Chemical composition of *Mentha pulegium* L. (Pennyroyal) plant as influenced by foliar application of different sources of zinc

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Background and objective
Mentha pulegium L. is commonly known as pennyroyal and it is highly aromatic than any other mint. The essential oil could be considered as a possible candidate for human cancer chemotherapy. This study was carried out to evaluate the effect of different sources of zinc (algae extract, zinc sulfate, zinc multi, and zinc chelated) on herb yield, nutrient contents and their uptake, carbonic anhydrase, and essential oil production of M. pulegium plant.

Materials and methods
A field experiment was carried out under drip irrigated sandy soil at the Experimental Station of National Research Centre in Nubaria district, El-Behira Governorate, Egypt. Macronutrients and micronutrients contents of herb, nutrient uptake, carbonic anhydrase activity, and essential oil content were determined. Essential oil constituents were analyzed by chromatography-mass spectrometry.

Results
The results showed that algae extract followed by zinc multi significantly increased herb fresh and dry weight yield, nutrients content and their uptake, as well as showed the stimulatory impact on carboxylation enzyme activities. The highest essential oil yield (0.93 ml/plant and 20.67 l/ha) was recorded with algae extract, followed by zinc multi (0.80 ml/plant and 17.78 l/ha) than zinc chelate (0.50 ml/plant and 11.11 l/ha). Chromatography-mass spectrometry analyses of the essential oil showed that the essential oil composition was characterized by a high percentage of oxygenated compounds (96.83–97.33%) while the nonoxygenated compounds ranged from 2.48 to 2.89%. The major constituents of oxygenated compounds were found to be pulegone (67.75–74.43%) followed by neomenthone (10.66–17.12%). Algae extract and zinc multi produced the highest relative concentration of neomenthone (17.12 and 16.72%) and the lowest concentration of pulegone (67.75 and 67.97%). In contrast, foliar application of zinc chelated and zinc sulfate increased the biosynthesis of pulegone (74.43 and 73.98%) and decreased the percent of neomenthone (11.39 and 10.66%).

Conclusion
It might be concluded that the foliar application of zinc as algae extract followed by zinc multi chelated gave remarkable higher increases in herb yield, nutrients content and their uptake, carbonic anhydrase and essential oil production of *M. pulegium* plant.

Keywords:
essential oil, *Mentha pulegium*, pulegone, zinc sources

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Introduction

Mentha pulegium L. is popularly known as pennyroyal. The composition of *M. pulegium* has been studied and the three chemo types have been established as pulegone-type, piperitenone/piperitone-type, and isomenthone/neoisomenthol-type [1]. Pennyroyal is recognized as a source of pulegone, Aziz and Craker [2] stated that pulegone (88.05%) was the main constituent of pennyroyal oil under desert agro-system in Egypt, which is in accordance of some previously studies. Pulegone was the major components in Massachusetts, USA (82.61%) [3], Uruguay (73.4%) [4], and Bulgarian (45.4%) [5]. *M. pulegium* L. pose antiseptic, antispasmodic, insect repellent, carminative, diaphoretic and anti-inflammatory [6], antimicrobial [7], antioxidant [8], and abortifacient [9] properties. Herb was used for the treatment of fibrosis and cervical tumors [10]. Essential oil might be considered as a potentially toxic agent on human cancer cell lines, and a possible candidate for human cancer chemotherapy. However, further biological tests on the efficacy and side effects of this plant are necessary before its use for human [11].

Many investigators studied the effect of organic and biofertilizers as compared with chemical fertilizers on different plants. In this connection, Nofal *et al*. [12] showed that using algae as biofertilizers, improve the physiological performance of the plant. The effect of biofertilizers on vegetative growth, yield, and oil productivity of mint plant had been emphasized [13]. However, algae are considered as an important group of microorganisms capable of fixing atmospheric nitrogen, however, extracts of algae naturally contain auxin, cytokinins, and gibberellic acid [14].

Numerous studies found positive responses for applied micronutrients (Fe, Mn, and Zn) as a foliar application on growth, chemical composition, and flowering of different medicinal and aromatic plants; *Rosmarinus officinalis* [15]and *Pelargonium graveolens* [16]. In contrast, it was cleared that micronutrients, especially Zn and B either acted as metal components of various enzymes, as functional, structural, or regulatory cofactors. In addition, the role of zinc in the metabolism of RNA and ribosomal content in the plant cells led to the stimulation of carbohydrates, protein, and DNA formation [17].

Micronutrients are added to foliar fertilizers, to compensate for their deficiencies, especially in arid and semi-arid regions with calcareous soils [18]. Using some rich sources of zinc as the foliar application might be considered as a specific management for improving growth and physiological status of the plants, which is reflected on the crop yield and its quality. Therefore, the current study aimed to investigate the effect of zinc foliar application from different sources, comprising algae extracts, zinc sulfates, zinc multi, and zinc chelated on herb yield, nutrients content, carbonic anhydrase, and essential oil production of *M. pulegium* L. plant.

Materials and methods

This study was performed at the Experimental Station of National Research Centre in Nubaria district, El-Behira Governorate, Egypt, using a drip irrigation system during 2017 season to study the effect of different sources of zinc, that is, algae extract, zinc sulfate, zinc multi, and zinc chelated on herb yield, nutrients content and their uptake, carbonic anhydrase activity, and essential oil production of *M. pulegium* plant.

Representative surface soil samples (0–30 cm) from the experimental site were taken and analyzed [19]. Physical and chemical analyses were as follows : sand 91%, silt 2%, clay 7% (Sandy texture), pH 7.9, E.C. 0.93 mmhos/cm, calcium carbonate 4%, organic matter 1% while, N, P, K, Ca, Mg, Na contents were 35, 2.9, 11, 14, 9, and 44 mg, respectively, in 100 g/soil. Whereas, Fe, Mn, Zn, and Cu contents were 4.43, 4.30, 1.10, and 1.60 ppm, respectively.

During the soil preparation for cultivation, organic manure (40 m^3/ha), calcium superphosphate (300 kg/ha), and potassium sulfate (150 kg/ha) were added as recommended for the region.

The layout of the experiment was in randomized complete blocks design with three replications including five treatments, which were control, algae extract, zinc sulfate, zinc multi, and zinc chelated (Table 1). The four fertilizers were added as a foliar application at a rate of 45 ppm zinc at the end of May.

The plants were established from cuttings originally secured from the Medicinal Plant Program in the Department of Plant, Soil, and Insect Sciences at the University of Massachusetts, Amherst, USA. A sufficient number of plants were kept in the nursery of the National Research Center as the source of the cuttings used in this experiment. The cuttings (8 cm long) were rooted in the sand during February in a greenhouse. On March 15, 2017, seedlings were transplanted into the experimental field at Nubaria district with 60 cm adjusted to dripper lines, which were 75 cm apart (the experimental unit area was 5.4 m^2 with 12 plants). Irrigation of the plants was done using the drip system (10 l/day).

Foliar application of the used treatments was performed on May 30, 2017. Two weeks later, fresh and dry herb (g/plant and t/ha), branches number/plant, and moisture % were recorded.

Macronutrients and micronutrients contents of the herb were analyzed and the nutrient uptake was calculated [19].

Table 1 The chemical analysis of different sources of zinc

Zinc sources	Chemical composition
Algae extract	13.3% N, 2.22% P, 2.13% K, 0.22% Mg, 0.01% Na, 0.44% Ca, 19.3% Fe, 6.8% Mn, 4.5% Zn, 1.8% Cu, total amino acids 15.89%, 13.7 mg/g indole acetic acid, 3.2 mg/g indole butyric acid, and 1.2 mg/g gibberellic acid
Zinc sulfate	22% Zn
Zinc multi (chelated)	14% N, 1.5% Fe, 3.0% Mn, and 4.5% Zn
Zinc chelated	13% Zn

Determination of carbonic anhydrase activity assay was carried out [20].

Essential oil content of the fresh herb was determined by hydro-distillation using Clevenger-type apparatus in all the treatments. The resulted essential oil was separately dried over anhydrous sodium sulfate according to the Egyptian Pharmacopoeia, 1984 [21] and then kept in the refrigerator till chromatography-mass spectrometry (GC-MS) analyses. Then, the essential oil contents (percentage and ml/plant and l/ha) were calculated.

The essential oils were subsequently analyzed by GC-MS instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Centre with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., Waltham, Massachusett, USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m×0.25 mm internal diameter, i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1 : 10 and the following temperature program: 60°C for 1 min; rising at 3.0C/min to 240°C and held for 1 min. The injector and detector were held at 240°C. Diluted samples (1 : 10 hexane, v/v) of 0.2 μl of the mixtures were always injected. Mass spectra were obtained by electron ionization at 70 eV, using a spectral range of m/z 40–450. Most of the compounds were identified using the analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection, and NSIT library).

Statistical analysis

The obtained data were statistically analyzed for the least significant differences according to Sendecor and Cochran [22].

Results and discussion

Growth characters

Differences in herb fresh and dry weights (g/plant and t/ha), as well as a number of branches per plant of *M. pulegium,* were observed as a result of zinc foliar application, including algae extracts, zinc sulfate, zinc multi, and zinc chelated (Table 2). These parameters were affected by using all the treatments of zinc. However, algae extract gave the maximum response while zinc sulfate recorded the lowest one. In contrast, all treatments significantly decreased moisture content by 4% compared with the control, which is reflected on increasing the dry content. It has been stated that zinc is an essential micronutrient for metabolic processes in plants [23]. Furthermore, it has been reported that zinc is a component of carbonic anhydrase, as well as the function of zinc in CO_2 assimilation increases the fresh and dry weight of herbs [24]. Zinc also has many essential roles in the plant growth and production of biomass, chlorophyll production, pollen function, fertilization, metabolism of RNA, proteins and DNA formation, and it is required for the synthesis of tryptophan, a precursor of IAA that acts as a growth promoting substance [24,25].

The beneficial effect of algae extracts on several plants had been confirmed [12]. In this connection, it had also been concluded that seaweed extracts contain naturally occurring auxin, cytokines, and gibberellic acid, enhancing the shoot growth and promoting nutrient uptake [26].

Essential oil content (%, ml/plant and l/ha)

The foliar application with all zinc treatments increased positively the essential oil percentage over the control (Table 2). The highest essential oil percentages (1.44 and 1.39%) were obtained with zinc multi and algae extract when compared with the other zinc sources and control. Data also showed that oil yield (ml/plant and l/ha) varied between zinc sources in the following order: algae extract more than zinc multi more than zinc chelate more than zinc sulfate. In contrast, the highest essential oil yield (0.93 ml/plant and 20.67 l/ha) was recorded for algae extract, followed by zinc multi (0.80 ml/plant and 17.78 l/ha) and then zinc chelate (0.50 ml/plant and 11.11 l/ha). Whereas, zinc sulfate application recorded

Table 2 Effect of different zinc sources on growth, yield, and essential oil production of *Mentha pulegium* L

Character treatments	Herb F.W. (g/ plant)	Herb D.W. (g/plant)	Herb F.W. (t/ha)	Herb D.W (t/ha)	Moisture (%)	Branches no. (/plant)	Oil (%)	Oil yield (ml/ plant)	Oil yield (l/ha)
Control	41.3^a	14.3^{ab}	0.92^a	0.32^a	65.4^a	13^a	0.68^a	0.28^a	6.22^a
Algae extract	66.8^d	22.6^e	1.48^d	0.50^c	66.2^a	21^b	1.39^d	0.93^e	20.67^e
Zinc sulfate	43.7^a	13.5^a	0.97^a	0.30^a	69.2^b	14^a	0.91^b	0.40^b	8.89^b
Zinc multi	55.3^c	18.7^d	1.23^c	0.42^b	66.1^a	18^b	1.44^d	0.80^d	17.78^d
Zinc chelated	48.7^b	14.9^{bc}	1.08^b	0.33^a	69.4^b	19^b	1.03^c	0.50^c	11.11^c
LSD of 5%	4.10	1.30	0.10	0.06	3.2	3.00	0.11	0.11	2.15

All values with the same letter are insignificantly different at P value more than 0.05.

0.40 ml/plant and 8.89 l/ha of essential oil. The bioorganic and organic sources of zinc led to a remarkable increase in oil yield over the control and mineral sources of zinc. These results might be attributed to the fertilizer's composition and the reflection on the carbohydrate and protein formation and movement in plant tissues. It had been reported that the application of micronutrients increased essential oil percentage and its yield in peppermint [27]. The yield of the flowering heads, essential oil percentage, and essential oil yield of chamomile were increased by foliar application of Fe and Zn [18].

Nutrient contents

Macronutrients and micronutrients contents in pennyroyal plants have differed significantly with different zinc sources (Table 3).

Macronutrients content

Nitrogen and potassium content of *M. pulegium* varied according to the zinc sources. All zinc treatments significantly increased N and K content of pennyroyal herb as compared with control, while P, Ca, and Mg were insignificantly affected with the same treatments (Table 3). In addition, algae extracts showed the most pronounced effect on the macronutrient contents. These results might be attributed to the high N and K content in both algae extract and zinc multi. Moreover, it could also be caused by high amino acids content in algae extracts and the reverse with zinc chelated.

Micronutrient contents

Fe and Mn contents were significantly increased with algae and zinc multi. However, zinc content was positively affected with all zinc treatments when compared with the control, while the opposite trend was true with Ca, Mg, and Cu. These results may be due to the nutrient contents of zinc sources. It was obvious from previous data that the different zinc sources have a remarkable influence on the growth and yield characters, as well as nutrient contents of *M. pulegium* (pennyroyal). In this connection, the results revealed that micronutrients, such as zinc, have important roles in growth and nutrients content [28,29]. The results of macronutrients and micronutrients in the plant as a response to zinc foliar application may be a reflection to nutrient levels in the soil.

Nutrient uptake

Macronutrients and micronutrients uptake of *M. pulegium* as affected by foliar application with different zinc sources. Table 4 illustrated that the algae extract had significantly the highest nutrients' uptake and to a less extent the zinc multitreatment. The foliar application with zinc as the sulfate form was insignificant on the uptake of all nutrients. It might be concluded that all tested Zn sources had a positive effect on *M. pulegium*. Moreover, algae extract followed by zinc multi fertilizers were more effective than the sources on the studied characteristics.

Table 3 Effect of different zinc sources on nutrient contents of *Mentha pulegium* L.

Character treatments	Macronutrients (%)					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Control	1.75^{a}	0.14	1.6^{a}	0.42	0.33	201^{a}	25^{a}	39^{a}	1.8
Algae extract	2.15^{c}	0.13	2.4^{b}	0.41	0.35	285^{c}	36^{b}	44^{b}	2.0
Zinc sulfate	1.82^{ac}	0.15	2.3^{b}	0.45	0.34	210^{a}	27^{a}	42^{b}	2.2
Zinc multi	2.03^{b}	0.13	2.0^{b}	0.43	0.33	261^{b}	36^{b}	45^{b}	2.0
Zinc chelated	1.95^{b}	0.13	2.1^{b}	0.42	0.34	206^{a}	28^{a}	43^{b}	2.0
LSD of 5%	0.12	NS	0.4	NS	NS	19	6	2	NS

All values with the same letter are insignificantly different at *P* value more than 0.05.

Table 4 Effect of different zinc sources on nutrient uptake of *Mentha pulegium* L

Character treatments	Macronutrients (g/plant)					Micronutrients (mg/plant)			
	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Control	0.25^{a}	0.02^{a}	0.23^{a}	0.06^{a}	0.05^{a}	2.87^{a}	0.36^{a}	0.56^{a}	0.03^{a}
Algae extract	0.49^{c}	0.03^{a}	0.54^{c}	0.09^{b}	0.08^{b}	6.44^{c}	0.81^{b}	1.00^{d}	0.04^{a}
Zinc sulfate	0.25^{a}	0.02^{a}	0.31^{a}	0.06^{a}	0.05^{a}	2.38^{a}	0.36^{a}	0.57^{a}	0.03^{a}
Zinc multi	0.38^{b}	0.03^{a}	0.37^{b}	0.08^{a}	0.06^{a}	4.88^{b}	0.67^{b}	0.84^{c}	0.03^{a}
Zinc chelated	0.29^{a}	0.02^{a}	0.31^{a}	0.06^{a}	0.05^{a}	3.07^{a}	0.41^{a}	0.64^{b}	0.03^{a}
LSD of 5%	0.09	NS	0.12	0.02	0.02	1.45	0.28	0.07	NS

All values with the same letter are insignificantly different at *P* value more than 0.05.

Carbonic anhydrase activity

All the physiological and biochemical assign were significantly influenced by the implementation of organic (algae extract, zinc multi, and zinc chelated) and inorganic source of zinc (zinc sulfate). Carbonic anhydrase activity in pennyroyal leaves extract and zinc content under various sources of zinc is shown in Tables 2 and 5. It has been found that the foliar spraying with algae extracts and zinc multi showed stimulatory impacts on the zinc content. In contrast, the other sources of zinc, such as Zn SO_4 and Zn chelated did not show the stimulatory effect on the Zn content. This may be explained by the important role of Zn multi chelated in the chelation of metal ions, translocation, or utilization of zinc [30,31]. The carbonic anhydrase activity increased as a result of different Zn regimes and the algal extract treatment or Zn multi showed to be superior of carbonic anhydrase activity compared with foliar application of zinc sulfate and zinc chelated. The carbonic anhydrase can facilitate the synthesis of certain enzymes, which may be due to the nature of algae, which are rich sources of several fine chemicals of economic value, such as vitamins, carotenoids, phycobiliprotein, polysaccharides, and fatty acids [32].

Table 5 Effect of different zinc sources on carboxylation enzyme activities of *Mentha pulegium* L

Character variants	CA (EU/mg protein)
Control	7.47[a]
Algae extract	49.89[c]
Zinc sulfate	10.33[a]
Zinc multi (chelated)	19.26[b]
Zinc chelated	10.56[a]
LSD 0.05	3.37

All values with the same letter are insignificantly different at *P* value more than 0.05.

Zn plays an important role in auxin formation and in other enzyme systems and is recognized as an essential component in several carboxylating enzymes, dehydrogenases, proteinases, and peptidases [33]. It has been stated that algae contain nutrients and hormones that might invigorate enzymes or modify the permeability of a membrane. This could excite a cascading effect resulting in increased cellular metabolism and enhanced aggregation of distinct stringent intermediate [34]. The increased growth

Table 6 Effect of different zinc sources on essential constituents of *Mentha pulegium* L

N	Rt.	Compounds	Area (%)				
			Control	Algae extract	Zinc sulfate	Zinc multi	Zinc chelated
1	5.17	4-Methyl-1,6-heptadien-4-ol	0.24	0.15	0.12	0.22	0.26
2	5.65	Sabinene	Traces	0.04	0.05	0.03	0.05
3	5.82	α-Pinene	0.24	0.28	0.28	0.24	0.27
4	6.10	β-Pinene	0.22	0.23	0.26	0.21	0.22
5	6.42	3-Octanol	1.29	1.54	1.56	1.40	1.46
6	7.41	D-Limonene	0.81	0.77	1.08	0.91	0.95
7	8.55	3-octanyl acetate	0.09	0.08	0.09	0.09	0.09
8	9.48	α-Terpinolene	0.08	0.08	0.09	0.09	0.09
9	12.45	Neomenthone	11.56	17.12	10.66	16.72	11.39
10	12.82	D-Menthone	1.61	2.74	1.57	3.05	1.81
11	13.34	Isopulegone	4.86	4.63	5.69	4.92	4.91
12	14.09	α-Terpineol	0.46	0.42	0.47	0.42	0.42
13	14.84	Spiro[adamantane-2,5′-[1.2]dioxolan]-3′-one, 4′-methylene-	0.20	0.15	0.07	0.17	0.21
14	16.37	Pulegone	73.90	67.75	73.98	67.97	74.43
15	16.69	Piperitone	0.29	0.46	0.31	0.52	0.29
16	17.31	(+)-Isopiperitenone	0.20	0.16	0.20	0.16	0.16
17	20.18	Piperitenone	2.24	1.90	1.98	1.58	1.58
18	23.01	Trans-Caryophyllene	0.90	0.73	0.77	0.66	0.73
19	24.51	α-Caryophyllene	0.58	0.47	0.50	0.47	0.48
20	29.61	Caryophyllene Oxide	0.05	0.04	0.04	traces	0.04
Total of nonoxygenated compounds			2.75	2.48	2.89	2.49	2.65
Total of oxygenated compounds			97.07	97.22	96.83	97.31	97.14
Total of identified compounds			99.82	99.74	99.77	99.83	99.84

Traces=less than 0.04.

accomplishment of plants could be caused by the competence of microalgae to natural nutrient cycles. It can also be accomplished by enriching plants' performance with various nutrients, hormones, and secondary metabolites, which have the definitive effectiveness on growth [35,36].

Essential oil constituents

Data in Table 6 showed the effect of foliar spraying with different sources of zinc, that is, algae extract, zinc sulfate, zinc multi, and zinc chelated on the essential constituents of *M. pulegium* L. The essential oil composition *of M. pulegium* was characterized by high percentages of oxygenated compounds (96.74–97.22%) while nonoxygenated compounds ranged from 2.56 to 2.98%.The major constituents of oxygenated compounds were found to be pulegone (67.75–74.43%) followed by neomenthone (10.66–17.12%) and isopulegone (4.63–5.69%). These results agreed with several studies, which have reported that pulegone (88.05%) was the main constituent of pennyroyal oil under a desert agro-system in Egypt [2]. Moreover, pulegone was the major component in Massachusetts, USA (82.61%) [3] and Uruguay (73.4%) [4]. Algae extract and application of zinc multi produced the highest relative concentration of neomenthone (17.12 and 16.72%) and the lowest concentration of pulegone (67.75 and 67.97%).

In contrast, foliar application of zinc chelated and zinc sulfate increased the biosynthesis of pulegone (74.43 and 73.98%) and decreased the percent of neomenthone (11.39 and 10.66%).

The biosynthesis of secondary metabolites is affected intensely by ecological factors. Plant nutrition as an environmental factor affects essential oil production. CO_2 and glucose are precursors of monoterpene biosynthesis and are the most likely sources of carbon utilized in terpenoid biosynthesis. Carbohydrates are a source of energy and reducing power for terpenoid synthesis. CO_2 fixation, the content of primary metabolites and sucrose metabolism are closely linked with essential oil accumulation [37]. Zinc is involved in photosynthesis and carbohydrate metabolism. It 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.

Conclusion

It might be concluded that the foliar application of biofertilizer as algae extract followed by the organic source as zinc multi and chelated could be effective in increasing or improving the quantity and quality of *M. pulegium* plant grown under the sandy soil.

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Conflicts of interest

There are no conflicts of interest.

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