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Role of Cobalt Insweet Basil *(Ocimumbasilicum* L.) Plants A. herb Yield, Essential Oil Content and its Composition

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Abstract: The World Health Organization estimates that plant extracts or their active constituents are used as folkmedicine in traditional therapies of 80% of the worlds population (World Health Organization, 1993). Sweet basil has been used as aroma additives in food, pharmaceuticals and cosmetics. Thus, The present investigation has been carried out to estimate the effect of different concentration of cobalt (0.0, 7.5, 15.0 22.5, 30.0 ppm) on herb yield, essential oil content and its composition of *Ocimumbasilicum* plant grown in the Experimental Station of National Research Centre at Nubaria, Behira governorate, West of Nile Delta of Egypt under drip irrigation system.Cobalt at 15.0 ppm gave the greatest values of fresh and dry herb yield (66.20 and 13.19 ton ha^{1}). Increasing Co from 0.0 to 7.5, 15.0 and 22.5 ppm significantly increased the essential oil yield from 38.39 to 94.67, 266.78 and 181.49 Lha^{-1}. While the highest level of Co (30ppm) recorded 91.15 L ha^{-1}.The essential oil of *Ocimumbasilicum*were characterized by a high content of linalool (23.43-35.46%), methyl chavecol (27.68-29.77%), followed by eugenol (6.76-10.54%) then 1,8 cineol (2.34-9.65%). Cobalt at 15.0 ppm increased the principal components of linalool (35.46%), 1,8 cineol (9.65%), linalyl acetate (8.71%) and benzyl acetate (8.14), while cobalt at the high levels (30 ppm) havepossible role in the essential oil compounds and recorded the highest content of methyl chavecol and eugenol.Which were considered the main contributors of the antioxidant activity of volatile extract of basil.

Key words: Cobalt · Sweet Basil · Ocimumbasilicum · Herb · Essential Oil Composition

INTRODUCTION

Sweet basil (*Ocimumbasilicum* L.) is native to areas in Asia and Africa. Basil was brought from India to Europe through the Middle East in the sixteenth century and subsequently to America in the seventeenth century [1]. It is a popular herb in the US and Mediterranean diets. Herbs can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics [2, 3]. Plant extracts and essential oils have been widely used in traditional medicine for treatment of many diseases. *O. basilicum*extracts have been used to treat headaches, cough, diarrhea, warts, constipation, kidney mal-functions and microbial infections [2, 4]. Basil's essential oil has anti-vira l [5], anti-cancer [6], antioxidant activity [7, 8]. Aglycones found in *O. basilicum*have high antioxidant capacity [7], whereas antimutagenic properties of linalool, which induce DNA strand breaks have also been demonstrated [9]. Anti-oxidant and anti-microbial activities due to its phenolic and aromatic compounds [10, 11]. Ethanol extracts of *O.basilicum* L. decrease cholesterol and lipid accumulation in human macrophages [12]. Recently, methanol extracts showed anti-herpes activity [13]. *O. basilicum*are well known to contain flavonoids and have a strong antioxidant affect that is beneficial for serum antioxidant levels, leading to improved sperm health parameters via the reduction of oxidative stress, so it seems likely that

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long-term use of herbs can increase testosterone levels, improve sperm parameters and increase the chance of fertility [14].

Cobalt is considered a beneficial element for higher plants due to its direct role in their metabolism. Cobalt promoted many developmental processes including stem and coleoptile elongation opening of hypocotyl, leaf expansion and bud development [15]. It is an essential element for the synthesis of vitamin B, which is required for human and animal nutrition [16, 17]. Unlike other heavy metals, cobalt is saver for human consumption up to 8 mg can be consumed on a daily basis without health hazard [16]. Maryam Mirza *et al.* [18] stated that traces of elements (Cu, Zn, Mn, Fe, Co, Ni, Cd, Pb, Cr, Ag, Na and K) in indigenous medicinal diuretic plants (*Cymbopogoncitrates, Raphanussativus and Zea mays*) have possible role in human health and disease. Pan Zuewu *et al*., [19] reported that the addition of microelements (BO_3^{---} - MoO_4^{--}, Co^{++}, Cu^{++}, Fe^{++} and Zn^{++}) have important roles on the biosynthesis of comptothecin and growth of suspension cultures of *comptotheca acuminate*. Cobalt is a beneficial element for plant growth,Walser *et al*., [20] stated that cobalt application (2.7 kg Co/ha soil) increased tomato leaf number as well as surface of chloroplasts per unit leaf area, leaf chlorophyll content, leaf area and rate of photosynthesis. Cobalt is required in low levels for maintaining high yields of squash [21], tomato [22] groundnut [23] sweet potato [24]. Moreover Aziz *et al*., [25] found that low level of cobalt (7.5 ppm) recorded the highest content of L-(-)-menthol (28.54%) of peppermint oil. Thus, the relatively high level of menthol in the peppermint oil suggests that a marketable peppermint essential oil could be successfully produced in newly reclaimed land of Egypt. As well as Cobalt at 30 ppm have possible role in indigenous medicinal diuretic plants (*Cymbopogon citrates*) and recorded the relatively high level of neural and geranial, which represented about 65.43% of lemongrass oil [26].

The aim of this study was to investigate the influence of cobalt on herb yield, essential oil content and composition of *Ocimumbasilicum* plant.

MATERIALS AND METHODS

Field experiments were conducted during two successive seasons of 2008 and 2009 at Research and Production Station, National Research Centre, Nubaria, Behiragovernorate, west of the Nile Delta of Egypt to evaluate the effect of different cobalt levels on growth, yield, essential oil content and composition of sweet basil.

Physical and chemical properties of Nubaria Soil were determined and particle size distributions along with soil moisture were determined as described by Black *et al*., [27]. Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen and available P, K, Fe, Mn, Zn, Cu were run according to Black *et al*. [27]. Determination of soluble, available and total cobalt was determined according to method described by Cottenie *et al*., [28]. Some physical and chemical properties of Nubaria soil are shown in Table 1.

During the soil preparation for cultivation organic manure, at 40 $m^3 ha^{-1}$, calcium super phosphate at 300 kg ha^{-1} and potassium sulfate at 150 kg ha^{-1} were added as is customary for the region.

The seeds were sown on 1 March 2008 and 2009 in the nursery. On 15 April the uniform healthy basil seedlings (10 cm of length) were transplanted to the Experimental filed under drip irrigation system. Drip lines

Table 1: Some physical and chemical properties of Nubaria soil

Physical properties							
Particle size distribution%				Soil moisture constant%			
Sand	Silt	Clay	Soil texture	Saturation	FC	WP	AW
70.8	25.6	3.6	Sandy loam	32.0	19.2	6.1	13.1

Chemical properties											
				Soluble cations (meq^{-1} L)				Soluble anions (meq^{-1} L)			
pH 1:2.5	EC (dS m^{-1})	CaCO3%	OM%	Ca^{++}	Mg^{++}	K^{+}	Na^{+}	HCO_3^{-}	CO_3	Cl^{-}	$SO_4^{=}$
8.49	1.74	3.4	0.20	0.8	0.5	1.6	1.80	0.3	-	1.9	0.5

Cobalt			Total	Available		Availablemicronutrients			
Ppm			mg 100 g^{-1} soil			ppm			
Soluble	Available	Total	N	P	K	Fe	Mn	Zn	Cu
0.35	4.88	9.88	15.1	13.3	4.49	4.46	2.71	4.52	5.2

FC (Field capacity), WP (Welting point) and AW (Available water) with 2 liter h^{-1} discharge rate at a spacing of 50 cm apart in 1 m wide beds at about 5 cm from each dripper were put directly on surface of each soil bed. The seedlings were planted in one row parallel to the drip lines at adjacent to water sources on the irrigated beds.

The layout of the Experiment was randomized complete block design with three replicates. The experiment was consisting of 5 treatments i.e. 0.0, 7.5, 15.0, 22.5 and 30.0 ppm. Each treatment was represented by three plots. Each plot area was 5X3 meter, consisting of three rows. After one month from transplanted the seedlings were irrigated once with cobalt at the different levels.

The plants were harvested two times (in July and October) during two successive seasons. At each harvesting time, fresh and dry weights of herb (gm $plant^{-1}$ and ton ha^{-1}) were recorded. The obtained data were statistically analyzed according toSnedecorand Cochran [29].

The essential oil percentage of fresh herb was determined by hydro distillation in Clevenger's apparatus for 3 hour. The essential oil yield (ml $plant^{-1}$and L ha^{-1}) was calculated.

The resulted oil was dehydrated over anhydrous sodium sulfate andstored in glass vials at freezer in the absence of light till used for gas liquid chromatographic (GLC) analysis. The GLC analysis of the oil samples was carried out using Hewlett Packard gas chromatograph apparatus at the Central Laboratory of NRC with the following specifications: instrument: Hewlett Packard 6890 series, column: HP (Carbwax 20M, 25 m length x 0.32 mm I.D), film thickness: 0.3 mm,sample size:1 µl, oven temperature: 60-190°C, program: 60°C/2 min, 8°C/ min,190°C/25 min, injection port temperature :240°C, carrier gas: nitrogen, detectortemperature (FID): 280°C, flow rate: N_2 30 ml/min., H_2 30 ml/min., air 300 ml/min. Main compounds of the essential oil were identified by matching their retentiontimes with those of the authentic samples injected under the same conditions. The relative percentage of each compound was calculated from the peak areacorresponding to each compound.

RESULTS AND DISCUSSION

Herb Yield: The results in Table 2, showed that, fresh and dry herb yield of *Ocimumbasilicum* (gm $plant^{-1}$ and ton ha^{-1}) increased gradually by increasing cobalt concentration from 0.0to 7.5 and 15.0, ppm). Cobalt at 15.0ppm gave the greatest values of fresh and dry herb yield (66.20and 13.19 ton ha^{1}). No further increase was recorded with the highest level of cobalt (30 ppm). These data are harmony with those obtained withLiala and Nadia Gad [30] whostated that plant growth of parsley i.e. plant height, number of leaves per plant as well as fresh and dry weight of leaves and root were significantly increased with low levels of Co (25 mg kg^{-1}soil). Nadia Gad *et al*. [31] pointed that cobalt is a promising element in the newly reclaimed soils such as Rass Seder, Egypt and had a significant promotive effect on olive trees (Manzanello and Arbicon) growth, yield and fruits quality. Aziz *et al*., [32] showedthat the low level of Co (20 mg kg^{-1}soil) caused significant increase in plant height, No. of branches and as well as fresh and dry weights of roselle calyces as compared with the high level (40 mg kg^{-1}soil). Nadia Gad [33] demonstrated that cobalt at 12.5 ppm gave a synergistic effect of canola growth and seed yield. Aziz *et al*. [25] showed that cobalt at 15.0 ppm gave the greatest fresh and dryherb yield (11.81 and 3.12 ton ha^{-1}) of peppermint as well asAziz andNadia Gad [26] stated that cobalt at 22.5ppm gave the greatest values of fresh and dry herb yield (8.97and 2.66 ton ha^{1}) of *Cymbopogon citrates*.

Table 2: Herb yield of*Ocimumbasilicum*as affected by cobaltnutrition (mean of two seasons).

Cobalt treatments ppm	Fresh weight gm $plant^{-1}$ 1 cut	Fresh weight gm $plant^{-1}$ 2 cut	Dry weight gm $plant^{-1}$ 1 cut	Dry weight gm $plant^{-1}$ 2 cut	Total fresh weight gm $plant^{-1}$	Total dry weight gm $plant^{-1}$	Fresh herb yield ton ha^{-1}	Dry herb yield ton ha^{-1}
Control	293.79	498.74	68.02	105.63	792.53	173.65	17.12	3.75
7.5	603.36	894.85	113.00	155.75	1498.21	268.75	32.36	5.81
15.0	1181.97	1882.94	244.82	365.87	3064.91	610.69	66.20	13.19
22.5	1031.37	1498.68	211.93	314.65	2530.05	526.58	54.65	11.37
30.0	681.09	935.63	142.35	189.94	1616.72	332.29	34.92	7.18
LSD 5%	64.14	26.80	1.60	1.93	72.10	2.37	1.56	0.05

Table 3: Essential oil content of*Ocimumbasilicum* as affected by cobalt nutrition (mean of two seasons)

Cobalt Treatments ppm	1 cut Oil%	1 cut ml plant^{-1}	2 cut Oil%	2 cut ml plant^{-1}	Total oil yield ml plant^{-1}	Oil yield l ha^{-1}
Control	0.20	0.60	0.24	1.18	1.78	38.39
7.5	0.27	1.61	0.31	2.77	4.38	94.67
15.0	0.39	4.57	0.41	7.78	12.35	266.78
22.5	0.34	3.51	0.33	4.90	8.40	181.49
30.0	0.25	1.73	0.27	2.49	4.22	91.15
LSD 5%	0.01	0.22	0.01	0.16	0.22	4.66

Table 4: Essential oil composition of *Ocimumbasilicum*as affected by cobalt nutrition

Essential oil constituents	Cobalt concentration (ppm) Control	7.5	15.0	22.5	30.0
α –pinene	0.35	0.32	0.92	0.29	-
β-pinene	1.18	1.07	2.53	0.73	0.61
1,8 cineol	2.73	4.26	9.65	3.65	2.34
Ocimene	2.14	2.21	0.32	0.78	0.70
Linalool	25.36	29.64	35.46	24.24	23.43
Linalyl acetate	2.63	3.11	8.72	2.77	2.52
Terpeniol	0.10	0.85	0.34	0.84	0.68
Methyl chavecol	27.68	27.19	28.56	29.77	29.71
Benzyl acetate	5.32	5.12	8.14	5.33	5.45
Geranyl acetate	0.98	1.95	0.37	2.97	3.46
Farnesol	3.07	3.40	0.34	3.91	4.29
Methyl eugenol	2.92	2.97	0.23	3.25	3.88
Eugenol	6.74	8.62	0.37	10.33	10.54
Total identified	81.2	90.71	95.95	88.86	87.61

Essential Oil Yield: Data in Table 3, showed that the essential oil percent and yield (ml plant^{-1} and Lha^{-1}) were significantly increased with the application of different concentration of cobalt. In the first and second cut Co at 15.0 ppm recorded the highest values of the essential oil percent (0.39 and 0.41), content (4.57and 7.78 ml plant^{-1}) and yield (266.78 Lha^{-1}). Increasing Co from 0.0 to 7.5,15.0 and 22.5ppm significantly increased the essential oil yield from 38.39 to94.67,266.78 and 181.49 Lha^{-1}). While the highest level of Co (30ppm) recorded 91.15 L ha^{-1}. These results agreed with that reported with Liala and Nadia Gad [30] who found that cobalt at 25 mg kg^{-1} soil significantly increased essential oil content of parsley leaves, moreover Nadia Gad *et al*., [31] pointed that cobalt is a promising element in the newly reclaimed soils such as Rass Seder, Egypt and had a significant promotive effect on olive trees (Manzanello and Arbicon) growth, yield, fruits quality and oil percent especial with organic fertilization. Moreover Nadia Gad [33] demonstrated that cobalt at 12.5 ppm gave a synergistic effect of canola oil compared to untreated plants as well as Aziz and Nadia Gad [26] stated that increasing Co from 7.5 to 15.0 ppm significantly increased the essential oil yield of *Cymbopogon citrates* from 43.02 to 59.19 Lha^{-1}. While cobalt at 22.5ppm gave greatest increase in the essential oil yield (63.07 L ha^{-1}). Moreover Aziz *et al*., [25] found thatcobalt at 15 ppm recorded the highest values of the essential oil yield (38.95 lha^{-1}) of peppermint plant.

Essential Oil Composition: Data in Table 4, showed that the essential oil of *Ocimumbasilicum* were characterized by a high content of linalool(23.43-35.46%), methyl chavecol (27.68-29.77%), followed byeugenol (6.76-10.54%) then 1,8 cineol (2.34-9.65%). Major volatiles detected in this study were consistent with those of previously published studies [34, 35] reported that the main components of *Ocimumbasilicum*were linalool (39.8%), estragole (20.5%), methyl cinnamate (12.9%), eugenol (9.1%) and 1,8-cineole (2.9%).

The obtained data showed that cobalt at 15ppm increased the principal components of linalool (35.46%), 1,8 cineol (9.65%),linalyl acetate (8.71%) and benzyl acetate (8.14%) of *Ocimumbasilicum*as compared with other treatment and control and this effect was companied with decreasing the relative content ofeugenol (6.76%). While cobalt atthe highlevels (30ppm) recorded the highest content of methyl chavecol and eugenol. These results agreed with Aziz *et al*., [25] showed that the highest level of cobalt (30ppm) increased the principal components ofmenthone (37.84%) and isomenthone (15.19%) of peppermint. While low level (7.5 ppm) of cobalt recorded the highest content of L-(-)-menthol (28.54%) as compared with other treatment and control. As well as Aziz and Nadia Gad [26] stated that the highest level of cobalt (30ppm) increased the principal components of neral (36.17%) and geranial (29.26%), which represented about 65.43% of lemongrass oil as well as the quality is generally determined by its content of citral (neral and geranial isomers).

CONCLUSION

Cobalt at 15 ppm increased the principal components of linalool (35.46%). While 30ppm have possible role in the essential oil compounds of *Ocimumbasilicum* and recorded the highest relative content of methyl chavecol and eugenol. Whichwere considered the main contributors of the antioxidant activity of volatile extract of basil [36] and these aroma compounds may help to prevent oxidative damage, such as lipid peroxidation, which is associated with cancer, premature aging, atherosclerosisand diabetes.

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