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The Effect of Foliar Application of Zinc on Essential Oil Content and Composition of Holy Basil [*Ocimum sanctum*] at First and Second Harvests

Zohreh Moghimipour [1], Mohammad Mahmoodi Sourestani [1]*, Naser Alemzadeh Ansari [1] and Zahra Ramezani [2]

[1] Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] Nanotechnology Research Center, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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Abstract: Holy basil is a perennial plant belongs to lamiaceae family. In order to evaluate the effect of foliar application of zinc on essential oil content and composition of holy basil, an experiment was conducted at research farm, based on randomized complete block design with six treatments and three replications. The treatments were nano zinc chelate (0, 0.5, 1 and 1.5 g/l) and zinc sulfate (1 and 1.5 g/l) fertilizers. Zinc content, glandular trichomes number, glandular trichomes size, essential oil content, yield and composition were measured at full bloom stage. The results showed that the effect of foliar application of zinc fertilizers on all measured traits were significant ($p \geq 0.01$). The highest and lowest values of zinc and essential oil content were obtained in plants sprayed with 1.5 g/l nano zinc chelate and control, respectively. Moreover, eugenol, 1,8-cineole and methyl chavicol were the most oil components of all treatments at both harvests. Other main compounds of essential oil were ocimene, bisabolol and α-bisabolene. Overall, there were no significant differences between 1 and 1.5 g/l nano zinc chelate and 1.5 g/l zinc sulfate treatments for essential oil content and yield of holy basil. Therefore, foliar application of 1.5 g/l zinc sulfate is recommended.

Key words: Holy basil, nano zinc chelate, glandular trichomes, essential oil, eugenol.

Introduction

In lamiaceae family 200 genera and 3200 species are found. *Ocimum* is one of the lamiaceae family genera. *Ocimum sanctum* is a medicinal crop characterized by its specific aroma. The plant is a perennial and thrives well in hot and humid climate. Among the two varieties of *O. sanctum* Linn, Krishna tulsi (leaves dark purple color) and Rama tulsi (leaves green color) are found in India [1]. Aerial parts of this plant have been using in food, pharmaceuticals, cosmetics and perfumery industries. Leaves and flowers contain 0.5-1.5 % essential oil [2]. Eugenol, carvacrol, methyl chavicol, linalool, 1,8-cineole and β-caryophyllene are the main oil constituents. Holy basil also contains methyl eugenol ester, terpineneol, cadinene, carene, α-pinene, β-pinene, camphor, luteolin, limatrol, decyladehyde, germacrene-D and cirsilineol [3]. Different parts of plant are used in systems of medicine for prevention and cure of many illnesses like common cold, headache, cough, flu, earache, fever, colic pain, sore throat, bronchitis, asthma, hepatic diseases, malaria fever, as an antidote for snake bite and scorpion sting, skin diseases, wound, insomnia and night blindness [2].

*Corresponding author (Mohammad Mahmoodi Sourestani)
E-mail: < mohammad.mahmoodi1360@gmail.com; m.mahmoodi@scu.ac.ir >

Essential oil of plants is strongly influenced by several integrated factors. A balanced fertilization program with macro and micronutrients is very important in produce of high quality yield [4]. Zinc is involved in carbon assimilation, saccharids accumulation, reactive oxygen radicals scavenging and finally carbon utilization in volatile oil biosynthesis [5]. Furthermore, zinc stimulates IAA production, chlorophyll biosynthesis and is necessary for DNA, RNA and protein synthesis [6]. Throughout the world especially in arid and semi arid regions, foliar application has been doing for compensating of microelements deficiency [7]. Essential oil biosynthesis of *Ocimum sanctum* is strongly affected by Fe and Zn [8]. Peppermint oil is also increased by foliar application of zinc chloride [9]. Misra and Sharma reported the same results by zinc application on the fresh and dry matter production, essential oil and menthol concentration of Japanese mint [10]. Misra *et al.*, reported that essential oil biosynthesis of geranium was strongly influenced by zinc deficiency [4]. The purpose of this research was to investigate the effect of zinc foliar application with different concentrations on zinc content of leaves, essential oil content and composition of holy basil.

Material and methods

Edapho-climatic conditions

This experiment was carried out in 2013 at research farm of Horticultural Science, Shahid Chamran University (31°20'N latitude and 48°40'E longitude and 22.5 m mean sea level), Ahvaz (Iran), a site characterized by a semidry climate. During the experimentation period (spring-summer), the average maximum temperature was 46.5°C, the minimum was 17.8°C and the average precipitation of the region was 31.42 mm. The physicochemical properties of soil are also given in Table 1.

Plant material and treatments

The experiment was arranged in a randomized complete block design with six treatments and three replications. The treatments were nano zinc chelate (0, 0.5, 1 and 1.5 g/l) and zinc sulfate (1 and 1.5 g/l) fertilizers. Nano zinc chelate was purchased from Khazra Company (Tehran, Iran). Land preparation consisted of disking and the formation of raised beds (15 cm high and 45 cm wide across the top) using a press-pan-type bed shaper. The NPK fertilizer (100 kg/ha) was added to soil before seed sown. Holy basil seeds were sown on two rows on each bed, with 15 cm in-row and 40 cm between-row spacing. The field was irrigated immediately after sowing. The plants were also irrigated weekly as needed. No pests or diseases were observed on any of the holy basil plants. The common weeds removed by hand several times during the growing seasons. Foliar application of zinc fertilizers were done at six-eight leaf stage and were repeated with interval 15 days until the end of the study.

At full bloom stage, some mature leaves were randomly selected from each block of treatments to determine their zinc content. Zinc was measured by atomic absorption spectrophotometer [11]. The number and size of glandular trichomes of adult leaves were also measured by light microscope at full bloom stage. Holy basil was harvested 10 cm above the soil two times over the growing season (June 6 and July 27). The plants were dried in a shade place 30°C for 5 days. Harvests were down at full bloom stage and after first harvest, when the plants were regrown, zinc spraying was continued.

Essential oil extraction

Essential oil of leaves and flowers was extracted base on hydro-distillation for 180 minutes by Clevenger-type apparatus. The amount of oil was

Table 1. Physicochemical properties of the soil (0-30 cm)

Soil texture	pH	EC (ds/m)	Organic carbon	Total N (%)	Available P (mg/kg)	Available K (mg/kg)	Cu	Mn	Zn	Fe
Sandy loam	7.85	7.0	0.93	0.03	34	355	0.6	2.6	1.6	1.8

measured after direct recovery from the distilling unit without any addition of solvent. The oil was dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) before analysis.

Essential oil analysis

GC analyses were performed using a Varian 3800 equipped with flame ionization detector (FID) and a CP-Sil 8-CB column (30 m × 0.32 mm Id × 0.25 µm df). Temperature was programmed from 40 to 300°C at a ramp rate of 10°C/min/ with a final hold time of 11 minute. Injector and detector temperature were 280°C and 300°C, respectively. Helium gas (99.999 %) was used as carrier gas at constant flow rate 1.5 ml/min.

GC-MS analyses were performed using an Agilent 5975 equipped with mass spectrometer and a HP-5 ms column (30 m × 0.25 mm Id × 0.25 µm df). Temperature was programmed from 40 to 300°C at a ramp rate of 10°C/min with a final hold time of 11 minute. Injector and detector temperature were 280°C and 300°C, respectively. Helium gas (99.999 %) was used as carrier gas at constant flow rate 1.5 ml/min. A scan interval of 1 seconds and an electron ionization system with ionizing energy of 70 eV was used.

The identification of the oil components was performed by their retention indices (RI) and their mass spectra, by comparison with a NIST database mass spectral library [12], as well as with literature data [13]. RIs were calculated using an n-alkane series (C4-C28) under the same GC conditions as for samples. The relative amount (RA) of individual components of the oil were expressed as percent peak area relative to total peak area from the GC analyses of the whole extracts, without the use of correction factors.

Statistical analysis

All data were subjected to analysis of variance using the statistical analysis system software package (SAS Institute, Cary, NC, USA). Duncan's multiple range tests were performed to determine the differences among the mean values.

Result and discussion

Zinc content

The result showed that zinc application caused a significant ($p \geq 0.01$) increase in zinc content of leaves. Zinc content of leaves was from 21.37 to 110.53 mg/kg dry weight. The highest zinc content was observed in plant treated with 1.5 g/l nano zinc chelate. There was no significant difference between 1.5 g/l nano zinc chelate, 1 g/l nano zinc chelate and 1.5 g/l zinc sulfate. The lowest value was obtained in control plants. There was no significant difference between 1 g/l zinc sulfate and control plant (table 2). The results obtained of this experiment were the same with the results of other researchers on *Ocimum basilicum* [5], *Mentha japonica* [10], *Rosmarinus officinalis* [14], *Coriandrium sativum* and *Pimpinella anisum* [15]. The optimal zinc content is 100-200 mg/kg of leaves dry weight, and deficiency threshold is less than 15 mg/kg of leaves dry weight [16]. Misra *et al.*, stated that zinc content of holy basil was 11 to 61 mg/kg of dry weight in various genotypes [8]. The effect of nano zinc chelate on zinc content of leaves could be due to the relatively small size of the particles (about 40 nm) and unique characteristics such as more surface energy and activity[17].

Number of glandular trichomes

The results showed that effect of foliar application of zinc fertilizers on number of glandular trichomes were significant ($p \geq 0.01$). The number of glandular trichomes of holy basil at lower epidermis was more than upper epidermis. The highest numbers of glandular trichomes at lower and upper epidermis were obtained in plants treated with 1.5 g/l nano zinc chelate. There were non-significant differences between 1.5 g/l nano zinc chelate, 1 g/l nano zinc chelate and 1.5 g/l zinc sulfate for glandular trichomes number at lower epidermis. The lowest number of glandular trichomes at lower and upper epidermis was obtained in control plants (table 2). Increase of zinc content of leaves had positive and significant correlations with number of the glandular trichomes at lower ($r = 0.89$) and upper ($r = 0.92$) epidermise (table 3).

Size of glandular trichomes

The results showed that effect of foliar application of zinc fertilizers on size of glandular trichomes were significant ($p \geq 0.01$). The highest

Total 2. Measured traits of *O. sanctum* as influenced by zinc treatments at two harvests

Zinc Treatments		Zinc content (mg/kg DM)	Number of glandular trichomes		Size of glandular trichomes (μm²)		Essential oil content* (%)		Essential oil yield (Kg/ha)	
			Lower epidermis	Upper epidermis	Lower epidermis	Upper epidermis	First cut	Second cut	First cut	Second cut
Sulfate	Control	21.37 c	10.44 d	8.33 e	112 d	93 d	1.03 c	1.05 c	2.57 b	2.13 b
	1.0 g/l	35.70 c	15.44 c	10.53 d	114 d	103 d	1.53 b	1.57 b	3.94 b	2.57 b
	1.5 g/l	106.27 a	20.33 a	15.00 b	160 b	170 b	2.03 a	2.06 a	13.30 a	12.86 a
Nano Chelate	0.5 g/l	62.90 b	17.78 b	13.43 c	130 c	140 c	1.57 b	1.64 b	5.33 b	4.77 b
	1.0 g/l	107.90 a	20.56 a	16.13 ab	180 b	180 b	2.09 a	2.12 a	14.19 a	13.03 a
	1.5 g/l	110.53 a	21.56 a	17.37 a	210 a	200 a	2.11 a	2.14 a	14.67 a	12.43 a

Letters with similar alphabets within a column are not significantly ($p \geq 0.01$) different

*: Essential oil content was measured base on dry weight

glandular trichomes size at lower and upper epidermises were obtained in plants treated with 1.5 g/l nano zinc chelate. 1.5 g/l nano zinc chelate treatment had significant difference with 1 g/l nano zinc chelate and 1.5 g/l zinc sulfate in size of glandular trichomes at both epidermises. There are no significant differences between 1 g/l nano zinc chelate and 1.5 g/l zinc sulfate at both epidermises for the size of glandular trichomes. The lowest size of glandular trichomes was obtained in control plants (table 2). Increase of zinc content of leaves had positive and significant correlations with size of the glandular trichomes at lower ($r = 0.92$) and upper ($r = 0.91$) epidermises (table 3). Since zinc has an important role in chloroplast activity, it can improve net photosynthesis and plant growth. So, zinc supplement can stimulate size and number glandular trichomes of leaves [18].

Essential oil content

According to table 2, foliar application of zinc had significant ($p \geq 0.01$) effect on essential oil content for both harvests. Essential oil content of plants at first harvest was from 1.03 to 2.11 % base on dry weight, whereas it was 1.05 to 2.14 % base on dry weight at second harvest. Moreover, essential oil content at second harvest was slightly higher than first harvest. The highest essential oil content was obtained in plant sprayed with 1.5 g/l nano zinc chelate at first and second harvests. There were no significant differences in essential oil content between 1.5 g/l nano zinc chelate, 1 g/l nano zinc chelate and 1.5 g/l zinc sulfate at first and second harvests. The lowest essential oil content at first and second harvests was observed in control plant. The same results were reported by researchers on holy basil [8], *Ocimum basilicum* [5], *Mentha piperita* [9], *Mentha japonica* [10], *Rosmarinus officinalis* [14], *Coriandrium sativum* and *Pimpinella anisum* [15]. There was positive and significant correlation between zinc content with glandular trichomes number ($r = 0.89$), glandular trichomes size ($r = 0.92$) and essential oil content ($r = 0.95$) of holy basil (table 3). Saccharides considered as source of energy for the biosynthesis of terpenoids. According to the role of the zinc in increase of photosynthesis, stomatal conductance and polysac-

Total 3. Correlation between measured traits of *O. sanctum*

No.	Traits	1	2	3	4	5	6	7
1	Zinc content	1						
2	No. of glandular trichomes at lower epidermis	0.89**	1					
3	No. of glandular trichomes at upper epidermis	0.92**	0.94**	1				
4	Size of glandular trichome at lower epidermis	0.92**	0.92**	0.91**	1			
5	Size of glandular trichomes at upper epidermis	0.91**	0.90**	0.98**	0.94**	1		
6	Essential oil content	0.95**	0.91**	0.87**	0.95**	0.85**	1	
7	Essential oil yield	0.96**	0.90**	0.95**	0.95**	0.95**	0.91**	1

* * means significant difference at 1 % level ($P< 0.01$)

charide metabolism, this element has an important effect on essential oil production and accumulation [5].

Essential oil yield

Result showed that foliar application of zinc had significant ($p\geq0.01$) effect on essential oil yield at both harvests (table 2). Essential oil yield at first harvest was slightly higher than second harvest. Essential oil yield of plants at first harvest was from 2.57 to 14.67 kg/ha, whereas it was from 2.13 to 12.43 kg/ha at the second harvest. The highest essential oil yield at two harvests was obtained in plant sprayed with 1.5 g/l nano zinc chelate. There was no significant difference for essential oil yield between 1.5 g/l nano zinc chelate, 1 g/l nano zinc chelate and 1.5 g/l zinc sulfate. The lowest essential oil yield was observed in control plant. There was no significant difference in essential oil yield at first and second harvests between 1 g/l zinc sulfate, 0.5 g/l nano zinc chelate and control plant. The same results have been reported for some herbs such as *Ocimum basilicum* [5], *Mentha piperita* [19], *Mentha japonica* [10], *Rosmarinus officinalis* [14]. There was positive and significant correlation between zinc content with glandular trichomes number (r = 0.89), glandular trichomes size (r = 0.92), essential oil content (r = 0.95) and essential oil yield (r = 0.96) of holy basil (table 3). Zinc has indirect effect on biosynthesis of terpenoids by increasing of photosynthesis. Moreover, the probable enhanced in biosynthesis of auxin, and cell division with zinc application can cause an increase of leaf area and plant photosynthesis [20]. El-Sawi and Mohamed reported that zinc foliar application had no significant effect on essential oil content of cumin, whereas oil yield increased due to enhance of seed yield [21].

Essential oil composition

The list of essential oil components of *Ocimum sanctum* subjected to zinc foliar application are presented in tables 4 and 5 for first and second harvests, respectively. Overall, thirty one components were identified in the essential oil of six treatments at both harvests. Eugenol was the most component of all treatments. Eugenol ranged from 28.14 % (1 g/l zinc sulfate) to 30.64 % (control plant) at first harvest. The highest and lowest amounts for this component at second harvest were observed in 1.5 g/l nano zinc chelate and 1 g/l zinc sulfate treatments, respectively. Meanwhile, increased of zinc from 0 to 1.5 g/l had not strongly effect on eugenol. In total, eugenol of holy basil at second harvest was higher than first harvest. Eugenol ($C_{10}H_{12}O_2$) is a phenylpropanoid, derived from phenylalanine in shikimic acid pathway. At first stage phenylalanine transmuted into the cinnamic acid. Then, cinnamic acid with cinnamic-4-hydroxylase transformed into p-coumaric acid. p-coumaric acid transmuted into eugenol

Table 4. Oil composition (%) of holy basil (*O. sanctum*) as influenced by zinc treatments at first harvest

No.	Compounds	KI	Control	1 g/l Zinc sulfate	1.5 g/l Zinc sulfate	0.5 g/l Nano chelate	1 g/l Nano chelate	1.5 g/l Nano chelate
1	Isovaleric acid	789.6	0.16	0.12	0.10	0.10	0.11	0.14
2	α-Pinene	908.8	1.40	1.16	0.94	0.98	1.01	1.23
3	β-Phellandrene	967.2	1.18	1.12	0.93	0.99	1.00	0.93
4	Camphene	969.4	0.58	0.59	0.47	0.51	0.52	0.57
5	β-Pinene	972.9	2.57	2.53	2.15	2.28	2.30	2.49
6	β-Myrcene	989.2	0.75	0.79	0.64	0.67	0.68	0.82
7	1,8-Cineole	1039.2	24.79	23.86	22.11	22.48	23.47	20.53
8	Ocimene	1052.5	5.24	4.65	3.93	4.02	4.28	3.92
9	Carene	1064.7	0.16	0.16	0.15	0.13	0.13	0.14
10	Sabinene hydrate	1037.7	0.43	0.51	0.42	0.53	0.53	0.51
11	Linalyl acetate	1104.0	0.18	0.13	0.15	0.18	0.15	0.20
12	Limonene	1138.5	0.21	0.27	0.23	0.24	0.25	0.26
13	Terpineol	1174.8	0.40	0.36	0.35	0.32	0.33	0.53
14	Terpinen	1185.5	0.37	0.30	0.34	0.28	0.31	0.31
15	α-Terpinyl	1198.5	1.36	1.23	1.38	1.30	1.35	1.39
16	Methyl chavicol	1207.9	11.37	12.53	13.40	12.60	12.89	12.21
17	Cosmene	1210.8	0.10	0.11	0.11	0.13	0.12	0.12
18	Chavicol	1257.6	1.01	1.91	1.01	0.80	0.94	0.98
19	Eugenol	1375.9	30.29	28.14	30.64	29.67	29.92	29.13
20	Methyl eugenol	1409.2	1.06	1.17	1.21	1.26	1.19	1.09
21	Caryophyllene	1439.0	0.86	0.99	0.95	1.11	0.98	0.94
22	α-Farnesene	1138.5	0.27	0.32	0.31	0.35	0.32	0.31
23	β-Sesquiphellandrene	1455.3	0.34	0.46	0.37	0.42	0.38	0.56
24	β-Farnesene	1463.2	1.68	1.87	1.88	1.96	1.87	1.97
25	Germacrene-D	1500.8	0.94	0.93	1.05	1.07	0.92	0.98
26	Bisabolol	1523.2	5.77	6.89	7.16	7.87	7.07	7.50
27	α-Bisabolene	1568.3	4.43	4.75	4.79	5.50	4.81	4.84
28	Allylphenol	1601.0	0.16	0.35	0.29	0.27	0.24	0.44
29	Caryophyllene oxide	1605.0	0.18	0.29	0.29	0.32	0.28	0.31
30	Cyclohexene	1632.6	0.10	0.07	0.13	0.13	0.11	0.13
31	Spatulenol	1673.6	0.19	0.19	0.24	0.24	0.20	0.26
	Total		98.53	98.75	97.71	98.71	98.66	97.63

(with cinnamoyl-CoA reductase enzyme) or chavicol (with cinnamyl alcohol dehydrogenase enzyme) during several stages. Moreover, chavicol with chavicol O-methyltransferase enzyme transmuted into methyl chavicol [22].

1,8-Cineole, a fragrant monoterpene was the second major volatile oil component. The greatest amounts of this compound at first and second harvests were obtained in control plant and 1 g/l zinc sulfate, respectively. Whereas 1.5 g/l nano zinc chelate had least amounts of this compound at first and second harvests. Moreover, 1,8-cineole at first harvest was higher than second harvest.

Methyl chavicol was the third major volatile oil component which 1.5 g/l zinc sulfate and 1 g/l

Table 5. Oil composition of holy basil (*O. sanctum*) as influenced by zinc treatments at second harvest

No.	Compounds	KI	Control	1 g/l Zinc sulfate	1.5 g/l Zinc sulfate	0.5 g/l Nano chelate	1 g/l Nano chelate	1.5 g/l Nano chelate
1	Isovaleric acid	789.6	0.14	0.15	0.13	0.15	0.11	0.13
2	α-Pinene	908.8	1.21	1.50	1.20	1.43	1.15	1.19
3	β-Phellandrene	967.2	0.75	0.98	0.85	0.97	0.96	0.78
4	Camphene	969.4	0.40	0.51	0.42	0.46	0.46	0.37
5	β-Pinene	972.9	1.77	2.28	1.99	2.13	2.10	1.74
6	β-Myrcene	989.2	0.53	0.69	0.60	0.65	0.63	0.52
7	1,8-Cineole	1039.2	18.40	22.40	21.37	19.94	20.81	17.54
8	Ocimene	1052.5	3.43	4.87	4.64	4.35	5.07	3.61
9	Carene	1064.7	0.11	0.15	0.17	0.15	0.17	0.12
10	Sabinene hydrate	1037.7	0.47	0.41	0.28	0.34	0.30	0.40
11	Linalyl acetate	1104.0	0.32	0.20	0.20	0.16	0.18	0.18
12	Limonene	1138.5	0.21	0.26	0.29	0.28	0.22	0.29
13	Terpineol	1174.8	0.18	0.26	0.28	0.25	0.27	0.24
14	Terpinen	1185.5	0.24	0.30	0.37	0.31	0.34	0.25
15	α-Terpinyl	1198.5	0.99	1.18	1.20	1.13	1.17	1.08
16	Methyl chavicol	1207.9	12.05	13.03	12.08	12.50	12.56	10.48
17	Cosmene	1210.8	0.09	0.07	0.04	0.07	0.05	0.05
18	Chavicol	1257.6	1.10	0.88	0.98	1.04	1.02	1.18
19	Eugenol	1375.9	37.85	33.83	37.62	36.57	37.19	41.42
20	Methyl eugenol	1409.2	0.99	0.83	0.86	0.91	0.87	0.93
21	Caryophyllene	1439.0	1.03	0.78	0.81	0.85	0.76	0.93
22	α-Farnesene	1138.5	0.35	0.26	0.25	0.28	0.23	0.30
23	β-Sesquiphellandrene	1455.3	0.29	0.46	0.29	0.32	0.29	0.34
24	β-Farnesene	1463.2	1.57	1.31	1.36	1.45	1.34	1.51
25	Germacrene-D	1500.8	0.73	0.65	0.62	0.71	0.72	0.71
26	Bisabolol	1523.2	7.73	6.03	5.64	6.28	5.50	6.99
27	α-Bisabolene	1568.3	4.60	3.76	3.58	4.16	3.74	4.40
28	Allylphenol	1601.0	0.36	0.22	0.23	0.26	0.19	0.34
29	Caryophyllene oxide	1605.0	0.59	0.31	0.31	0.36	0.23	0.51
30	Cyclohexene	1632.6	0.09	0.06	0.05	0.09	0.05	0.09
31	Spatulenol	1673.6	0.24	0.17	0.24	0.21	0.14	0.24
	Total		98.81	98.79	98.95	98.73	98.82	98.86

zinc sulfate had the greatest amounts for this compound at first and second harvests, respectively. The lowest amount of methyl chavicol was observed in control plant and 1.5 g/l nano zinc chelate at first and second harvests, respectively. In contrary to eugenol, methyl chavicol was higher at first harvest than second harvest.

Ocimene (3.43-5.24 %), bisabolol (5.50- 7.87 %) and α-bisabolene (3.58-5.50 %) were other main oil components of holy basil. Regarding to the main components of essential oil, it seems that there was some discrepancy and/or similarity between present study and other researches. The GC-MS analysis of two varieties of *O. sanctum* showed that eugenol was one of the major constituent (about 50 %). Other main components were β-elemene (39.27 %), germacrene-D (2.31 %) and α-humulene (1.41 %)[23]. Misra *et al*., identified four compounds; methyl chavicol (30-50 %), eugenol (11.2-30.5 %), linalool (11-20 %) and geraniol (1-4 %) as major compounds in the essential oil of holy basil's genotypes[8].

Zheljazkov *et al*., reported that main oil components of three basil cultivars (German, local and mesten) were linalool (30.7-37 %), cadine (insignificant or 3.4 %), 1,8-cineole (2.7-5.4 %) and eugenol (0- 18 %)[24]. In another research, Zheljazkov *et al*., showed that main oil components of holy basil at four locations were different. However, methyl chavicol (7.02-25.1 %), 1, 8-cineole (6.72-23.4 %) and eugenol (0-17 %) were main oil components[25].

In present research, zinc application had slight effect on quantities and qualities of holy basil oil. The influence of zinc application on essential oil composition was evaluated by many scholars. Dehabadi *et al*., observed that total content of monoterpenes of *Mentha spicata* essence increased with zinc application, whereas sesquiterpenes were declined. Zinc treatment also caused a decrease in oxygenated monoterpenes and hydrogenated monoterpenes of essence[26]. Hassanpouraghdam *et al*., found that methyl chavicol as main compound oil of *Ocimum basilicum* increased by zinc sulfate treatment[27]. The same result was reported by Pirzad *et al*., on *Pimpinella anisum*[18]. These researchers expressed that zinc influences on primary metabolic pathways that ultimately lead to the biosynthesis of active components of essential oil. Gerjtovsky *et al*. reported that soil application of zinc slightly affected chamazulene and (E)-β-farnesene of chamomile oil. Moreover, zinc supply had no effect on flavones, apigenin, coumarin and herniarin[28]. Misra and Sharma observed that zinc application stimulated the menthol concentration in *Mentha japonica*[10]. Misra *et al*., stated that there was a strong relationship between primary metabolic pathways and biosynthesis/accumulation of secondary metabolites of *Pelargonium graveolens*[4]. Those authors declared that proper correlation of carbon assimilation pathways and accumulation of secondary metabolic needs association of several intrinsic and extrinsic factors, particularly optimum levels of micronutrients such as zinc.

Conclusion

In summary, the results showed that foliar application of zinc fertilizers could lead to increases in number and size of glandular trichomes, zinc content, essential oil content and yield of holy basil. Zinc content showed positive and significant correlations with glandular trichomes number (r = 0.89), glandular trichomes size (r = 0.92), essential oil content (r = 0.95) and essential oil yield (r = 0.96). The highest and lowest values of mentioned traits were obtained in plant sprayed with 1.5 g/l nano zinc chelate and control, respectively.

The effect of nano zinc chelate on zinc content of leaves could be due to the relatively small size of the particles (about 40 nm) and unique characteristics such as more surface energy and activity[17]. Moreover, GC-MS analyses of essential oil were identified thirty one components in the essential oil of treatments at both harvests. Eugenol, 1,8-cineole and methyl chavicol were the most oil components of all treatments at both harvests. Other main compounds of essential oil were ocimene (3.43-5.24 %), bisabolol (5.50-7.87 %) and α-bisabolene (3.58-5.50 %). There was no significant difference between 1 and 1.5 g/l nano zinc chelate and g/l zinc sulfate treatments for important traits such as essential oil content and yield of holy basil. So, foliar application of 1.5 g/l zinc sulfate is recommended.

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References

1. **Kothari, S.K., Bhattacharya, A.K. and Ramesh, S. (2004).** Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L. (syn. *O. sanctum* L.) grown in south India as influenced by method of harvest. J. Chromatogr. 1054: 67-72.
2. **Singh, V., Amdekar, S. and Verma, O. (2011).** *Ocimum sanctum* bio-pharmacological activities.

Webmedcentral Pharmacol., 1(10): 1-7.

3. **Prakash, P. and Gupta, N. (2005).** Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian J. Physiol. Pharmacol. 49: 125-131.
4 **Misra, A., Srivastava, A.K., Srivastava, N.K. and Khan, A. (2005).** Zn-acquisition and its role in growth, photosynthesis, photosynthetic pigments and biochemical changes in essential monoterpene oil(s) of *Pelargonium graveolens*. Photosynthetica. 43(1): 153-155.
5. **Said Al-Ahl, H. and Mahmoud, A. (2010).** Effect of zinc and iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum*) under salt stress. Ozean J. Appl. Sci. 3(1): 97-111.
6. **Nahed, G.A. and Balbaa, L.K. (2007).** Influence of tyrosine and zinc on growth, flowering and chemical constituents of *Salvia farinacea*. J. Appl. Sci. Res. 3(11): 1479-1481.
7. **Kaya, M., Atak, M., Mahmood, Kh., Ciftci, C.Y. and Ozcan, S. (2005).** Effect of pre-sowing seed treatment with zinc and foliar spray of humic acids on yield of common bean (*Phaseolus vulgaris* L.). Int. J. Agri. Biol. 6(7): 875-878.
8. **Misra, A., Dwivedi, S.A., Srivastava, K.D., Tewari, K., Khan, A. and Kumar, R. (2007).** Analysis of growth, physiology, photosynthesis, essential monoterpene oil(s) yield and quality in *Ocimum sanctum* L genotypes. Biosci. Res. 4(1): 01-05.
9. **Akhtar, N., Sarker, A.M., Akhter, M.H. and Nada, M. (2009).** Effect of planting time and micronutrient as zinc chloride on the growth, yield and oil content of *Mentha piperita*. Soil Sci. J. 44(1): 125-130.
10. **Misra, A. and Sharma, S. (1991).** Critical Zn concentration for essential oil yield and menthol concentration of Japanese mint. Nutr. Cycl. Agroecosys. 29(3): 261-263.
11. **Holon, E.A. (1998).** Elemental determination by atomic absorption spectrophotometer (AAS). In: V.P. Kalra (ed). Handbook of Reference Method for Plant Analysis. Soil and Plant Analysis Council. CRC Press, Florida, USA, pp: 157-164.
12. **NIST, EPA, NIH (2000).** Mass Spectral Database, Version 2.1. Perkin Elmer Instrument LLC.
13. **Adams, R.P. (2007).** Identification of essential oil components by gas chromatography/mass spectroscopy. Allured: Carol Stream, IL, USA.
14. **Tarraf, Sh., El-Sayed, A.A. and Ibrahim, M.E. (1994).** Effect of some micronutrients on *Rosmarinus officinalis*. J. Physiol. Sci. 18(1): 201-208.
15. **Khalid, Kh.A. (1996).** Effect of fertilization on the growth, yield and chemical composition of some medicinal Umbelleferous plant. MSc. Thesis, Al-Azhar University, Cairo, 152 p.
16. **Plank, C.O. (1993).** Plant analysis handbook for Georgia, University of Georgia Cooperative Extension Services, pp. 60-64.
17. **Ma, X., Lee, J.G., Deng, Y. and Kolmakov, A. (2012).** Interactions between engineered nanoparticles and plants: Phytotoxicity, uptake and accumulation. Sci. Total Environ. 408: 3053-3061.
18. **Pirzad, A.R., Tousi, P. and Darvishzadeh, R. (2013).** Effect of Fe and Zn foliar application on plant characteristics and essential oil content of anise (*Pimpinell aanisum*). Iranian J. Crop Sci. 15(1): 12 -23 (In Persian).
19. **Zehtab-Salmasi, S., Heidari, F. and Alyari, H. (2008).** Effect of micronutrients and plant density on biomass and essential oil production of peppermint (*Mentha piperita* L.). Plant Sci. Res. 1(1): 24-26.
20. **Derakhshani, Z., Hassani, A., Sadaghiani, M.H.R., Hassanpouraghdam, M.B., Khalifani, H. and Dalkani, M. (2011).** Effect of zinc application on growth and some biochemical characteristics of costmary (*Chrysanthemum balsamita*). Commun. Soil Sci. Plan. pp. 37-41.
21. **El-Sawi, S. and Mohamed, A. (2002).** Cumin herb as a new source of essential oil and its

response to foliar spray with some micro-elements. Food Chem. 77: 79-80.

22. **Guy, P., Kamatou, V. and Alvaro, M. (2012).** Eugenol-from the Remote Maluku islands to the international market place: a review of a remarkable and versatile molecule. Molecules, 17: 6953-6981.
23. **Sharma, V., Gupta, R.C., Singh, B., Singh, H. and Kumar Srivastava, D. (2014).** Volatile oil composition and antimicrobial activity of essential oil of two varieties of *Ocimum sanctum* (L.) from dhameta (kangra)-a north Indian region. Int. J. Recent Sci. Res. 5(1): 142-147.
24. **Zheljazkov, V.D., Cantrell, C., Tekwani, B. and Khan, S. (2008a).** Content, composition, and bioactivity of the essential oil of three basil genotypes as a function of harvesting. J. Agr. Food Chem. 56: 380-385.
25. **Zheljazkov, V.D., Cantrell, C., Evans, W., Ebelhar, M. and Coker, C. (2008b).** Yield and composition of *Ocimum basilicum* and *O.sanctum* growth at four locations. HortScience, 43(3): 737-740.
26. **Dehabadi, S.Z., Asrar, Z. and Mehrabani, M. (2010).** Biochemical changes in terpenoid compounds of *Mentha spicata* essential oils in response to excess zinc supply. Iranian J. Plant Biol. 2(1): 1-10 (In Persian).
27. **Hassanpouraghdam, M.B., Gohari, G.R., Tabatabaei, S.J., Dadpour, M.R. and Shirdel, M. (2010).** NaCl salinity and Zn foliar application influence essential oil composition of basil (*Ocimum basilicum* L.). Acta Agric. Slov. 97(2): 93-95.
28. **Gerjtovsky, A., Markusova, K. and Eliasova, A. (2006).** The response of chamomile (*Matricaria chamomilla* L.) plants to soil zinc supply. Plant Soil Environ. 52(1): 1-7