitrogen metabolism and the role of iron in the chlorophyll synthesis and nitrogen fixation (Marschner, 1995; Miller et al, 1995).

On the other hand, increment of flower yield might be due to the increased number of flowers per plant as a result of positive effects of iron and zinc application that mentioned in the previous section. Increment of flowers number is directly responsible for higher flower yield in chamomile. These results are in agreement with those obtained by Grejtovský et al. (2006) and Nasiri et al. (2010) on chamomile, Said-Al Ahl and Omer (2009) on coriander and Said-Al Ahl and Mahmoud (2010) on basil. They stated that soil or foliar application of iron and zinc led to the increment of flowering parameters and plant yield.

In the case of essential oil percent and essential oil yield of chamomile plant, results showed significantly addition in their amount with application of Fe and Zn. The maximum amount of these parameters was observed in Fe + Zn soil and foliar application treatments, respectively.

The increase in essential oil due to zinc and /or iron was also reported in Japanese mint (Misra and Sharma, 1991), cumin (El-Sawi and Mohamed, 2002), peppermint (Akhtar et al., 2009) and sweet basil (Said-Al Ahl and Mahmoud, 2010) and chamomile (Nasiri et al., 2010).

Previous studies indicated that biosynthesis of secondary metabolites is not only controlled genetically but also affected intensely by ecological effects (Naghdi-Badi et al., 2004; Said-Al Ahl and Mahmoud, 2010). Plant nutrition as an environmental variable affects essential oil of medicinal plants. CO₂ and glucose are precursors of monoterpene biosynthesis. Carbohydrates are a resource of energy and reducing power for terpenoid synthesis. CO₂ fixation, content of primary metabolites and sucrose metabolism are closely linked with essential oil accumulation (Srivastava et al., 1997). As zinc is involved in photosynthesis and carbohydrate metabolism and CO₂ and glucose are the most likely sources of carbon utilized in terpenoid biosynthesis, the role

of Zn in influencing of essential oil accumulation seems particularly important. Moreover, iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration and the glycolate pathway (Marschner, 1995).

Increase of EOY previously reported by Nasiri et al. (2010) in chamomile at field conditions and Said-Al Ahl and Mahmoud (2010) in sweet basil. They found that combined application of Fe + Zn gave the highest values of essential oil yield under normal soil conditions. This increment seems may be due to the raise of flower yield and essential oil percentage as a result of positive effects of Fe and Zn application. Since the EOY is directly associated with the flower yield and EO %, so any increase in these two traits led to the increase of essential oil yield.

Micronutrients such as Fe, Cu, Mn and Zn are essential for growth and development of the living plants. As they are found in the most redox reactions and are fundamental for cellular processes and in proteins and enzymes for structural and catalytic enzyme activities (Hall and Williams, 2003). These nutrients are known to be required for all higher plants and shortage of them in culture media causes deficiency symptoms and reducing plant growth (Marschner, 1995).

Fe and Zn act as metal components of various enzymes and also are associated with saccharide metabolism, photosynthesis, and protein synthesis. Iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration, the glycolate pathway and chlorophyll content. Zinc is an essential micronutrient for synthesis of IAA, cell division and the maintenance of membrane structure and function. Zn deficiency reduces plant growth, pollen viability, flowering, number of fruits and seed production (Sharma *et al.*, 1990; Marschner, 1995). Therefore, sufficient amount of these nutrients in the plant is necessary for normal growth and obtain a satisfactory product.

Many studies have reported that micronutrients such as Fe, Mn and Zn have important roles in plant growth and yield of aromatic and medicinal plants (Abd El- Wahab, 2008). Since, the soil application of micronutrients fertilizers in the cultivation may not meet the crop requirement for growth and nutrient use, thus the alternative effective approach is to apply these micronutrients as a foliar application (Saedh et al., 2009).

The positive influence of application of micronutrients on crop growth may be due to the improved ability of the crop to absorb nutrients, photosynthesis and better sink-source relationship as these play vital role in various biochemical processes (Kalidasu, et al, 2008).

Fe and Zn are absorbed by plant root and shoot as Fe^{2+} , Fe^{3+} and Zn^{2+} , respectively. The mobility and remobilization of these micronutrients in plants are low. The Fe and Zn concentrations in the soil solution are very low. The availability and solubility of Fe and Zn in soils were dependent on pH, organic matter content, texture, redox potential, moisture content, calcium carbonate equivalent percent, interactions with other elements, climate conditions and plant factors. The availability and solubility of Fe and Zn decrease with increased soil pH. So, the Fe and Zn deficiencies in plants can be observed in alkaline calcareous soils. At alkaline pH, Fe and Zn fertilizers used in soils precipitate as insoluble $ZnCO_3$, $FeCO_3$, $ZnFe_2O_4$ and $ZnSiO_4$. Fe and Zn adsorption on the surface of $CaCO_3$, clay minerals and Al/Fe oxides could also reduce the availability and solubility of these nutrients. As a result the effectiveness of these fertilizers is low when applied to soils (Marschner, 1995; Towfighi and Najafi, 2001; Havlin et al., 2004). So, when problems of soil fixation of these nutrients exist, foliar spraying constitutes an effective means of fertilizer application. Foliar fertilization needs lower amounts of fertilizers and provides for more rapid utilization of nutrients and permits the correction of observed deficiencies in less time than would be required by soil treatments (Havlin et al., 2004).

4 CONCLUSIONS

In this study it was found that Fe and Zn had beneficial effect on yield and essential oil production of chamomile plant. The obtained results also showed that the application of these two elements in combination had more positive and significantly effects on yield and essential oil of chamomile compared to the their individual applications (Tab. 2). Although there was no significant effect between two methods of fertilizer application in any of the studied parameters, however, soil application of iron and zinc was

slightly more effective than use of them by foliar application, but this difference was not significant. With this interpretation, since the foliar application is low-costly technique of feeding plants by applying liquid fertilizer directly to their leaves (Baloch et al., 2008; Yassen et al., 2010), so the use of this method to compensate of micronutrients deficiency like iron and zinc and to improve of chamomile performance especially in arid and semi-arid regions with calcareous soils would be justified.

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