*J. Appl. Environ. Biol. Sci.*, 4(12S)170-176, 2015



ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
*www.textroad.com*

# Effect of Foliar Application of Methanol and Chelated Zinc on the Quantities and Qualities Yield of Marigold (*Calendula officinalis* L.)

**Shahram Yazdi Far[1], Pejman Moradi[1], Mojtaba Yousefi Rad[2]**

[1]Department of Horticulture science, college of Agriculture, Saveh Branch, Islamic Azad University, Saveh, Iran
[2]Department of Agronomy, college of Agriculture, Saveh Branch, Islamic Azad University, Saveh, Iran

*Received: November 21, 2014*
*Accepted: January 5, 2015*

## ABSTRACT

For investigation of the effect of foliar application of methanol and chelated zinc on quantities and qualities of marigold medicinal plant factorial experiment based on randomized complete block design with three replicate was conducted. The first factor was foliar application of methanol at 0 (control), 10, 20, 30 and 40% concentrations and second factor was foliar application of chelated zinc at 0 (control), 0.5 and 1 gr per thousand. The results of variance analysis showed that methanol has significant effect ($P<0.01$) on measured indices except arytenoids. Also, chelated zinc has significant effect ($P<0.01$) on the number of capitol, flavonoids, essential oil content and gaiol percentage and has significant effect at $P<0.01$ on plant height and carotenoids. Also, the interaction of methanol with chelated zinc was not significant effect on measured indices. According to the mean comparison foliar application of methanol and chelated zinc increased measured indices, so that 30% methanol and 0.5 gr per thousand has most effect on measured indices.

**KEYWORDS**: Chelated zinc, Chlorophyll, Foliar application, Marigold, Methanol.

## INTRODUCTION

The harmful effects of chemical drugs on healthy cause to more attention of human to uses of medicinal plant and extract ingredients from these plants for treated of many diseases. Iranian plateau have been introduced as an origins of many medicinal plants and according to the needs of pharmaceutical, food and cosmetic industries to medicinal plants as a raw materials, necessity of cultivation of medicinal plant species in the country is quite clear, because dependency to natural products and the indiscriminate exploitation of them will be extinction of this species (Omidbaigy, 2001). A marigold (Calendula officinalis L.) is annual and perennial plant and belongs to the Asteraceae family. The origin of this plant is Mediterranean region. Marigold flowers are containing a small amount of essential oils, volatile oils, saponin, resins, a bitter substance, organic acid, calendoline, enamel material, albumin, salicylic acid, lauric acid and cholesterol and its roots are containing inulin (Samsam Shariat, 2004). Flowers of this plant containing compounds used in industry (production of color and nylon) and pharmacy (production of creams and lotions) (Kalvatchev et al., 1997). Recently, in Europe much attention for this plant as an oil plant. Marigolds seed has 15-20% oil that is containing 45-60% calendic acid (Adas, 2002). If this oil extracted by pressure and cold, it has anti-inflation effect (Bemath, 2000). Some research results have shown that organic extract of marigold flower have anti-AIDS virus (HIV) activity (Kalvatchev et al., 1997).

One of the important strategies for increasing of carbon dioxide concentration in plants is using compounds such as methanol, ethanol, propanol and butanol (Nonomura and Benson, 1992). Among these compounds methanol is well-known for plants, because this substance is one of the simplest herbal products and produce during leaf growth and demethylation of the cell walls pectins (Mudgett and Clarke, 1993; Fall and Benson, 1996; Haston and Raj, 2001). After the production of this volatile organic substance in plants, some of it go out from leaves and enters to the boundary layer and then enters to the atmosphere and the other parts of it converted to formaldehyde, formic acid and finally converted to $CO_2$. Produced $CO_2$ can affect $CO_2$ stabilization in plants (Galbal and Kristine, 2002). After foliar application of methanol on wheat and oats leaves chlorophyll content was increased (Ramadant and Omran, 2005). Methanol cause to increases of glucose metabolism in leaves and as a result, swelling pressure, velocity stabilization and growth in treated plant increases (Rajala et al., 1998). It is known that methanol increased stomatal conductance, leaf area index, leaf durability and decreased leaf temperature and transpiration (Makhdum et al., 2002). Hernandez et al. (2000) reported that foliar application of methanol at sunflower increases stem length, leaf area and shoot dry weight. Ramirez et al. (2006) reported that foliar application of methanol increases tobacco fresh weight. Also, their study showed that methanol cause to delay in leaves senescence, more photosynthetic activity and increase of yield. In the study that was conducted on tomato, observed that foliar application of

*** Corresponding Author:** Shahram Yazdi Far, Department of Horticulture science, college of Agriculture, Saveh Branch, Islamic Azad University, Saveh, Iran. shahram_yazdifar@yahoo.com

methanol increases stem and root weight (Rowe et al., 1994). Vyshghay et al. (2008) found that foliar application of methanol at 20% concentration on peanut shoots increasing grain yield, thousands seed weight, number of mature pods and number of peanut proteins. Zbiec et al. (2003) reports that tomato, beans, sugar beet and canola plants treated by 30% methanol produce 12-13% yield higher than the control, and these plat were less sensitive to water deficit and in some cases their product was same with supplemented irrigated plants.

Medicinal plants are needs to adequate amounts of micronutrients for growth and produce active substance (Leilah et al., 1988). Using of micronutrients in peppermint increases number of essential oil secretion glands and essential oil production (Evans, 1996). Foliar application of micronutrients on peppermint increases plant height, dry matter and essential oil yield (Heidari et al., 2008). Foliar feeding is a method for increasing the efficiency of the uses of chemical fertilizers and the reducing of their environmental risks (Broadley et al., 2007). In young leaves, the nutrient solution absorbs through tiny fibers on the surface of the leaf, stomata and hydrophilic pores in the leaf cuticle (Jiang et al., 2007). Zinc is an essential micronutrients and the existence of which is necessary for metabolic in plants (Hassegawa et al., 2008). The researchers found that with foliar application of zinc on leaf destroyed this problem (Gao et al., 2005). Typically in zinc deficiency conditions, the concentration of this element in plant is less than 20 ppm (Doberman and Fairhurst, 2000). The researcher also reported that use of zinc sulfate increases the plant height, thousand seed weight and seed yield (Zou et al., 2007). In addition, the uses of zinc increases zinc and protein concentration in the seed and shoot and increases quality of yield (Bayvordi, 2006). Several studies showed that application of micronutrients increases quality and quantity of crops yield and some of medicinal plants (Nagaraj, 1987; Mosavi et al., 2007).

The aim of this study was investigation of the effect of chelated zinc and methanol on some quality and quantity of essential oil production in marigold medicinal plant.

## MATERIALS AND METHODS

The study was performed in 1392 at the Agricultural Research Station of Azad University Qom branch in factorial based on randomized complete block design with three replicate. The site at longitude: 50˚, 50′ and 3″ N and latitude: 34˚, 35′ and 33″ E at 956 meters above sea. In this study the effect of foliar application of methanol at five levels (0, 10, 20, 30, and 40%) and chelated zinc at three levels (0, 0.5 and 1 g per thousand) was evaluated. Seeds of marigold with 11.6 g thousand weight obtained from Pkan Bazr Company. For this purpose, a piece of treasury area about 200 $m^2$ was ready for planting and according to the laboratory results twice heavy irrigation was performed and then completely rotted farmyard manure, sand and pabyl (two times disturbing and perpendicular to 0-30 cm depth) were added.

**Table 1. Physicochemical analysis results of soil**

| Soil texture | EC (ds/m) | S.P (%) | pH | O.C (%) | N (%) | P (ppm) | K (ppm) | T.N.V (%) |
|---|---|---|---|---|---|---|---|---|
| **Sandy/Loam** | 4.9 | 33 | 8.7 | 0.53 | 0.057 | 15.8 | 250.4 | 27 |

For planting in pots the amount of soil was added to pots and then the desired soil (two parts) was mixed with rotten farmyard manure and mixed with sand (one part) and then in the 13$^{th}$ of Shahrivar one seed in each pot planted. After planting, irrigation of pots were performed daily in morning and evening and four days after planting seeds were begin to germinate.

By observing a four to six leaves on the seedling, seedlings planted in the field with 15 cm space on the row and 25 cm between the rows and was attempting to irrigation every four days and weeds were removed by hand weeding. One day before treatment leaves were washed by water. In 6$^{th}$, 27$^{th}$ Aban and 11$^{th}$ Azar foliar applications were conducted at noon. To prepare the methanol solution with purity of more than 99.5% (Dr. Mojalali, Iran) and chelated zinc (JH Biotech, USA) twice distilled water was used. Determining the amount of alcohol and distilled water for each treatment was performed with volumetric flask and determining the amount of chelated zinc was performed by 0.0001 accurate digital scales. Then each plot treated so that the plant is completely wet. In the mid-term, the leaf miner pests observed on leaves but according to the expert opinion of Agriculture Plant Protection Organization of Qom spraying did not used. Many aphids were observed during the flowering stage, but after the first picking according to the expert opinion of Agriculture Plant Protection Organization of Qom, all plants sprayed with 1:1000 diazinon and seven days after spraying other flowers picked up. For evaluate the chlorophyll and carotenoids content 0.2 g samples prepared from middle part of the fourth leaf and were completely worn. Then 10 mL acetone (90%) was added and at the end, the absorbance was read at 470, 663 and 645 nm wavelengths by spectrophotometer (Optizen 3220UV) and chlorophyll and carotenoids content calculated by following formula (Arnon, 1967):

V= 10
W= 0.2
Chlorophyll a = [(12/7 ×A663)-(2/69 ×A645)]V/(1000×W)
Chlorophyllb = [(22/9 ×A645)-(4/69 ×A663)]V/(1000×W)
Total Chlorophyll = [(20.2 ×A645)+(8.02 ×A663)]V/(1000×W)
Carotenoids =[(100× A470) – (3.27× cola) –(104× Chlb)]/227

Essential oil percentage was evaluated by Clevenger apparatus. The samples essential oil was extracted by distillation with water for 4 h method. Extracted essential oil was dehumidifier by dry sodium sulfate and then essential oil percentage was calculated. To analysis of essential oil and accurate measurement of its ingredients gas chromatography was used. For this purpose, gas chromatograph (Hewlett-Packard 6890) with Splitless injector and capillary column with 30 m length, 0.25 mm internal diameter and 25 mm thickness (Agilent/J and W Scientific, Folsom, Ca, USA) was used. Detector of its ionizing and its radiation was with 210˚C, in which hydrogen and air was passed at 40 ml per minute rate. The firs temperature was 80˚C for 2 minutes and the increased 10˚C per minutes to 140˚C. After 1 minutes temperature changes 4˚C per minute to 190˚C and kept for 2 minutes and the temperature changes 2˚C per minutes to 210˚C. Ultra-pure helium was used as the carrier gas with flow rate of 1 ml per minutes. Output peaks based on retention times compared with standard samples ad determined the identity and determined the concentration based on the area under the curve (Young-Cheol et al., 2005). Analysis of variance was performed with Spss software and mean comparison was conducted by Duncan's multiple range tests at 5% probably.

## RESULTS

**Plant height and number of the capitol:** according to analysis of variance results (Table 2) methanol at 1% probability had significant effect on plant height and number of the capitol and chelated zinc at 1% probability had significant effect number of the capitol and at 5% probability on plant height, but the interaction of methanol with chelated zinc did not significant effect on plant height and number of the capitol. Methanol was significantly increased plant height and number of the capitol and by using of methanol, plant height and number of the capitol was increased from 16.66 cm and 10.56 capitols at without methanol to 23.83 cm 13.89 capitols at 30% methanol treatment. Application of chelated zinc was significant effect on plant height and number of the capitol and the highest plant height (20.73 cm) and capitol (12.73 capitols) was obtained a 0.5 g per thousand chelated zinc treatment (Table 3 and 4). Mean comparison indicated that the highest plant height and number of the capitol was 25.83 cm and 16 capitols at 30% methanol with 0.5 g per thousand chelated zinc.

**Total chlorophyll and carotenoid content:** according to the analysis of variance results methanol had significant effect ($P<0.01$) on total chlorophyll content and chelated zinc had significant effect ($P<0.05$) on carotenoid. But the effect of methanol on carotenoid content and the effect of chelated zinc on chlorophyll content and the effect of interaction of methanol with chelated zinc on chlorophyll and carotenoid content was not significant (Table 2). Methanol cause to increases of total chlorophyll content, in which by using methanol chlorophyll content increased from 0.41 mg/g fresh weight at control to 1.11 mg/g fresh weight at 30% methanol (Table 3). Also observed that chelated zinc decreases carotenoid content than the control. In which the highest carotenoid content (0.39 mg/g fresh weight) at control and the lowest amount of that (0.27 /g fresh weight) at 0.5 g per thousand zinc sulfate (Table 4). The mean comparison results (Table 5) showed that the highest total chlorophyll content was 1.17 and 1.12 mg/g per fresh weight at 30% methanol with 0.5 and 1 g per thousand chelated zinc respectively and the highest carotenoid was 0.46 and 0.42 mg/g fresh weight at 10% methanol without chelated zinc and 20% methanol with 1 g per thousand chelated zinc, respectively.

**Flavonoid:** the analysis of variance results indicated that methanol and chelated zinc significantly affected ($P<0.01$) flavonoid content, but the interaction of methanol and chelated zinc did not significant effect on flavonoid content (Table 2). Methanol significantly increased flavonoid content than the control and by using methanol flavonoid content increased from 0.12% at control treatment to 0.22% at 30% methanol treatment (Table 3). Application of chelated zinc increased flavonoid content than the control. By using of chelated zinc the flavonoid content increased from 0.15% at control treatment to 0.2% at 0.15 g per thousand chelated zinc (Table 4). According to the mean comparison results (Table 5) the highest flavonoid content was 0.25% at 0.5 g per thousand chelated zinc with 30% methanol and the lowest amount of that was 0.107% at control treatment.

**Essential oil and gaiol percentage:** the analysis of variance results showed that methanol and chelated zinc had significant effect ($P<0.01$) on essential oil and gaiol percentage, also interaction of methanol with chelated zinc had significant effect ($P<0.01$) on essential oil and gaiol percentage (Table 2). Different levels of methanol significantly increased gaiol percentage and the essential oil percentage increased from 0.89% at control treatment to 1.54% at 30%

methanol. Also, gaiol percentage increased from 4.19% at control to 5.65% at 30% methanol. Application of chelated zinc increased essential oil and gaiol percentage significantly. The essential oil and gaiol percentage increased from 1% and 4.68% at control treatment to 1.44% and 5.21% at 0.5 g per thousand chelated zinc (Table 3 and 4). The mean comparison of interaction of methanol and chelated zinc showed that the highest essential oil and gaiol percentage was 1.88% and 6.07% at 30% methanol with 0.5 g per thousand chelated zinc (Table 5).

**Table2.Variance analysis results of methanolandchelate zinc effects on the quantitative and qualitative characteristics of marigold**

| S.O.V | df | Plant height | Number of capitol | Total chlorophyll | Carotenoid | Flavonoid | Essential oil | Gaiol |
|---|---|---|---|---|---|---|---|---|
| **Block** | 2 | $1.14^{ns}$ | $0.54^{ns}$ | $0.05^{ns}$ | $0.001^{ns}$ | $0.001^{ns}$ | $0.12^{**}$ | $0.7^{**}$ |
| **Methanol (a)** | 4 | $79.62^{**}$ | $14.71^{**}$ | $0.66^{**}$ | $0.01^{**}$ | $0.01^{**}$ | $0.57^{**}$ | $2.91^{**}$ |
| **Chelated zinc (b)** | 2 | $4.36^{*}$ | $8.63^{**}$ | $0.002^{ns}$ | $0.05^{*}$ | $0.009^{**}$ | $0.72^{**}$ | $1.01^{**}$ |
| **a*b** | 8 | $1.85^{ns}$ | $1.95^{ns}$ | $0.05^{ns}$ | $0.009^{ns}$ | $0.001^{ns}$ | $0.02^{ns}$ | $0.08^{ns}$ |
| **Error** | 28 | 1.2 | 1.06 | 0.02 | 0.012 | 0.001 | 0.02 | 0.05 |
| **CV%** | | 5.52 | 8.71 | 14.57 | 14.91 | 4.73 | 8.02 | 4 |

**, * and ns are significantly at 1%, 5% and not significant, respectively

**Table3. The mean comparison of methanol different levels effects on the quantitative and qualitative characteristics of marigold**

| Methanol (%) | Plant height (cm) | Number of capitol | Total chlorophyll ($mg.g^{-1}FW$) | Carotenoid ($mg.g^{-1}FW$) | Flavonoid (%) | Essential oil (%) | Gaiol (%) |
|---|---|---|---|---|---|---|---|
| **0** | $16.66^{d}$ | $10.56^{c}$ | $0.41^{d}$ | $0.32^{a}$ | $0.12^{d}$ | $0.89^{d}$ | $4.19^{d}$ |
| **10** | $17.55^{c}$ | $11.3b^{c}$ | $0.5^{cd}$ | $0.37^{a}$ | $0.16^{c}$ | $1.07^{c}$ | $4.61^{c}$ |
| **20** | $21.8^{b}$ | $12.22^{b}$ | $0.68^{b}$ | $0.38^{a}$ | $0.19^{b}$ | $1.33^{b}$ | $5.09^{b}$ |
| **30** | $23.81^{a}$ | $13.89^{a}$ | $1.11^{a}$ | $0.29^{a}$ | $0.22^{a}$ | $1.54^{a}$ | $5.65^{a}$ |
| **40** | $20.71^{b}$ | $11.33b^{c}$ | $0.62^{bc}$ | $0.29^{a}$ | $0.18^{b}$ | $1.29^{b}$ | $5.26^{b}$ |

Each value is the mean of three replicates. Values followed by different letters in each column are significantly different at P≤5

**Table4. The mean comparison of chelated zinc effects on the quantitative and qualitative characteristics of marigold**

| Chelated zinc (g per thousand) | Plant height (cm) | Number of capitol | Total chlorophyll ($mg.g^{-1}FW$) | Carotenoid ($mg.g^{-1}FW$) | Flavonoid (%) | Essential oil (%) | Gaiol (%) |
|---|---|---|---|---|---|---|---|
| **0** | $19.85^{b}$ | $11.47^{b}$ | $0.66^{a}$ | $0.39^{a}$ | $0.15^{c}$ | $1^{c}$ | $4.69^{c}$ |
| **0.5** | $20.72^{a}$ | $12.73^{a}$ | $0.66^{a}$ | $0.27^{b}$ | $0.2^{a}$ | $1.44^{a}$ | $5.21^{a}$ |
| **1** | $19.75^{b}$ | $11.38^{b}$ | $0.68^{a}$ | $0.33^{ab}$ | $0.18^{b}$ | $1.24^{b}$ | $4.98^{b}$ |

Each value is the mean of three replicates. Values followed by different letters in each column are significantly different at P≤5

**Table5. The mean comparison of chelated zinc and methanol effects on the quantitative and qualitative characteristics of marigold**

| Methanol (%) | Chelated zinc (g per thousand) | Plant height (cm) | Number of capitol | Total chlorophyll ($mg.g^{-1}FW$) | Carotenoid ($mg.g^{-1}FW$) | Flavonoid (%) | Essential oil (%) | Gaiol (%) |
|---|---|---|---|---|---|---|---|---|
| **0** | **0** | $16.31^{d}$ | $9.78^{e}$ | $0.55^{cde}$ | $0.34^{ab}$ | $0.107^{g}$ | $0.79^{e}$ | $4.03^{b}$ |
| **0** | **0.5** | $16.51^{d}$ | $11.22^{cde}$ | $0.35^{e}$ | $0.32^{ab}$ | $0.112^{fg}$ | $0.95^{e}$ | $4.35^{fgh}$ |
| **0** | **1** | $17.17^{d}$ | $10.67^{de}$ | $0.33^{e}$ | $0.29^{ab}$ | $0.15^{defg}$ | $0.92^{e}$ | $4.2^{gh}$ |
| **10** | **0** | $17.6^{d}$ | $11^{cde}$ | $0.46^{de}$ | $0.46^{a}$ | $0.133^{efg}$ | $0.85^{e}$ | $4.31^{fgh}$ |
| **10** | **0.5** | $17.9^{d}$ | $11.67^{cde}$ | $0.47^{de}$ | $0.31^{ab}$ | $0.183^{bcd}$ | $1.27^{bcd}$ | $4.81^{def}$ |
| **10** | **1** | $17.15^{d}$ | $11.22^{cde}$ | $0.56^{cde}$ | $0.33^{ab}$ | $0.157^{cdef}$ | $1.09^{cde}$ | $4.71^{efg}$ |
| **20** | **0** | $21.59^{bc}$ | $11.78^{cd}$ | $0.78^{bc}$ | $0.41^{ab}$ | $0.153^{cdefg}$ | $1.09^{cde}$ | $4.71^{efg}$ |
| **20** | **0.5** | $22.25^{b}$ | $12.78^{bc}$ | $0.53^{cde}$ | $0.29^{ab}$ | $0.223^{ab}$ | $1.54^{b}$ | $5.44^{bc}$ |
| **20** | **1** | $21.56^{bc}$ | $12.11^{bcd}$ | $0.74^{cd}$ | $0.42^{a}$ | $0.18^{bcde}$ | $1.36^{bc}$ | $5.13^{cde}$ |
| **30** | **0** | $22.78^{b}$ | $13.78^{b}$ | $1.03^{ab}$ | $0.41^{ab}$ | $0.2^{bc}$ | $1.26^{bcd}$ | $5.18^{bcde}$ |
| **30** | **0.5** | $25.83^{a}$ | $16^{a}$ | $1.17^{a}$ | $0.21^{a}$ | $0.25^{a}$ | $1.88^{a}$ | $6.07^{a}$ |
| **30** | **1** | $22.82^{b}$ | $11.89^{cd}$ | $1.12^{a}$ | $0.25^{ab}$ | $0.213^{ab}$ | $1.49^{b}$ | $5.7^{ab}$ |
| **40** | **0** | $20.97^{bc}$ | $11^{cde}$ | $0.46^{de}$ | $0.32^{ab}$ | $0.143^{defg}$ | $1^{de}$ | $5.23^{bcde}$ |
| **40** | **0.5** | $21.13^{bc}$ | $12^{bcd}$ | $0.78^{bc}$ | $0.21^{a}$ | $0.21^{ab}$ | $1.55^{b}$ | $5.37^{bcd}$ |
| **40** | **1** | $20.04^{c}$ | $11^{cde}$ | $0.63^{cde}$ | $0.36^{ab}$ | $0.18^{bcde}$ | $1.32^{bc}$ | $5.17^{bcde}$ |

Each value is the mean of three replicates. Values followed by different letters in each column are significantly different at P≤5

## DISCUSSION

Alcohol treatments are effective to the growth and development of the vegetative organs of plants. Also, alcohol treatments can increase the carbohydrate accumulation and carbon dioxide concentration (Zbiec et al., 1999). Therefore, the use of substance that can increase the carbon dioxide concentration cause to fixation of plant yield. Also, foliar application of methanol same with methanol that produce at leaves by pectin methyl esterase enzyme in cell wall development processes can increase cytokinin production and stimulate plant growth and corresponded with Holland (1997) results. Nonimura and Benson (1992) studies showed that treated plants with methanol can increase net photosynthesis and improve their performance. They also said that methanol increases efficiency of carbon conversion. Researchers reported that increasing of nitrate reductase and alkaline phosphates enzymes activity increases plant yield quality and quantity (Zbiec et al., 1999). Also, reports indicate that increases of growth and yield of plant by foliar application of methanol duo to deterrent effects of this organic matter on photorespiration (Nonimura and Benson, 1992). According to the reports methanol convert to formaldehyde by rapid oxidation and then to the carbon dioxide and the carbon dioxide concentration increases increase at leaves and compensation point of carbon dioxide increases. Methanol increases turger pressure, sugar content, cell swelling and help to leaves development and increasing of chlorophyll and carotenoid content (Zbiec et al., 2003). Methanol is smaller than the $CO_2$ and can be easily used by $C_3$ plants for increases of dry matter and as a carbon source (Ramirez et al., 2006). Foliar application of methanol increases chlorophyll and carotenoid content which was corresponded with our results (Ramberg et al., 2002; Theodoridou et al., 2002). However, the foliar application of methanol increased chlorophyll content and photosynthetic capacity and dry matter. Increasing of methanol concentration after 30% decreased quality and quantity of yield, because higher levels of methanol cause to further damage of photosystem II reaction centers, this can happen in drought, heat and light stress conditions. Foliar application of methanol lead to increasing of FBPase enzyme activity such as enzymes that's control photosynthetic process (Andreas et al., 1990). The results of study on the effect o foliar application of ethanolic solutions on lettuce growth showed that, ethanol convert to formaldehyde after penetrating to plant tissue and finally convert to carbon dioxide. Produced carbon dioxide increases internal concentration of that at leaf and cause to increases of photosynthetic efficiency (Morales et al., 1997). Zinc plays an important role in the auxin metabolism (Marschner, 1995). Zinc is involved at many enzymes activity, tryptophan construction, protein structure and precursor of plant growth hormones. Decreasing of growth hormone production in zinc deficient plants decreasing of the internodes length. Zinc deficiency cause to disturbances in some important tasks on the cell surface and consequently decreases plant growth and development (Brown et al., 1993). The positive effect of micronutrients on dry matter yield may be cause to increases of the nitrogen and phosphor uptake in the presence of zinc, which is similar with the findings of other researchers (Sharafi et al., 2002). Foliar application of micronutrients on peppermint increases dry matter (Zehtab-Salmasi et al., 2008). The positive effect of micronutrients on dry matter yield, plant height, number of seeds and capitol and thousand seed weight may be cause to increases of auxin biosynthesis by zinc, increases chlorophyll concentration, increases of phosphor insole pyruvate carboxylase and ribulose bio phosphate, decreases of sodium accumulation in plants tissue and increases of the nitrogen and phosphor uptake in the presence of zinc, which is similar with the findings of other researchers (Sharafi et al., 2002). This element plays multiple roles in plants and regulates the activity of some enzymes and plays structural role in some plant enzymes. In fact, zinc exists in the structure or cofactor of several major enzymes such as dehydrogenase, also zinc plays an important role in grain quality of cereal (Behl et al., 2003). This element has a major role in many cellular functions such as protein metabolism, gene expression, stability of the structure and function of biological membrane, photosynthesis and auxin metabolism (Marschner et al., 1995). Zinc has important role in the enzyme structure; therefore, it has three tasks include catalytic, common catalytic and construction (Vallee and Falchuk, 1993). Zinc has major role in DNA and RNA production, chromatin structure and gene mutations (Williams, 1988). Compared with other micronutrients, zinc concentration in biological systems, particularly in cell membrane is important, thus zinc has key role in development and protection of membrane structure against injuries, protein production and carbohydrate metabolism (Williams, 1988). Micronutrients and heavy metals concentrations in the soil, one of the main criteria in the production of pharmaceutical compounds in new cultured plant. This reflects the fact that, the absorption amount and entry of the concentration and have high effect at drug compounds biosynthesis (Weckx and Clijsters, 1997). Zinc is an essential nutrient and in low concentration it is important in growth and development of plants and involved in many metabolic processes. This element acts as an activator and cofactor for several enzymes such as carbonic anhydrates, dehydrogenase, alkaline polymerases in RNA metabolism, phosphateses, phospholipases, proteins, sugars, nucleic acids, lipids and auxin biosynthesis as a plant growth hormone, whereas at high concentration acts as a heavy metal lead to metabolic disturbances and growth inhibition in most plant species (Rout and Das, 2000). Foliar application of zinc at basil increases essential oil (Said-Al Ahl and Abeer, 2009). Abd El-

Wahab and Mohamed (2008) and Zehtab-Salmasi et al. (2008) reported that intake of micronutrients such as iron and zinc increased growth, aromatic compounds and essential oil in plants such as basil. The application of zinc increases nitrogen efficiency and phosphor increases essential oil, gaiol, carotenoid, quality and quantity of yield.

## REFERENCES


1. Abd El- Wahab, Mohamed. A. 2008. Effect of some trace elements on growth, yield and chemical constituents of *Trachyspermum ammi* L. (Ajowan) plants under Sinai conditions. Res. Agric Biol. J. 4(6): 717-724.
2. Adas consulting Ltd. 2002. Calendulla as Agronomic Raw material for industrial application (CARMINA). (final projectreport). ADAS terrington, terrington ST clement, king's lynn, Norfolk.
3. Andreas, A. R., Lazaro, J. J., Chueca, A., Hermoso, R. and Lopez Gorge, J. 1990. Effect of alcohols on the association of photosynthetic FBPase to thylakoid membrances. Physiology Plant. 78: 409-413.
4. Arnon, A.N. 1967. Method of extraction of chlorophyll in the plants. Agronomy Journal, 23:112-121.
5. Bayvordi, A. 2006. Zinc in soils and crop nutrition. Paivar press. Tabriz, Iran. 180 pp. (In Persian).
6. Behl, R.K., Osaki, M., Wasaki, J., Watanabe, T., Shinano, T.2003. Breeding wheat for zinc efficiency improvement in semi-arid climate. A Review. *TROP*. 12: 295-312.
7. Bernath, J. 2000. Medicinal and aromatic plants. Mezo. Publ. Budapest, PP. 667.
8. Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I., Lux, A. 2007. Zinc in plants. New Phytologist 173: 677-702.
9. Brown, P.H., Cakmak, I., Zhang, Q.1993. Form and Function of Zinc in Plants.
10. Doberman, A., Fairhurst, T. 2000. Rice nutrient disorders and nutrient management. Potash and Phosphate Institute of Canada and International Rice Research Institute, Los Baños, Philippines.
11. Evans, W.C.. 1996. Trease and Evans' Pharmacognosy. 14th ed., Chapter 21, "Volatile oils and resins", Wiley, New York, 450p.
12. Fall, R., Benson, A.A. 1996. Leaf methanol, the simplest natural product from plants. Trends in Plant Science 1: 296-301.
13. Galball, E., Kristine, W. 2002. The production of methanol by flowering plants and the global cycle of methanol. Journal of Atmospheric Chemistry 43: 195-229.
14. Gao, X.P., Zou, C.Q., Zhang, F.S., Vander Zee, S.E., Hoffland, E. 2005. Tolerance influenced by sources and times of zinc application. Indian Journal of Agricultural IRRI, Philippines, PPI, U.S.A., and PPIC.
15. Hassegawa, R.H., Fonseca, H., Fancelli, A.L., da Silva, V.N., Schammass, E.A., Reis, T.A., Correˆa, B. 2008. Influence of macro-and micro nutrient fertilization on fungal contamination and fumonisin production in corn grains. Food Control. 19: 36-43.
16. Haston, A.D., Roje, S. 2001. One carbon metabolism in higher plants. Annual Review of Plant Biology 52: 119-138.
17. Heidari, F., Zehtab Salmasi, S., Javanshir, A., Aliari, H., Dadpoor, M.R. 2008. The effects of application of microelements and plant density on yield and essential oil of peppermint (*Mentha piperita* L.). Iranian J. Med. Arom. Plants. 24:1-9. [In Persian with English Summary].
18. Hernandez, L.F., C.N. Pellegrini, L.M. Malla. 2000. Effect of foliar application of methanol on growth and yield of sunflower (Helianthus annuus L.). Phyton–Revista Internacional de Botanica xperimental. 66: 1-8.
19. Holland, M. A. 1997. Occam's rezor applied to hormonology. Are cytokinins produced by plants? Plant Physiol. 115: 865- 868.
20. Jiang, W., Struik, P.C., Jin, L.N., Van Keulen, H., Zhao, M., Stomph, T.J. 2007. Uptake and distribution of root-applied or foliar-applied 65Zn after flowering in aerobic rice. Annals of Applied Biology150: 383-391.
21. Kalvatchev, Z., Walder, R., Garzaro, D. 1997. Anti- HIV avtivity of extracts from calendulla. Biomedicine & pharmacotheraphy, 51 (4): 176- 180.
22. Leilah, A.A., Badawi, M.A., EL-Moursy, S.A., Attia, A.N. 1988. Response of soybean plants to foliar application of zinc and different levels of nitrogen. J. Agric. Sci. (Mansoura Univ., Egypt). 13: 556-563.
23. Makhdum, I.M., Nawaz, A. Shabab, M., Ahmad, F., Illahi, F. 2002. Physiological response of Cotton to methanol foliar application. Pakistan Journal of Research Science. 13: 37-43.
24. Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. Academic Press. 2th ed. Harcourt Brace and Company Publishers.

25. Morales, J. P. and Santos, B.M. 1997. Effects of different ethanol concentration on the initial growth of lettuce (Lactuca sativa). Proceeding of the Caribbean Food Crop Society., 33: 442-447.
26. Mudgett, M.E., Clarke, S. 1993. Characterization of plant L-isoaspartyl methyltransferases that may be involved in seed survival. Purification, characterization and sequence analysis of the wheat germ enzyme. Biochemistry 32: 1100-1111.
27. Nagaraj, G. 1987. Effect of foliar spray of micro nutrients on yield and chemical composition of peanut in calcareous soils. Annals of Plant Physiology 1: 196-202.
28. Nonomura, A.M., Benson, A.A. 1992. The path of carbon in photosynthesis: Improved crop yields with methanol. National Academy Science. 89: 9794-9798.
29. Omidbaigi, R. 2007. Production and processing of medicinal plants (Volume 1). Behnashr Publications. Tehran. 347 pp. (In Persian).
30. Rajala, A., karkkainen, J., Peltonen, & Peltonen-Sainio, P. 1998. Foliar applications of alchols failed to enhance growth and yield of C3 crop. Indust Crop Prod. 7: 129-137.
31. Ramadant, T., Omran, Y. 2005. The effects of foliar application of methanol on productivity and fruit quality of grapevine cv. flame seedlees. Vitis Journal. 44: 11-16.
32. Ramberg, H.A., Bradley, J.S.C., Olson, J.S.C., Nishio, J.N., Markwell, J., Osterman,J.C. 2002. The role of methanol in promoting plant growth: An update. Rev. Plant Biochem. Biotechno.1:113-126.
33. Ramirez, I., Dorta, F., Espinoza, V., Jimenez, E., Mercado, A., pen q-cortes, H. 2006. Effects of foliar and root applications of methanol on the frowth of Arabidopsis, tobacco and tomato plants. Plant growth regulation. 25: 30- 44.
34. Rout, G.R., Das, P. 2003. Effect of metal toxicity on plant growth and metabolism; Zinc.Agronomy Journal. 23: 3-11.
35. Rowe, R.N., Farr, D.J., Richards, B.A.J. 1994. Effects of foliar and root applications of methanol or ethanol on the growth of tomato plants (*Lycopersicon esculentum* Mill) New Zealand Journal of Crop and Horticultural Science. 22: 335-337.
36. Said-Al Ahl, H.A.H., Abeer.A.M. 2010. Effect of zinc and / or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. Appl. Sci. J. 3(1): 97-111.
37. Samsam Shariat, S.H. 2004. Collection of medicinal herbs. Rozbahan Publications. 393 pp. (In Persian).
38. Theodoridou, A., Dornemann, D., Kotzabasis,K.2002. Light-dependent induction of strongly increased microalgal growth by methanol. Biochim. Biophys. Acta. 1573: 189-198.
39. Vallee, B.L., Falchuk,K.H. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73:79-118.
40. Vyshgahy, M., Noormohammadi, Gh, Majidi, A., Rubii, B. 2008. Effect of mrthanol on the growth function peanuts. Special issue journal of Agricultural sciences. 1: 102- 87. (In Persian with English summary).
41. Weckx, J.E.J., Clijsters, H.M.M. 1997. Zn phytotoxicity induces oxidative stress in primary leaves of phaseolus vulgaris. Plant Physiology. 35:405-410.
42. Williams, R.J.P. 1988. An introduction to the biochemistry of zinc. London, UK: Springer-Verlag. 15-31.
43. Young-Cheol, Y., Hoi-Seon, L., Si Hyeock Lee, J., Marshall, C., Young-Joon, A. 2005. Ovicidal and adulticidal activities of Cinnamomum zeylanicum bark essential oil compounds and related compounds against Pediculus humanus capitis (Anoplura: Pediculicidae). International Journal for Parasitology. 35: 1595–1600.
44. Zbiec, I., Karczmarczyk, S., Podsiadlo, C. 2003. Response of some cultivated plants to methanol as compared to supplemental irrigation. Electronic Journal of Polish Agricultural Universities. 6(1):1-7.
45. Zbiec, I.I., Karczmarczyk, S., Koszanski, Z. 1999. Influence of methanol on some cultivated plants. Department of Plant Production and Irrigation. Agricultural University of Szczecin.Poland,73: 217-220.
46. Zehtab-Salmasi, S., F. Heidari, and H. Alyari. 2008. Effect of micronutrients and plant density on biomass and essential oil production of peppermint (*Mentha piperita* L.). Plant Sci. Res. 1(1): 24-28.
47. Zou, C.Q., Gao, X.P., Zhang, F.S. 2007. Micronutrient deficiencies in crop production in China. In: Alloway, B. (Ed) Micronutrient Deficiencies in Global Crop Production (in press).