Influence of Copper and Zinc on Growth, Metal Accumulation and Chemical Composition of Essential Oils in Sweet Basil (*Ocimum basilicum* L.)

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Abstract

Background: **Biosynthesis and metabolism of phytochemicals in medicinal and aromatic plants are vigorously affected by different abiotic elicitors including chemicals.**

Objective: **This experiment was designed to investigate the effects of three levels of copper sulfate (Cu: 0, 5, 25 mg kg^{-1}) and zinc sulfate (Zn: 0, 10, 50 mg kg^{-1}) and their combinations on yield, chemical compositions of essential oil and metals accumulation in sweet basil (*Ocimum basilicum* L.).**

Methods: **The amount of employed metals dissolved in 200 ml distilled water and sprayed over each pot, layer by layer as evenly as possible. Physical and chemical properties and concentration of the elements in soil samples were measured. The essential oil was isolated by hydro-distillation and analyzed by GS and GS-MS technique.**

Results: **Results showed that the dry weight of root, shoot and essential oil yield were increased in treatments of Cu_0Zn_{10}, Cu_5Zn_0 and Cu_5Zn_{10}, as compared to control. The increase of Zn levels in soil enhanced the concentration of Zn in shoot and root of plant when compared to control. Also, Zn and Cu antagonized the uptake of together at the high levels. Root tissues always showed greater concentration of both Cu and Zn than those of shoot. The content of linalool and methyl chavicol was significantly increased by application of employed metals in comparison to control plants.**

Conclusion: **Findings indicate that the growth and essential oil yield of *O. basilicum* was significantly enhanced by application of low levels of Cu and Zn. However, the highest levels of Cu and Zn were accumulated in root, without significant reduction in growth and biomass values.**

Keywords: ***Ocimum basilicum* L., Copper, Essential oil, Linalool, Zinc**

Introduction

In this century, heavy metal pollution is one of the main environmental concerns due to its impact on human health through the food chain and its high persistence in the environment [1]. It is known that accumulation of certain micronutrients as heavy metal in soils can be toxic for most plants [2]. In this context, copper (Cu) and Zinc (Zn), are the main heavy metals contaminants that their 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 [3-5]. Moreover, Cu and Zn are essential micronutrient for normal plant growth and metabolism. In plants, Cu and Zn play 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 [2, 5, 6, 7, 8]. However, an excess of these metals may inhibit plant growth and development [3], induces oxidative stress by catalyzing the formation of harmful reactive oxygen species (ROS) [9], changes membrane integrity and permeability [10], as well as affects the uptake of other nutrient elements [2].

Among the non-food crops, essential oil bearing plants are widely grown in Iran. Basil (*Ocimum basilicum* L.), belonging to the family Lamiaceae, is an annual, herbaceous and white-purple flowering plant [11], generally known under the name “Reyhan” in Iran. It is native to Iran and commonly used as spices, flavouring agent and traditional herb [12]. The essential oil of basil (*Ocimum basilicum* L.) has also been popularly used in manufacturing of cosmetic and pharmaceutical products or pesticides [13]. The effects of metal application on yield of food and non-food crops were extensively studied. Moreover, several studies have demonstrated the ability of *O. basilicum* to tolerate and accumulate high amounts of heavy metals in its tissues [14, 15], and have also shown that some positive effects of soil amended with microelements on the growth and yield of medicinal plants [16, 17]. However, the effects of Cu and Zn on growth, uptake of mineral elements and essential oil production of medicinal plants are poorly understood. Thus, the aim of this study was to examine the effects of Cu and Zn on growth, nutrient uptake and essential oils content and constituents of *O. basilicum* under greenhouse conditions.

Materials and methods

Characterization of the soil

A pot experiment was conducted in the greenhouse conditions (temperature 20±2, light and dark photoperiod 16/8, and relative humidity 70%) with soil which collected from the top 30-cm soil layer in a research farm from campus of agriculture and natural resources, university of Tehran, Karaj, Iran. Immediately after collection, soil samples were air dried, crushed, ground to pass through a 2-mm sieve and mixed uniformly. Physical and chemical properties and concentration of the elements in samples were measured. Total nitrogen of soil was determined by Kjeldal method [18], available phosphorus by Olsen method [19], available potassium by normal ammonium acetate [19], organic carbon [20], pH on saturated extract [21], electrical conductivity by Rhoades method [22], equal calcium carbonate by Bouyoucos method [23], texture of the soil by hydrometric method [23], and cation exchange capacity by Bower method [24]. Also, available concentration of Cu, Zn, Fe and Mn were extracted through DTPA method [25] and measured through atomic absorption spectrometery (AAS). The result of soil analysis was presented in Table 1.

Plant materials

Basil seeds (*Ocimum basilicum* L.) were provided from Zardband pharmaceutical company, Tehran, Iran. Seeds were cultivated in chest and after reaching to three-leaf stage, four uniform plants were transferred to plastic pots (6 L) containing 4 kg soil. Pots with plants were weekly rotated randomized on the greenhouse bench to minimize any localized environmental effects on plant growth or development. Plants were irrigated daily with 70-80% maximum field capacity (FC), and fertilized by some of necessary elements including N-P-K (urea, triple superphosphate, and sulphate of potassium, respectively) based on soil test results to have optimal conditions for plant growth. Plants were harvested after 12 weeks at the fully flowering growth stage. The aboveground plant parts were harvested by cutting the foliage at 2-3 cm above the pot level. Half of the aboveground shoots were shade dried for 14 days at room temperature (20-25°C) and distilled for essential oil extraction. The remaining half of the shoot washed, air dried in the shade and were placed in the special packets and oven dried at 70°C to a constant weight. Pots were emptied on a clear plastic sheet, the roots were carefully separated, and after washing with distilled water, they were dried in oven at 70°C and weighed. After recording dry-matter yield, the samples were ground and sieved by a 0.5 mm screen and stored in polyethylene bottles at room temperature for further analyses.

Table 1- Physical and chemical properties of soil used in this experiment before adding copper and zinc.

Characteristic	Quantity	Characteristic	Quantity
Soil texture	Sandy loam	CEC (Cmol (c) kg^{-1})	10.77
Clay (%)	17.46	total nitrogen (%)	0.044
Silt (%)	18	available phosphate (mgkg^{-1})	8.79
Sand (%)	64.56	available potassium (mgkg^{-1})	180
pH	7.4	Fe (mgkg^{-1})*	12.3
EC (dS/m)	1.28	Mn (mgkg^{-1})*	9.32
$CaCO_3$%	6.77	Cu (mgkg^{-1})*	0.63
OC%	0.63	Zn (mgkg^{-1})*	0.71
SP%	29.1		

* DTPA-Extractable

Application of copper and zinc

The treatments consisted of three levels of Cu (supplied as $CuSO_4.5H_2O$) and Zn (supplied as $ZnSO_4.2H_2O$). The experiment was laid out in a completely randomized design (CRD) with 9 treatments and three replicates (n=3). The amount of reference metals dissolved in 200 ml distilled water and sprayed over each pot, layer by layer as evenly as possible. Treatments consisted of control (Cu_0Zn_0), 10 mg Zn kg^{-1} with no Cu (Cu_0Zn_{10}), 50 mg Zn kg^{-1} with no Cu (Cu_0Zn_{50}), 5 mg Cu kg^{-1}with no Zn (Cu_5Zn_0), 5 mg Cu kg^{-1} and 10 mg Zn kg^{-1}(Cu_5Zn_{10}), 5 mg Cu kg^{-1} and 50 mg Zn kg^{-1}(Cu_5Zn_{50}), 25 mg Cu with no Zn ($Cu_{25}Zn_0$), 25 mg Cu kg^{-1} and 10 mg Zn kg^{-1} ($Cu_{25}Zn_{10}$), and 25 mg Cu kg^{-1} and 50 mg Zn kg^{-1}. After applying the employed treatments and watering to the FC level, in order to obtain balance of Zn and Cu with soil, pots were incubated for two months.

Essential oil extraction

The essential oil of *O. basilicum* plants (one half of the air dried shoot) was isolated by hydro-distillation for 3 h, using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia [26]. Oil yield was computed by multiplying the dry shoot yield by the oil content. The isolated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C to determine of essential oil constituents.

GC and GC-MS analysis procedure

GC analysis was performed using Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out on fused silica capillary DB-5 column (30 m × 0.25 mm i.d.; film thickness 0.25 µm). The injector and detector temperatures were kept at 250°C and 300°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.1 mL/min; oven temperature program was 60-250°C at the rate of 4°C/min and finally held isothermally for 10 min; split ratio was 1:50.

GC-MS analysis was carried out using Thermoquest-Finnigan gas chromatograph equipped With fused silica capillary DB-5 column (60 m × 0.25 mm i.d.; film thickness 0.25 µm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200°C and 250°C, respectively. Mass range was from 35 to 456 amu. Oven temperature program was the same as mentioned above for the GC.

Identification of the components

The constituents of the essential oils were identified by calculating their retention indices (RI) under temperature-programmed conditions for n-alkanes (C6-C24) and the oil on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparing their mass spectra with those of the internal reference mass spectra library (Adams and Wiley 7.0) or with authentic compounds and confirmed by com-paring their retention indices with authentic compounds or with those of reported in the literature [27]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Elemental analyses

The remaining half of the shoot materials and root samples were used to determine Cu and Zn concentration in the plant tissues. The concentration of elements in plant tissues (shoot and root) were determined by dry ashing the tissue in chloric acid as described by Cottenie [28]. Cu and Zn were analyzed using atomic absorption spectrophotometry apparatus (Shimadzu-AA 6400, Japan).

Statistical analysis

Data were processed by the analysis of variance (ANOVA) on the basis of completely randomized design (CRD) in a factorial experiment with three replicates (n=3). The data were analyzed using computer SAS software (version 9.1; CoHort Software), and the means were compared by Duncan's Multiple Range Test (DMRT) at probability (P) level of < 0.05.

Results

Plant biomass and essential oil yield

Shoot and root dry matter and essential oil yield of Sweet basil were significantly affected by application of the employed treatments. Supplying Cu and Zn separately at low levels increased root dry weight and essential oil yield (Table 2). However, the highest values of shoot, root and essential oil yield in basil plants were obtained by the combined application of these elements at 5 mg Cu + 10 mg Zn kg^{-1} soil. The increase in dry matter of shoot (16.04%), root (23.55%) and essential oil yield (28.66%) were obtained over the control with application of $Zn_{10}Cu_5$. Whereas, further increase in the levels of Cu and Zn were not significantly changed shoot, root and essential oil yield as compared to control. The lowest shoot and root dry weight and essential oil content was observed in combination of 25 mg Cu and 10 mg Zn kg^{-1}. In this study, it has been observed that low application of Cu and Zn in alone or combination together (Cu_0Zn_{10}, Cu_5Zn_0 and Cu_5Zn_{10}) had a positive impact on the shoot and root dry matter and essential oil yield. In contrast, high levels of Cu and Zn (Cu_0Zn_{50}, $Cu_{25}Zn_0$ and $Cu_{25}Zn_{50}$) application to soil was not significantly reduced these parameters.

Essential oil constituents

The oil composition of the aerial parts of basil plant was listed in Table 3. Totally, 20 constituents were identified for all essential oils. The chemical composition of essential oils was modified by application of employed treatments. Linalool content was the dominant constituent of the essential oil for all nine treatments tested, ranging from 49.18% to 61.04%. We observed an increase in content of linalool in volatile oil of Sweet basil by application of all treatment, although treatment of Cu_0Zn_{10} and $Cu_{25}Zn_{10}$ had no significant effect on linalool content. The increase in content of linalool was 11.18% in Cu_0Zn_{50}, 15.81% in Cu_5Zn_0, 24.11% in Cu_5Zn_{10}, 17.22% in Cu_5Zn_{50}, 11.95% in Cu_0Zn_{50} and 14.96% in $Cu_{25}Zn_{50}$ as compared to the control. The second major constituent of essential oil was methyl chavicol that increased with the treatment of Cu_0Zn_{50} and $Cu_{25}Zn_{10}$, and other treatment had no significant effect on the value of this compound. The increases in methyl chavicol content were 32.96% and 43.79% over the control with the application of Cu_0Zn_{50} and $Cu_{25}Zn_{10}$, respectively. The levels of eugenol and humulene oxide in *O. basilicum* oil significantly increased in plants treated with Cu_0Zn_{10}, $Cu_{25}Zn_{50}$ and $Cu_{25}Zn_{10}$ treatments. However, application of other treatments had no significant effect or showed adverse effect on eugenol and humulene oxide content. Application of Cu_0Zn_{10} and Cu_5Zn_{50} treatment did not alter the trend of major constituent compared to control. However, when plants treated with Cu_0Zn_{50} and Cu_5Zn_0, E-citral becomes one of the four main compounds instead of eugenol in control treatment. Morover, 1,8-Cineol and pulegone appeared as main compounds under $Cu_{25}Zn_{10}$ and $Cu_{25}Zn_{50}$ treatments over the control, respectively. The greatest amount of some other main constituent such as pulegone, α-pinene, sabinene, O-methoxy cinnamaldehyde and caryophylene oxide were obtained in combined treatment of Cu and Zn at high level ($Cu_{25}Zn_{50}$), whereas application of other treatment had no significant effect as compared to the control. However, the content of beta-elemene, *cis*-alpha-bergamotene and trans- alpha- bergamotene were significantly increased with application of Cu and Zn at low level as treated singly or in their combination. Also, the content of *cis*-epoxyocimene and germacrene D decreased with the application of high levels of these elements. The other essential oil constituents altered by application of employed treatments, however without a clear trend.

Classification of the identified compounds based on functional groups was summarized in Table 3. It is to note that the oxygenated monoterpenes were the main group of essential oil at different Cu and Zn levels. The maximum amount of oxygenated monoterpenes (81.28%) was obtained in Cu_5Zn_{10} treatment. However, oxygenated sesquiterpenes, the second group of essential oils, increased under all treatments except in $Cu_{25}Zn_0$ and $Cu_{25}Zn_{10}$. The remaining fractions such as sesquiterpene hydrocarbons and monoterpene hydrocarbons and phenols formed the minor classes.

Accumulation of copper and zinc in plant tissues

The metal accumulation in shoot and root tissues of sweet basil was significantly changed by increasing Cu and Zn to soil (Table 2). At all of the treatment, more Cu and Zn accumulated in root than in shoot. High amount of Cu and Zn was accumulated in root tissue of basil, indicating a slow rate of translocation and suggesting metal tolerance in this plant. Compared to the control, Cu content in shoot was significantly increased by 10 mg Zn kg^{-1} and decreased in treatments of 50 mg Zn, but Cu concentration in root tissue was increased with increasing Zn levels. On the other hand, Zn content in shoot was not significantly altered by 5 mg Cu, but it decreased significantly at 25 mg Zn kg^{-1}. However, in root, Zn concentration increased with increasing Cu levels. However, when Cu and Zn were supplied in combination treatment, concentration of Cu and Zn in root

Table 2- Effect of copper (Cu) and zinc (Zn) levels on root and shoot dry weight (g pot^{-1}), essential oil yield (mg pot^{-1}), root and shoot Cu and Zn accumulation (mg kg^{-1}DW)

Treatment	Root dry weight	Shoot dry weight	Essential oil yield	Root Cu concentration	Shoot Cu concentration	Root Zn concentration	Shoot Zn concentration
Cu_0Zn_0	3.9 ± 0.06 bc	11.5 ± 0.3 bc	47.02 ± 2.6 bc	9.05 ±0.59 h	6.20±0.37 fg	123.36±2.48 f	81.23±1.10 c
Cu_0Zn_{10}	4.5 ± 0.09 ab	12.8 ± 1.0 ab	56.40 ± 2.9 ab	11.40±0.47 g	9.96±0.48 e	147.33±2.02 e	105.76±3.08 b
Cu_0Zn_{50}	3.3 ± 0.34 c	10.8 ± 0.5 bc	43.45 ± 2.0 bc	17.60±0.45 e	5.43±0.18 g	184.74±2.64 c	150.43±2.64 a
Cu_5Zn_0	4.0 ± 0.33 bc	12.5 ± 0.6 bc	50.8 ± 2.4 abc	13.60±0.43 f	12.31±0.46 d	131.76±2.82 f	82.91±3.66 c
Cu_5Zn_{10}	4.9 ± 0.18 a	13.3 ± 0.6 a	60.50 ± 5.0 a	18.46±0.49 e	14.16±0.31 c	155.33±4.80 e	109.73±3.98 b
Cu_5Zn_{50}	3.5 ± 0.15 c	10.4 ± 0.5 c	40.33 ± 4.2 c	24.20±0.24 d	7.40±0.41 f	202.70±2.88 b	153.43±0.99 a
$Cu_{25}Zn_0$	3.2 ± 0.11 c	11.1 ± 1 abc	43.38 ± 5.9 bc	26.23±0.44 c	20.96±1.03 b	145.66±4.33 e	69.30±2.25 d
$Cu_{25}Zn_{10}$	3.9 ± 0.40 bc	10.9 ± 0.7 bc	41.69 ± 2.5 c	35.13±0.86 b	25.53±0.61 a	170.80±4.35 d	87.40±0.40 c
$Cu_{25}Zn_{50}$	3.3 ± 0.20 c	10.2 ± 0.2 c	38.21 ± 6.3 c	42.20±1.15 a	14.63±0.40 c	263.33±4.91 a	106.53±2.50 b

Data with similar letters are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Table 3- Effect of copper (Cu) and zinc (Zn) levels on the constituents of essential oils in *Ocimum basilicum* L.

No.	compound	RI	Cu_0Zn_0	Cu_0Zn_{10}	Cu_0Zn_{50}	Cu_5Zn_0	Cu_5Zn_{10}	Cu_5Zn_{50}	$Cu_{25}Zn_0$	$Cu_{25}Zn_{10}$	$Cu_{25}Zn_{50}$
1	α-pinene	936	1.34 ± 0.03bc	1.24 ± 0.06 c	1.22 ± 0.12c	-	0.73 ± 0.06d	0.90 ± 0.05d	1.61 ± 0.20b	-	2.18 ± 0.09a
2	sabinene	975	1.90 ± 0.27 ab	1.51 ± 0.03 b	1.81 ± 0.12 ab	1.77 ± 0.14 ab	1.86 ± 0.10 ab	1.87 ± 0.16 ab	0.98 ± 0.02 c	1.62 ± 0.18 b	2.23 ± 0.11 a
3	1,8-cineol	1037	2.67 ± 0.10 c	2.80 ± 0.03 c	3.40 ± 0.180 b	2.72 ± 0.18 c	2.92 ± 0.19 bc	-	3.37 ± 0.17 b	4.72 ± 0.33 a	-
4	*cis*-ocimene	1048	0.72 ± 0.04 abc	0.56 ± 0.07cd	0.53 ± 0.02 d	0.88 ± 0.04 a	0.85 ± 0.05 a	0.75 ± 0.07 ab	0.51 ± 0.08d	0.59 ± 0.02bcd	0.77 ± 0.03 a
5	linalool	1113	49.18 ± 0.66 d	51.43 ± 1.19 cd	54.68 ± 1.46 bc	56.96 ± 1.96ab	61.04 ± 1.89 a	57.65 ± 1.18 ab	55.06 ± 1.61bc	47.56 ± 1.36 d	56.54 ± 1.30 ab
6	*cis*-epoxyocimene	1144	1.45 ± 0.13 a	1.29 ± 0.12 ab	1.06 ± 0.11 ab	1.28 ± 0.11 ab	1.11 ± 0.26 ab	0.91 ± 0.05bc	1.06 ± 0.12 ab	0.57 ± 0.04 c	-
7	methyl chavicol	1210	6.37 ± 0.69 b	5.92 ± 0.17 b	8.47 ± 0.51 a	6.15 ± 0.32 b	6.44 ± 0.25 b	6.46 ± 0.21 b	6.64 ± 0.20 b	9.16 ± 0.14 a	5.90 ± 0.26 b
8	nerol	1230	1.03 ± 0.09 a	1.10 ± 0.20 a	-	1.03 ± 0.07a	-	-	1.13 ± 0.25 a	1.08 ± 0.07 a	-
9	pulegone	1237	3.18±0.03 c	2.61 ± 0.21cd	3.21 ± 0.30 c	-	4.19 ± 0.08b	3.23 ± 0.04 c	2.26± 0.28 d	2.83 ± 0.35cd	5.25 ± 0.57a
10	e-citral	1248	1.43 ± 0.04d	2.80 ± 0.12c	4.33 ± 0.18 a	3.32 ± 0.23 b	3.68 ± 0.10 b	0.48 ± 0.15 e	0.80 ± 0.02 e	0.88 ± 0.10e	-
11	eugenol	1356	3.29 ± 0.06c	5.75 ± 0.15 b	1.78 ± 0.09 de	1.66 ± 0.12 e	1.90 ± 0.29 de	3.53 ± 0.12 c	2.21 ± 0.22 d	3.17 ± 0.33 c	9.07 ± 0.27 a
12	*cis*-alpha-bergamotene	1398	0.51 ± 0.02 cd	1.10 ± 0.10 cd	1.26 ± 0.19 bc	2.06 ± 0.21 a	1.89 ± 0.57 ab	0.38 ± 0.06d	1.18 ± 0.10 bc	1.08 ± 0.16 cd	0.69 ± 0.05 cd
13	beta-elemene	1375	2.39 ± 0.25 ab	2.92 ± 0.27 a	1.42 ± 0.23c	3.06 ± 0.18 a	2.50 ± 0.15 ab	1.88 ± 0.22 bc	2.11± 0.35 bc	1.55 ± 0.18 c	0.67 ± 0.10 d
14	trans- alpha- bergamotene	1434	0.89 ± 0.09cd	1.71 ± 0.15a	1.02 ± 0.07 bc	1.32 ± 0.06b	1.24 ± 0.13b	1.06 ± 0.09 bc	0.54 ± 0.03 e	0.62 ± 0.02de	0.72 ± 0.06 de
15	germacrene D	1489	1.22 ± 0.22 ab	1.68 ± 0.36 a	0.63 ± 0.04 c	1.27 ± 0.13 a	1.70 ± 0.13a	1.37 ± 0.06 a	0.73 ± 0.11 bc	0.60 ± 0.05 c	0.30 ± 0.05 c
16	trans-nerolidol	1562	1.61 ± 0.10 a	1.22 ± 0.22abc	1.50 ± 0.36 ab	0.87 ± 0.04 bcd	0.74 ± 0.32 cd	0.46 ± 0.02 d	1.29 ± 0.10 abc	1.52 ± 0.24ab	1.10 ± 0.06abcd
17	o-methoxycinnamaldehyde	1569	2.34 ± 0.15c	2.43 ± 0.14bc	2.56 ± 0.06 abc	2.46 ± 0.10 abc	-	2.54 ± 0.06 abc	2.21 ± 0.03 c	2.76 ± 0.15ab	2.81 ± 0.11 a
18	caryophylene oxide	1589	0.93 ± 0.11b	0.95 ± 0.01b	0.98 ± 0.11ab	0.96 ± 0.10b	1.10 ± 0.10ab	1.19 ± 0.10ab	1.07 ± 0.03ab	1.12 ± 0.09ab	1.32 ± 0.18a
19	humulene oxide	1627	6.91 ± 0.52b	5.92 ± 0.05c	4.89 ± 0.08d	5.55 ± 0.19cd	4.86 ± 0.26d	3.73 ± 0.17e	7.15 ± 0.08ab	7.83 ± 0.30a	3.75 ± 0.22e
20	t-muurolene	1632	0.37 ± 0.01d	0.40 ± 0.01d	0.51 ± 0.00b	0.39 ± 0.00d	0.46 ± 0.00c	0.53 ± 0.00b	0.59 ± 0.01a	0.59 ± 0.02a	0.59 ± 0.02a
	hydrocarbonate monoterpenes		3.96	3.31	3.56	2.65	3.44	3.52	3.10	2.21	5.18
	oxygenate monoterpenes		68.6	73.7	76.93	73.12	81.28	72.26	72.53	69.97	76.76
	hydrocarbonate sesquiterpenes		5.01	7.41	4.63	7.45	7.33	4.95	4.56	3.85	2.38
	oxygenate sesquiterpenes		12.16	10.92	10.44	10.23	7.16	8.45	12.31	13.82	9.57
	Total identified		89.73	95.34	95.56	93.45	99.21	89.18	92.5	89.85	93.89

RI: retention index; data with similar letters are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

and shoot was more than those of control. The maximum concentration of Cu in shoot was recorded from the $Cu_{25}Zn_{10}$ treatment with a value of 25.53 mg kg^{-1} and for root 42.20 mg kg^{-1} in $Cu_{25}Zn_{50}$ treatment. However, the maximum concentration of Zn in shoot was recorded from the Cu_5Zn_{50} treatment with a value of 153.43 mg Zn $kg^{-1}$and for root 263.33 mg Zn kg^{-1} in $Cu_{25}Zn_{50}$ treatment. Cu and Zn as trace elements are necessary for plant nutrition, but it can become toxic at high concentrations for plants.

Discussion

Trace elements (e.g. Zn and Cu) concentrations in the soils are crucial for healthy plant growth and development as plants require a proper balance of all the essential nutrients for normal growth and optimum yield [29]. It has been reported that low level of Cu and Zn had a beneficial effect on plant growth, absorption of micro and macronutrient, photosynthesis, quantity and quality of essential oil because of their roles in stimulate enzyme activity and possible involvement utilization of photosynthetic which may be providing additional carbon skeletons for terpene biosynthesis and accumulation [3,16,30]. The adverse effects of Cu and Zn in sole or in combination treatment on shoot and root biomass and essential oil yield might be attributed to the accumulation toxic levels of Cu and Zn in plant tissue and interfere of them with essential nutrient uptake and transport and thereby disturb mineral nutrition composition of plants, chlorophyll content, photosynthetic rate, and root growth [8, 31].

Many researchers studied the chemical composition of the essential oil of basil plants, and the results were found to be very different [32, 33]. However, our findings are in agreement with those of Prasad et al. [14] who noted that linalool, methyl chavicol and eugenol were as the main constituent. Essential oil-producing plants are commonly grouped into chemotypes based on the identity of the prevalent maximum components of total essential oil [33]. As a consequence there are several chemotypes of basil oils identified according to their major compounds. One of them is based on the content of linalool and methyl chavicol [32]. For this reason, the sample of basil plants in the most employed treatments showed high content of linalool and methyl chavicol, which could be grouped into linalool- methyl chavicol chemotype. In this study, it has been found that the principle constituent of essential oil in *O. basilicum* was altered by application of the treatments, however, only in $Cu_{25}Zn_{50}$ treatment the chemotype was altered to linalool- eugenol. In addition, E-citral, 1,8-Cineol and pulegone replaced by eugenol or humulene oxide under application of some treatment. E-citral and 1,8-cineol revealed antifungal, antiinflammatory and antinociceptive potential, respectively [34,35]. Whereas, pulegone particularly incerased under high amounts of Cu and Zn ($Cu_{25}Zn_0$ and $Cu_{25}Zn_{50}$) which is a toxic compound with potentially lethal hepatotoxic effects [36]. However, increasing in pulegone content could be considered as negative effect on essential oil quality.

To the best of our knowledge, compositional changes in the essential oil of *O. basilicum* following Cu and Zn application is scanty, and only a few papers concerning a limited choice of species have been published up to now. The significant variation in the content of chemical compounds in the volatile oils with the application of Cu and Zn indicate that these metals had a significant impact on the quality of the oil produced. The mechanisms changes by which the various constituents in the essential oil of basil resulting from application of Cu and Zn are unknown, but it might be attributed to change the uptake of macro and micronutrient in plant tissues [16], as well as enzymatic activity and carbon metabolism [30]. In some enzymes, metals perform its catalytic function by forming an enzyme–substrate metal complex [7]; thus, metals play an obvious role in synthesis of monoterpene classes, which can produce several compounds [37]. Also, differences in the content of mono- and sesquiterpens as affected by metals probably could be due to the roles of these metals to different enzymes used in terpene synthesis. Also, the accumulation of monoterpene under abiotic constraint and stimulation of them by stressful environment in plant suggested that monoterpenes has physiological and ecological role such as a photorespiration-like protection [38]. Several authors have been reported the changes in chemical composition of essential oil in medicinal plants following micronutrients and heavy metals application [39] including Cu, Cd, Mo, and Zn. According to Prasad et al. 2011 the content of linalool in basil oil was decreased and that of methyl chavicol was increased by application of Cr, Cd, and Pb. Also, Rai et al. [40] reported that chromium stress induced the production of eugenol, a major component of essential oil of *Ocimum tenuiflorum*. Elicitation of some oil components such as α-pinene, β-pinene, sabinene, β-myrcene, limonene and linalyl acetate in *Mentha arvensis, Mentha piperita* and *Mentha citrata* were considerably affected through application of chromium and lead to soils as compared to control plants [14]. However, the observed modification in the synthesis of some essential oil constituents in metal treated plants considered as a defense response to metal stresses.

In our current study, we observed that the high amount of Cu and Zn was accumulated in root tissue of basil, indicating a slow rate of translocation and suggesting metal tolerance in this plant. This result is comparable with the reports of previous studies [5,41]. In both root and shoot, concentration of Cu and Zn increased with sole supply of Cu and Zn as compared to the control. In our current experiment, soil is relatively poor in clay and cation exchange capacities (Table 1), which may reduce Cu and Zn immobilization in soil and increase Cu and Zn concentration in plant tissues [42]. However, in root, Zn concentration increased with increasing Cu levels. This may be due to a synergistic relationship between Cu and Zn uptake by root and in shoot at low level of these metals, while further addition of them showed antagonistic effect in shoot [3]. Moreover, WHO standard limit for these metals has not been established yet. According to Olowoyo et al. [41] the value of Cu and Zn in agricultural products should not exceed 15 and 200 mgkg^{-1}, respectively. Cu was much higher than that of recommended as maximum limit for Cu by Olowoyo et al. [41], but Zn did not accumulate in excess value of the suggested. However, this study indicates that the root might act as a storage organ for Cu and Zn after uptake from the soil before transferring to the other part of the plants.

Conclusion

Our findings indicate that the growth and essential oil yield of Sweet basil (*Ocimum basilicum* L.) was significantly enhanced by application of low levels of Cu and Zn. In contrast, basil plants accumulate high levels of Cu and Zn in their tissues at excessive levels of Cu and Zn, which mostly concentrated in root, without significant reduction in growth and biomass production, showing tolerance to these metals. It is noteworthy; however, the significant changes in the content of different constituents in essential oils with application of Cu and Zn indicate that Cu and Zn contamination in soils had a significant impact on the quality of the essential oil produced. Altogether, the results presented here support the hypothesis that Cu and Zn potentially modify the production of essential oil, but for a more comprehensive understanding the exact mechanism of such modifications in plant metabolism required to be elucidated. However, treatment of medicinal plants such as basil with Cu and Zn or with their certain combination can be introduced as an efficient technique in order to produce more desired pharmaceutically active compounds such as linalool for drug industries and medical supplies.

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