Journal of Medicinal Plants and By-products (2021) 1: 21-28

Original Article

Application of Chelate and Nano-Chelate Zinc Micronutrient Onmorpho-physiological Traits and Essential Oil Compounds of Peppermint (*Mentha piperita* L.)

Zahra Nemati Lafmejani[1], Ali Ashraf Jafari[2*], Pejhman Moradi[3] and Alireza Ladan Moghadam[4]

[1]*Department of Horticulture, Islamic Azad University, Science and Research Branch, Tehran, Iran*
[2]*Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran*
[3]*Department of Horticulture, Islamic Azad University, Saveh Branch, Saveh, Iran*
[4]*Department of Horticulture, Islamic Azad University Garmsar Branch, Garmsar, Iran*

Article History: Received: 24 April 2019/Accepted in revised form: 03 July 2019

Abstract

Peppermint (*Mentha piperita* L.) from Lamiaceae family is one of the most important medicinal plants, used in food, sanitary and cosmetic industries. The aim of this study was to examine the effects of Nano Zinc and Zinc-Chelate on morpho-physiological traits and essential oil composition of peppermint. A factorial experiment was conducted based on Randomized Complete Block Design (RCBD) with three replications in Karaj, Iran, in 2016. Treatments were zinc micronutrient in two levels (Zinc-Chelate and zinc nanoparticles) and concentration in four levels (0, 0.5, 1 and 1.5 g/l). The zinc micronutrient was applied in foliar spraying in three times of the interval of 15 days up to flowering stages. Results showed that both of Nano zinc and Zinc-Chelate micronutrient significantly increased all of morpho-physiological traits than that for control. The higher values were obtained in Zinc-Chelate (1.5 g/l) and Nano zinc (1 g/l), respectively. The effects of treatments were significant at 21 out of 34 compounds. The higher compounds with average values of 19.7%, 7.8%, 5.9%, 5.7%, 4.9% and 4.1% were obtained in Menthol, Menthone, Menthofuran, Piperitone, Menthyl acetate and Beta-caryophyllen, respectively. Nano zinc (1.5 g/l) had increased Menthol, Menthone and Menthofuran content up to 28%, 61% and 237% higher than the control, respectively. t. It was concluded that foliar spraying of Nano zinc (1 g/l) in flowering stage had increased dry matter yield and essential oil content and spraying of Nano zinc (1.5 g/l) had increased essential oil composition of peppermint.

Keywords: Essential oil, Leaf pigments, Yield, Foliar spray, Zinc Nanoparticles, *Mentha piperita*

Introduction

Peppermint (*Mentha piperita* L.) belongs the family Lamiaceae, is one of the most important medicinal plants which used in food, sanitary and cosmetic industries. The leaves of peppermint are strongly scented due to the presence of essential oils. Peppermint is widely used for its medicinal properties such as anti-spasmodic, anti-sickness, anti-helminthic, carminative, stomachic, etc.[1]. Peppermint cultivated in the temperate, Mediterranean and subtropical regions of the world [2]. Peppermint is not native to Iran, but it cultivated in most provinces. According to Sefeidcon, the cultivated area of peppermint in the country is about 117 ha, This species is ranked in a high priority for breeding improved varieties [3].

The highest yield and quality of the aerial part of this plants is achieved by cultivation in long day period and humid regions where temperatures are 18 to 20 °C. Due to having surface roots, it needs more irrigation with short intervals. This plant is grown in loamy-sand soil with high amount of homes and acidity ranged (pH=5-8) [4]. Peppermint (*Mentha piperita* L.) which is tetraploid (2n = 72), is a sterile natural hybrid of *M. aquatic* L. (2n= 96) and *M. spicata* L. (2n=48) [5]. The plant is a perennial with 50-60 cm tall. The square stems are usually reddish-purple and

*Corresponding author: Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran
Email Address: aajafari@rifr-ac.ir

smooth. The leaves are short, oblong-ovate and serrate. The flowers are purple-pinkish and appear in the summer months. The plant has runners above and below ground [6].

Considering increased needs medicinal plants and the effect of micronutrient fertilizers on quantity and quality of essential oils, it is essential to use the best fertilizers in order to increase the efficiency of medicinal plants Various researchers reported that essential oil yield and its components in medicinal and aromatic plants in general is primarily related to their genetic, climatic, edaphic, elevation and topography [7,8].

Zinc (Zn), is the one of heavy metal contaminants that its accumulation in soils caused from using fertilizers, metaliferous mining, metal processing, agricultural and land applications of sewage sludge and discharge of untreated urban and industrial residues and other human activities [9]. Moreover, Zn is an essential micronutrient for normal plant growth and metabolism. In plants, Zn plays a vital role in various metabolic processes, namely cell wall metabolism, also act as structural elements in regulatory proteins, photosynthetic electron transport and mitochondrial respiration, biosynthesis of plant hormones, and function as cofactors for a variety of enzymes [10,11]. 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 [12]. Zinc also has important roles in the plant growth and development, including production of biomass, chlorophyll production, pollen function, fertilization, metabolism of RNA, proteins and the DNA formation [13,14]. Zinc plays an important role in protein and carbohydrate synthesis and takes part in the metabolism of saccharides, nucleic acid, and lipid metabolism. Zinc deficiency causes an inhibition of cell growth and proliferation. Toxic concentrations of Zn negatively affect photosynthetic electron transport and photophosphorylation. One of the primary mechanisms of Zn toxicity may increase the permeability of the root membranes, which will cause nutrients to leak out from the roots [15,16].

The use of Nano fertilizer led to an increased efficiency of the elements, reduce the toxicity of the soil, to at least reach the negative effects caused by the consumption of excessive use of fertilizers and reduce the frequency of spraying of fertilizers [17]. With production of Nano fertilizers, this Nano compounds rapidly and completely absorbed by plants and fix its nutrient shortages and growing needs [18]. Application of Zinc-Chelate has been reported to have significant positive effects in most cases on the growth measurements and chemical composition of lupine [19], cumin [20], soybean [21, 22], sunflower [23], mustard [24], and rice [25]. However, the effects of Zinc Nanoparticles on growth and essential oil production of peppermint are poorly understood. Thus, the aim of this study was to examine the effects of both Zinc-Chelate and Zinc Nanoparticles on growth and essential oil content and its compounds in peppermint (*M. piperita* L.) in field conditions.

Material and Methods

The present study was conducted in research farm of Jihad Daneshgahi, Karaj, Iran. Karaj has a semi-dry, continental climate. The soil of the field was silty loam with pH (7.9), contains total N (0.08%), total P_2O_5 (36.2 ppm) and total K_2O (49.8 ppm) and Fe (5.74 ppm) with an EC of 0.93 (dS/m) (Tables 1 and 2).

Field was established using tiller propagation of peppermint in May 2015 in density of 16 plants/m^2 with an interspace of 25 cm. A factorial experiment was conducted using two forms of Zinc micronutrient as Nano Zinc and common Zinc-chelate as main factor and micronutrient fertilizer concentration in three levels (0.5, 1.0 and 1.5 g/l) and control (no fertilizer) as the second factor based on Randomized Complete Block Design (RCBD) with three replications. The fertilizer treatments were applied in form of foliar spraying. The fertilizer treatments were sprayed on the plant as foliar in three times of the interval of 15 days up to flowering stages. Then an area of 1 m^2 in the center of each plot was selected for data sampling. Data collected as follows:

- The aerial part of plants were harvested in full flowering stage and weighed and dried under a shadow and open air flow and weighed as the aerial biomass yield.
- The stems number of each plot were counted.
- The chlorophyll a, b, and total were measured on new leaves using spectrophotometer [26].
- For extraction and measuring of essential oil, the herb, collected at the full flowering stage, then 80 g of dried material was grind to measuring of essential oil by Kelvenger Instrument. About 100g of each dried sample (aerial parts) was separated, triturated and steam-hydro distilled for 2 hours. The extraction of oil was carried out according to method of Hungarian pharmacopoeia [27] as follows:
- $\text{Essential oil content } \% = \frac{\text{Essential oil weight g}}{\text{Aerial biomass yield g}} \times 100$
- Essential oil yield was calculated by essential oil percentage × aerial biomass yield as g/plot.
- The essential oil compositions were detected using GC/MS a Varian 3400 GC-MS system equipped with a db-5 fused silica column [28].

Table 1 The chemical and physical properties of the soil in research farm

Soil texture	Clay %	Silt %	Sand %	Lime %	N %	C %	K ppm	P ppm	EC ds/m	pH	Fe ppm	Zn ppm	S ppm	Mn ppm	Cu ppm
Silty loam	16	22	62	8.5	0.08	0.82	49.8	36.2	0.93	7.9	5.74	0.60	6.3	11.2	0.7

Table 2 The Basic characterization of research area

Location of research	Sunny Hours Average	Annual Rainfall	Average Annual Temperature	Altitude	Latitude	Longitude
Research farm of Jihad Daneshgahi, Karaj	2899 hours	251 mm	21.13 °C	1426 m	36°35″ N	50°56″ E

The data of morph-physiological traits in three replications and essential oil composition in two replications were statistically analyzed using Minitab software and the means of the treatments compared using the Duncan's test at the $p<0.05$ confidence level. The Excel software was used for drawing histograms of treatments comparison.

Results and Discussion

Morpho-physiological Traits and Essential Oil Content

Result showed significant effects of treatments for all of Morpho-physiological traits ($p<0.01$). The higher values of these traits were obtained by applications of Nano Zinc fertilizer (Table 3 and Fig. 1). Results showed that Nano zinc fertilizer had significantly increased both chlorophyll a and b contents. The higher values of chlorophyll (a, b and total) were obtained in common Zinc Chelate 1.5 g/l and Nano Zinc 1 and 1.5 g/l. with no significant differences between them (Fig. 1). In other word, the Zinc Chelate 1.5 g/l and Nano Zincs 1 g/l and 1.5g/l had increased chlorophyll a content up to 58%, 56% and 58% higher than in control, respectively. For total chlorophyll the corresponding values were 61%, 59% and 60%, respectively (Fig. 1). Similar to our study, Mahmoodi *et al* in Borago officinalis found that both Zinc Chelate, Nano Zinc had increased chlorophyll indices in comparison to control [29].

There was a significant effect of treatments on stem number (Table 3). Results indicated that, the Nano zinc 1 g/l had increased the stem number up to 30% higher than that for control (Fig. 1). For aerial part fresh weights the lower level of Nano zincs 0.5 g/l had increased up to 74% higher than in control (Fig. 1). For aerial part dry weights the Nano zinc 1 g/l had increased dry yield value up to 30% higher than in control.Similar to our study Pirzad and Barin suggested that foliar spraying of 4g/l zinc enhanced the biological yields of Pimpinella anisum than that for control [30].

For leaf essential oil content all of treatments had significantly increased essential oil than that for control. The higher value of 3.57% was obtained using Nano zincs 1 g/l that it was up to 34% higher than in control. This value was a bit higher than 2.96% that was obtained by foliar spraying of in common Zinc Chelate in peppermint [31]. In the present study all of treatments had significantly increased essential oil than that for control. However, the Nano zincs 1 g/l had increased essential oil yield up to 76% higher than for control. Yazdani Chamheidary *et al*., in study effects of Zn foliar spraying on *Cuminumcyminum* found 2 g/l Zinc Chelate had increased essential oil and oil production up to 13 and 24%, respectively [32].

The use of Nano fertilizer has many benefits. It leads to increased nutrient utilization, reduction of soil toxicity, minimizing negative effects of too much fertilizer. Using Nano fertilizer, the time and speed of release of the nutrients are matched with the nutritional requirement of the plant. Therefore, the plant is able to absorb the highest amount of nutrients and consequently, reducing the leaching of the elements and increasing dry matter yield [33].

Our finding indicated that there were no significant differences between the low and high concentration of Nano Zinc for essential oil, so that, Nano Zinc (0.1 g/l) had increased the essential oil content and oil production up to 34 and 76% than in control, respectively.

Similar to our finding Mohamadipoor *et al*. to determine the suitable type of iron fertilizer and their application in two methods 'foliar and soil' on growth characteristics of Spathyphyllum illusion ornamental plant found that foliar application of Nano iron fertilizer was superior than iron-chelate because it reduced harmful effect of chemical fertilizers into the environment and its lower cost [34].

Essential Oil Compounds

In total, 34 compounds were identified in the essential oil from the aerial parts peppermint. But, the effect of Zn fertilizer was significant for 21 out of 34 compounds. The higher compounds with average values of 19.7%, 7.8%, 5.9%, 5.7%, 4.9% and 4.1% were obtained in Menthol, Menthone, Menthofuran, Piperitone, Menthyl acetate and Beta-caryophyllen, respectively (Table 4).

The effects of Zinc fertilizers were not similar to the essential oil compounds. For example the effects fertilizers were negative on α–Phellandrene, γ-Terpinene, Linalool, Carvone, Pulegone and β-Caryophyllen and higher values of these compounds were obtained in no foliar spraying (control). In other words, these compounds were decreasing by application zinc fertilizer than that for control (Table 4). In contrast, for other major compounds, the effect of zinc fertilizer were positive or non-significant (Table 4).

The results obtained in our study indicated that the major components in the essential oil were ranged as Linalool (1.95 to 3.20%), Menthone (5.9 to 9.5%), Menthofuran (3 to 7.1%), Menthol (17 to 21.7%), Carvone (1.45 to 2.95%), Piperitone (5.0 to 6.1%), Menthyl acetate (4.35 to 6.5%), β-Caryophyllen (2.95 to 6.5%). The presence and concentration of some of essential components were in the same range of other researchers [35, 36, 37, and 38].

Carmines found that peppermint essential oils contain many aromatic chemicals, like Menthol, Menthone, Isomenthone and Menthofuran, which are used in different industries, especially the two first mentioned components. Menthol is used in products of oral hygiene, pharmaceutical and cosmetic industries [39]. Studies have confirmed that this compound has high antifungal as well as antibacterial potential and is important in the scents and essences industry [40]. Result of mean comparisons of Linalool and β-Caryophyllen showed that application of Zinc fertilizer had significantly decreased their mean values lower than that for control (Table 4). For Menthone, the higher values were obtained by spraying of Nano zinc fertilizer; however, there were no significant differences between concentration levels of 1.0 and 1.5 g/l (Table 4). In the other word the 1.0 and 1.5 g/l Nano zinc fertilizer had increased Menthone content up to 48% and 61% higher than the control, respectively. The similar trend was obtained for Menthol, so that spraying Nano zinc of 1.5 g/l had increased Menthol content up 28% higher than in control (Table 4). Similar to our study, Hart *et al* by foliar application of $ZnSO_4$ on peppermint found increasing of Menthol biosynthesis up to 15% to 18% than in control [41].

For Menthofuran, the highest mean value was obtained by spraying both common Zinc Chelate (1.5 g/l) and Nano zinc fertilizer (1 and 1.5 g/l), so that, Menthofuran had strongly increased up to 235%, 237% and 233% higher than the control, respectively.

Dubey *et al*. showed that any increase of total plant carbohydrates that are the most important direct organic product of photosynthesis will be utilized in the biosynthesis of essential oil, consequently increasing the essential oil production [42].

Conclusion

It was concluded that both of Nano zinc and Zinc-Chelate micronutrient significantly increased all of morpho-physiological traits than that for control. The higher values all of the traits were obtained in Zinc-Chelate (1.5 g/l) and Nano zinc (1 g/l), respectively. The effects of treatments were significant at 21 out of 34 compounds. The higher compounds with average values of 19.7%, 7.8%, 5.9%, 5.7%, 4.9% and 4.1% were obtained for Menthol, Menthone, Menthofuran, Piperitone, Menthyl acetate and Beta-caryophyllen, respectively. Nano zinc (1.5 g/l) had increased Menthol, Menthone and Menthofuran content up to 28%, 61% and 237% higher than the control, respectively. It was concluded that foliar spraying of Nano zinc (1 g/l) in flowering stage had increased dry matter yield and essential oil content and (1.5 g/l) concentration had increased some essential oil compound of peppermint.

Table 3 Analysis of variance and mean of squares of some morphological, physiological traits and Essential Oil content and production of *Mentha piperita* L.

SOV	DF	MS							
		Chlorophyll			Stem	Fresh	Dry	Oil%	Oil
		a	b	Total	Number	Weight	Weight		Yield
Treatments	6	0.808 **	0.198 **	1.881 **	265.1 *	12224 **	81.23 *	0.305 **	0.181**
Replication	2	0.173 ***	0.042 *	0.405 *	111.4	2890	68.42	0.011	0.067
Error	12	0.120	0.017	0.250	101.5	1172	22.96	0.018	0.032
Total	20								
CV%		10.26	7.69	9.82	11.53	11.29	12.85	4.11	14.65

*, **: significant at P= 0.05 and 0.01 levels, respectively.

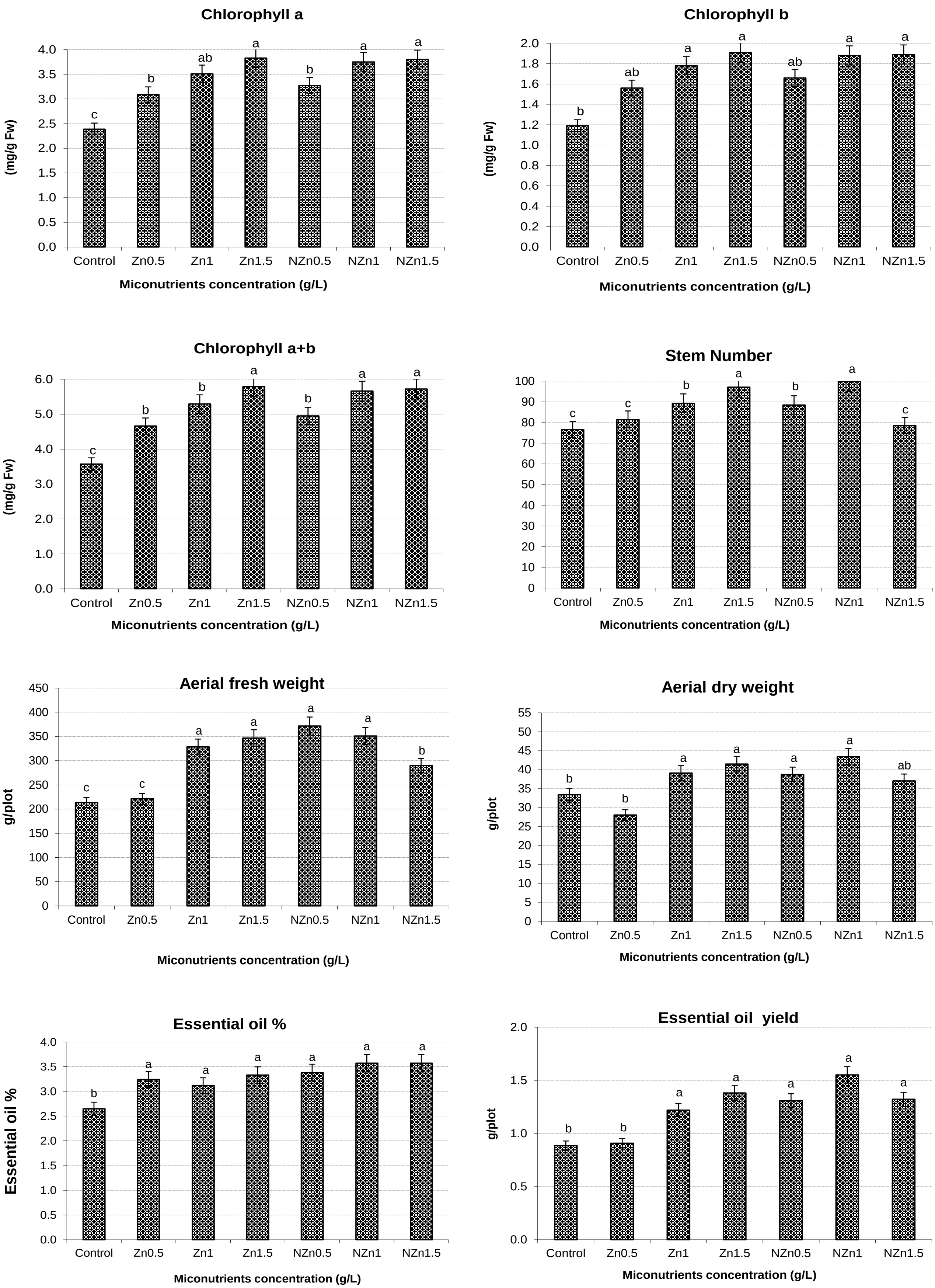

Fig. 1 Means of morphological, physiological traits and essential oil production of *Mentha piperita* L. in different levels of Zinc chelate and Nano zinc chelate fertilizer (Means of column followed by same letters has no significant differences based on Duncan method)

Table 4 Means of the essential oil components of *Mentha piperita* L. in different concentration of Zinc chelate and Nano zinc chelate

Oil components	Peak No.	Retention Index	Zn-chelate (g/l)			Nano-Zn (g/l)			Control	F test
			0.5	1.0	1.5	0.5	1.0	1.5		
α–Pinene	1	921	2.10 b	2.40 b	1.50 c	2.20 b	2.35 b	3.00 a	2.05 c	**
Camphene	2	933	1.80	1.80	1.40	1.95	1.85	1.60	1.45	ns
Sabinene	3	946	1.10 d	1.20 d	2.80 a	1.65 c	1.45 c	2.45 b	2.00 c	**
β-Pinene	4	957	2.25 b	2.70 a	2.05 c	1.70 c	2.65 ab	3.00 a	2.65 ab	*
1-octanol-3-ol	5	977	1.35 b	1.20 b	2.10 a	1.90 a	1.40 b	1.25 b	1.55 ab	*
Myrcene	6	986	2.00 ab	1.30 b	1.65 b	2.25 a	1.10 c	1.20 c	1.80 b	*
α–Phellandrene	7	998	1.70 b	2.30 a	1.70 b	1.55 b	1.30 c	0.90 c	2.55 a	**
α–Terpinene	8	1011	3.05 a	3.10 a	1.65 c	2.70 b	1.90 c	1.70 c	2.25 c	**
Para-Cymene	9	1028	1.65 c	1.80 c	2.35 a	2.00 b	1.85 c	2.05 b	2.00 b	*
Eucalyptol	10	1036	1.75	1.85	1.80	1.95	1.25	1.75	1.95	ns
Limonene	11	1044	2.60 a	3.05 a	1.95 b	1.45 b	2.65 a	1.90 b	2.85 a	**
1,8-Cineole	12	1057	3.00 a	2.05 b	2.65 a	1.90 b	1.90 b	1.25 c	1.90 b	**
β-Ocymene	13	1068	2.45 a	1.45 c	1.50 c	1.45 c	1.80 bc	2.15 b	1.50 c	**
γ-Terpinene	14	1080	1.30 b	1.75 b	1.40 b	2.55 a	1.20 b	1.20 b	2.30 a	**
Linalool	15	1093	2.55 a	2.05 b	2.15 b	1.95 b	2.30 b	2.30 b	3.20 a	*
Isopulegole	16	1112	1.85	2.20	1.85	1.80	2.75	1.85	1.95	ns
Menthone	17	1124	6.60 c	8.00 b	8.40 b	7.70 b	8.75 a	9.50 a	5.90 c	**
Isomenthone	18	1135	2.25	1.95	2.35	1.95	1.55	2.10	2.05	ns
Menthofuran	19	1144	5.00 b	6.20 b	7.05 a	5.60 b	7.10 a	7.00 a	3.00 c	**
Neomenthol	20	1153	1.70	1.50	2.40	1.95	1.85	1.65	1.95	ns
Menthol	21	1172	18.25 c	19.15b	19.95 b	19.30b	20.10 b	21.70 a	17.00 d	**
Myrtenal	22	1183	1.15 b	2.25 a	1.20 b	2.20 a	2.25 a	1.15 b	1.55 b	**
Dihydrocarvone	23	1194	1.60	1.40	1.70	1.30	1.65	1.90	1.15	ns
Carvone	24	1219	2.80 a	1.85 cd	2.45 b	1.45 d	2.10 c	1.70 cd	2.95 a	**
Pulegone	25	1228	1.40 c	2.05 b	1.60 b	2.95 a	1.45 c	1.00 c	2.75 a	**
Piperitone	26	1241	6.10	5.95	5.60	5.95	5.70	5.00	5.45	ns
Borneol	27	1256	1.20	1.40	1.90	1.45	2.00	1.80	1.65	ns
Menthyl acetate	28	1282	6.50 a	4.60 b	4.30 c	4.40 b	4.90 b	4.60 b	4.70 b	**
Carvacrol	29	1295	1.35	2.30	2.20	2.00	2.10	2.05	2.15	ns
β-Bourbonene	30	1364	1.80	1.40	1.10	1.30	1.25	1.70	1.86	ns
β-Caryophyllen	31	1396	5.00 b	3.60 c	2.85 c	4.45 b	3.00 c	2.95 c	6.50 a	**
α-Humulen	32	1419	1.10	0.85	1.00	1.30	1.20	1.35	1.75	ns
Dihydrocarvyl acetate	33	1430	0.95	1.50	1.20	1.10	1.00	1.10	0.90	ns
Germacren D	34	1465	2.05	1.80	1.25	1.90	1.65	1.55	1.70	ns

Means of Rows followed by same letters have no significant differences based on Duncan method

In the present study, all of treatments had significantly increased essential oil than that for control. However, the Nano zincs 1 g/l had increased essential oil yield up to 76% higher than for control.

In our study, only one cut was harvested in the establishment year. So, the essential oil yield was lower than those reported in the literature. The oil production expected to increase in the second and third years (having three cuts per year). Similar to our result, Amoie and Kameli in an entrepreneurship package of peppermint in Iran, suggested that the net profit of peppermint in the establishment year was negative, but in the following years, the net profit was increased for each investment unit to 36% per year [4].

References

1. Golparvar, A.R. and Hadipanah, A. Chemical compositions of the essential oil of peppermint (*Menthapiperita* L.) cultivated in Isfahan conditions. J. Herb Drug. 2013;4:75-80.
2. Ormancey, X., Sisalli, S. and Coutiere, P. Formulation of essential oils in functional perfumery. Parfums Cosmetiques Actualites. 2001;157:30–40.
3. Sefidkon, F. Strategic Medicinal Plants Research Program. Research Institute Forests and Rangelands Publication, Tehran, 40p. 2008. (In Persian).
4. Amoie, A., Kameli, M. Entrepreneurship Package of Peppermint (*Mentha piperita*, L.) in Iran. National council for science and technology. Development of medicinal and aromatic plants and traditional medicines. Tehran, Iran. 2015. (In Persian).
5. Tucker, A.O. The truth about mints. Herb Companion. 1992;4:51-52.
6. Mozaffarian, V. A pictorial dictionary of botany, botanical taxonomy Latin-English- French-Germany-Persian. Germany: Koeltz Scientific Books, 522 p. 2008.
7. Abdossi, V., Ghahremani, A., Hadipanah, A., Ardalani, H.R., Aghaee, K. Quantitative and qualitative responses in chemical composition of three ecotypes of fennel (*Foeniculum vulgare* Mill.) cultivated in Iran climatic conditions. J Biodiv Envir Sci. 2015;6:401-407.
8. Abedi, R., Golparvar, A.R., Hadipanah, A. Identification of the essential oil composition from four ecotypes of *Mentha longifolia* (L.) Huds. Growing wild in Isfahan province, Iran. J BioSci Biotec. 2015;4:117-121.
9. Kabata-Pendias A. Trace elements in soils and plants, CRC press. P: 548, 2010.
10. Todeschini V, Lingua G. Agostino GD, Carniato F, Roccotiello E, Berta G. Effects of high zinc concentration on poplar leaves: a morphological and biochemical study. Environ. Exp. Bot. 2011;71:50-6.
11. Marschner H. and Marschner P. Marschner's mineral nutrition of higher plants, Academic press, p: 672, 2012.
12. Grotz, N. and M. L. Guerinot. Molecular aspects of Cu, Fe and Zn homeostasis in plants'. Biochimicaet Biophysica Acta (BBA)-Molecular Cell Research. 2006;1763:595-608.
13. Pandey N., Pathak G.C., Sharma C.P. Zinc is critically required for pollen function and fertilization in lentil. J. Trace Elem. Med. Biol.. 2006;20:89–96.
14. Cakmak I. Enrichment of cereal grains with zinc: Agronomic or genetic bio-fortification. Plant Soil. 2008;302:1–17.
15. Auld, D. S. 'Zinc coordination sphere in biochemical zinc sites'. Biometals. 2001;14:271-313.
16. Genc, Y., McDonald G. K. and Graham. R. D. 'Contribution of different mechanisms to zinc efficiency in bread wheat during early vegetative stage'. Plant Soil. 2006;281:353-367.
17. Naderi, M., Danesh Shahraki, A.A. & Naderi, R. Application of nanotechnology in the optimization of formulation of chemical fertilizers Iran. J. Nanotechnol. 2011;165:21-23.
18. Pozveh Z T., Roya, R. and Fatemeh, R. Changes occurring in canola (*Brassica napus* L.) in response silver nanoparticles treatment under *in vitro* conditions. Indian J Fund Appl Life Sci. 2014;4:797-807.
19. Brennan, R. F., Bolland M. D. A. and Shea. G. 'Comparing how *Lupinus angustifolius* and *Lupinus luteususe* zinc fertilizer for seed production'. Nutr. Cycling Agroecosyst. 2001;59:209-217.
20. El-Sawi, S. A. and Mohamed. M. A. 'Cumin herb as a new source of essential oils and its response to foliar spray with some micro-elements'. Food Chem. 2002;77:75-80.
21. Gadallah, M. A. A. 'Effects of indole-3-acetic acid and zinc on the growth osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit'. J. Arid Environ 2000;44:451-467.
22. Heitholt, J. J., Sloanand J. J., Mackown. C. T. 'Copper, manganese and zinc fertilization effects on growth of soybean on a calcareous soil'. J Plant Nutr. 2002;25:1721-1740.
23. Mirzapour, M. H. and Khoshgoftar, A. H. 'Zinc application effects on yield and seed oil content of sunflower grown on a saline calcareous soil'. J Plant Nutr. 2006;29:1719-1727.
24. Chatterjee, C. and Khurana, N. 'Zinc stress-induced changes in biochemical parameters and oil content of mustard'. Commun. in Soil Sci. Plant Anal. 2007;38:751-761.
25. Wissuwa, M., Ismail A. M. and Graham, R. D. 'Rice grain zinc concentrations as affected by genotype native soil –zinc availability, and zinc fertilization'. Plant Soil. 2008;306:37-48.
26. Arnon, D. Copper enzymes in isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 1949;24;1-75.
27. Hungarian pharmacopoeia. VII Kiadas, Kotet, Medicine Publication, Hungary, 918p. 1984.
28. Adams, R.P. Identification of essential oil components by Gas Chromatography/Mass Spectroscopy, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA. 2007.
29. Mahmoodi, P., Yarnia, M., Rashidi, V., Amirnia, R. Tarinejhad. A. Effects of Nano and chemical *fertilizers on* physiological efficiency, and essential oil yield *of Borago officinalis* L. Appl Ecol Env Res. 2018;16:4773-4788.
30. Pirzad, A. and Barin, M. Iron and zinc interaction on leaf nutrients and the essential oil of *Pimpinella anisum* L. Iran. J. Plant Physiol. 2018;8:2507-2515.
31. Heidari F., Zehtab S., Javanshir A., Aliari H.,Dadpour M. The effect of micronutrients consumption and plant density on yield and essential oil of peppermint (*Mentha piperita* L.). Iran J Med. Arom. Plant Res. 2008;24:9-19. (In Persian).
32. Yazdani Chamheidary Y, Ramroudi M, Asgharipour M R. Evaluation the effects of drought stress on yield, yield components and quality of (*Cuminum cyminum* L.) under Fe and Zn foliar spraying conditions. Appl Res Plant Ecophysiology. 2014;1:81-96. (In Persian).
33. De Rosa MC, Monreal C., Schnitzer M., Walsh R., Sultan Y. Nanotechnology in fertilizers. Nat Nanotechnol. 2010;5:91.

34. Mohamadipoor, R., Sedaghathoor, S. and MahboubKhomami, A. Effect of application of iron fertilizers in two methods 'foliar and soil application' on growth characteristics of *Spathyphyllum illusion*. Eur. J. Exp. Biol. 2013;3:232-240.
35. Derwich, E., Benziane, Z., Taouil, R., Senhaji, O. and Touzani, M. Aromatic plants of morocco: GC/MS analysis of the essential oils of leaves of *Menthapiperita*. Adv Environ Biol. 2010;4:80-85.
36. Ka, M.H., Choi, E.H., Chun, H.S. and Lee, K.G. The antioxidative activity of volatile extracts isolated from *Angelica tenuissimae* roots, peppermint leaves, pine needles, and sweet flag leaves. J. Agric. Food Chem. 2005;53:4124-9.
37. Iscan, G., Demirci, F., Kirimer, N., Kurkcuoglu, M. and Baser, K. H. C. Antimicrobial screening *Mentha piperita* essential oil. J. Agric. Food Chem. 2002;50:3943-3946.
38. Abbaszadeh, B., Teymoori, M., Pouyanfar, M., Rezaei, M.B. and Mafakheri, S. Growth and essential oil of *Mentha longifolia* L. (var. amphilema) from different ecological conditions. Ann. Biol. Res.. 2013;4:85-90.
39. Carmines, E.L. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: cigarette design, testing approach, and review of results. Food Chem. Toxicol. 2002;40:77-91.
40. Souza M.P., Matos N.E.O., Matos F.J.A. Constituintes Químicos de Plantas Medicinais Brasileiras. Imprensa Universitária/UFC, Fortaleza. p. 416. 1991.
41. Hart J. M, Christensen NW, Mellbye ME and Gingrich GA. Nutrient and biomass accumulation of peppermint. Proceedings, Western Nutrient Management Conference. 2003;5:63-70.
42. Dubey, V. S., Bhalla, R. and Luthra, R. Sucrose mobilization in relation to essential oil biogenesis during palmarosa (*Cymbopogon martini* Roxb. Wats. Var. Motia) inflorescence development. Biosci. 2003;28:479-487.
43. Marotti, M., Dellacecca, V., Piccaglia, R. and Giovanelli, E., Effect of harvesting stage on the yield and essential oil composition of peppermint (*Mentha piperita* L.). Acta Hort. 1993;344:370-379.
44. Baranauskieneú, R., Venskutonis, P., Visÿkelis, P., Dambrauskiene, E. Influence of Nitrogen Fertilizers on the Yield and Composition of Thyme (*Thymus vulgaris*), J. Agric. Food Chem. 2003;51:7751-7758.
45. Kennedy, I. R., Choudhury, M. A., Kecskes, M. L. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited. Soil Biol Biochem. 2004;36:1229-1244.