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ORIGINAL ARTICLES

Physiological and Chemical Response of Lemongrass (*Cymbopogon Citratus* L.) to Cobalt Nutrition, A-Herb Yield, Essential Oil Content and Composition

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ABSTRACT

Trace elements in indigenous medicinal diuretic plants (*Cymbopogon citratus)* have possible role in human health and disease (Maryam Mirza *et al.,* 2004). 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 and essential oil composition of lemongrass 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 22.5ppm gave the greatest values of fresh and dry herb yield (8.97and 2.66 ton ha^{1}) as well as recorded the greatest increase in the essential oil yield (63.07 L ha^{1}). While 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). This effect was companied with decreasing the relative content of limonene and citronellol.

Key words: cobalt, *Cymbopogon citratus*, lemongrass, essential oil.

Introduction

Lemongrass (*Cymbopogon citratus*), family poaceae (Graminaceae). Lemongrass is a perennial herb widely cultivated in the tropics and subtropics. Its propagation is carried out by root or plant division. Lemongrass contains 1 to 2% essential oil on a dry basis with widely variation of the chemical composition as a function of genetic diversity, habitat and agronomic treatment of the culture (Carlson *et al.,* 2001). Lemongrass essential oil is characterized by a high content of citral composed of neral and geranial isomers (c. 69%), which is used as a raw material for the production of ionone, vitamin A and beta carotene (Paviani *et al.,* 2006).

As a medicinal herb, lemongrass has been considered as carminative, antimicrobial (Horne *et al.,* 2001) anti-oxidant (Dorman *et al.,* 2000), acts as central nervous system depressant, has antibacterial, antifungal activity and viruses (Chao and Young, 2000).

The volatile oils may also have some pesticide and mutagenic activities. The oil extracted from leaves of lemongrass is used for its spasmolytic, analgesic, anti-inflammatory, antipyretic, diuretic and tranquilizing properties in treating various digestive disorders, inflammation, diabetes, nervous disorders and fever (Onawunmi *et al.,* 1984 and Negrelle and Gomes, 2007). The main components of *C. citratus* oil are neral, geranial and citronellol represented about 80 % of the essential oil (Aziz and El-Ashry, 2002; Aziz *et al.,* 2010 and Koffi1 *et al.,* 2009). The essential oil of lemongrass is characterized by a high content of citral (>45%) (Khanuja *et al.,* 2005) and its quality is generally determined by its content of citral. Citral is a mixture of two stereoisomeric monoterpene aldehydes; geranial (trans-citral, called citral a) and neral (cis-citral, called citral b). Essential oil of *C. citratus* is mainly composed of citral (30-93.74%) with predominance of geranial (Negrelle and Gomes, 2007).

Plants have a natural ability to extract ions from soil and to distribute them between the roots and the shoot. Within a certain concentration range, some heavy metals are essential for the growth of higher plants (Breckle, 1991). In this context, long-distance root-to-shoot transport in the transpiration stream via the xylem as well as the transfer from the xylem to the phloem and the retrains location via the phloem must be considered as important processes for the redistribution of an element within a plant (Marschner, 1995). Maryam Mirza *et al.,* (2004) stated that trace elements (Cu, Zn, Mn, Fe, Co, Ni, Cd, Pb, Cr, Ag, Na and K) in indigenous medicinal diuretic plants (*Cymbopogon citratus, Raphanus sativus and Zea mays)* have possible role in human health and disease. Pan Zuewu *et al.,* (2004) 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*. Thus, cobalt is considered a beneficial element for higher plants in spite of the absence

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of evidence for direct role in their metabolism .It is an essential element for the synthesis of vitamin B, which is required for human and animal nutrition (Young, 1983 and Smith, 1991). Unlike other heavy metals, cobalt is saver for human consumption up to 8 mg can be consumed on a daily basis without health hazard (Young, 1983).

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

Materials and Methods

Field experiments were conducted during two successive seasons of 2008-2009 and 2009-2010 at Research and Production Station, National Research Centre, Nubaria, Behira governorate, West of the Nile Delta of Egypt to evaluate the effect of different cobalt levels on growth, yield, essential oil content and composition of *Cymbopogon citratus* plant.

Physical and chemical properties of Nubaria Soil were determined and particle size distributions along with soil moisture were determined as described by Blackmore (1972). 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.,* (1982). Determination of soluble, available and total cobalt was determined according to method described by Cottenie *et al.,* (1982). Some physical and chemical properties of Nubaria soil are shown in Table (1).

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		Available micronutrients				
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), AW (Available water).

On 15 on August, healthy rooted seedlings (45day old) of *Cymbopogon citratus were* transplanted to the Experimental filed under drip irrigation system. Drip lines 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.

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 prior to planting as is customary for the region. The plants were harvested two times (in June 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 to to Snedcor and Conchran (1982).

The essential oil percentage of fresh herb was determined by hydrodistillation in Clevenger's apparatus for 3 h. according to the Egyptian Pharmacopoeia (1984). The essential oil yield (ml $plant^{-1}$and L ha^{-1}) was calculated. The essential oil constituents were determined by gas chromatography (Perkin Elmer model 8500) coluemn 2m X 4 mm filled with 10 % carbowx K 20M, (nitrogen carriers gas at 30 ml/min.) equipped with FID, an electronic area integrator. The temperature program cycle was 90°C for 2 min., a rise of 8°C/min to 170°C, held at 170°C for 4 min., and a rise of 10°C/min. to 210°C, and held at 210 for 5 min. The qualitative determination of the different constituents of lemongrass oil was performed by comparing the relative retention times of different peaks with those of the pure authentic samples injected under the same conditions. The quantitative determination of each compound was calculated on the basis of peak area corresponding to each compound.

Results and Discussion

Herb Yield:

The obtained results in Table (2) showed that, fresh and dry herb yield of *Cymbopogon citratus* (gm plant^{-1} and ton ha$^{-1)}$ increased gradually by increasing cobalt concentration (0.0, 7.5, 22.5ppm). Cobalt at 22.5ppm gave the greatest values of fresh and dry herb yield (8.97and 2.66 ton ha^{1}).No further increase was recorded with the highest level of cobalt (30 ppm).These data are harmony with those obtained with Liala Helmy and Nadia Gad (2002) stated 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.,* (2006) 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.,* (2007) showed that 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 (2010) demonstrated that cobalt at 12.5 ppm gave a synergistic effect of canola growth and seed yield.

Table 2: Herb yield of *Cymbopogon citratus* as influenced with cobalt nutrition (mean of two seasons).

Cobalt treatments ppm	Fresh weight gm plant^{-1}		Dry weight gm plant^{-1}		Total fresh weight gm plant^{-1}	Total dry Weight gm plant^{-1}	Fresh herb Yield ton ha^{-1}	Dry herb Yield ton ha^{-1}
	1 cut	2 cut	1 cut	2 cut				
Control	71.20	106.40	24.06	35.01	177.60	59.07	3.84	1.28
7.5	135.38	173.40	44.04	51.78	308.78	95.82	6.67	2.07
15.0	151.60	212.90	49.91	64.34	364.50	114.24	7.87	2.47
22.5	181.15	234.13	54.85	68.20	415.28	123.05	8.97	2.66
30.0	109.60	182.01	35.30	55.45	291.61	90.75	6.30	1.96
LSD 5 %	1.31	1.44	1.48	1.49	1.56	2.70	0.03	0.06

Essential Oil Yield:

Data illustrated 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. Co at 22.5 ppm recorded the highest values of the essential oil percent, content (ml plant^{-1}) and yield (63.07 Lha^{-1}). Increasing Co from 7.5 to 15.0 ppm significantly increased the essential oil yield from 43.02 to 59.19 Lha^{-1}). No further increased in the essential oil yield were recorded with the highest level of Co (30ppm) which gave 37.97 L ha^{-1}, these results agreed with that reported with Liala Helmy and Nadia Gad (2002) found that cobalt at 25 mg kg^{-1} soil significantly increased essential oil content of parsley leaves, moreover Nadia Gad *et al.,* (2006) 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 (2010) demonstrated that cobalt at 12.5 ppm gave a synergistic effect of canola oil compared to untreated plants.

Table 3: Essential oil content of *Cymbopogon citratus* as influenced with cobalt nutrition (mean of two seasons).

Cobalt Treatment ppm	1 cut		2 cut		Total oil yield ml plant^{-1}	Oil yield l ha^{-1}
	Oil%	ml plant^{-1}	Oil%	ml plant^{-1}		
Control	0.51	0.36	0.61	0.65	1.01	21.73
7.5	0.55	0.75	0.72	1.24	1.99	43.02
15.0	0.67	1.02	0.81	1.72	2.74	59.19
22.5	0.69	1.25	0.71	1.67	2.92	63.07
30.0	0.53	0.58	0.65	1.18	1.76	37.97
LSD 5 %	0.02	0.03	0.02	0.03	0.05	1.04

Essential Oil Composition:

Data in Table (4) showed that the essential oil of *Cymbopogon citratus* were characterized by a high content of neral (36.17 - 33.65%), geranial (19.58 - 29.26 %), citronellol (22.02- 31.25 %) followed by limonene (4.24 - 6.87 %) then linalool (0.36 -- 1.31 %). These results agreed with Aziz and El-Ashry (2002), Aziz *et al.,* (2010) and Koffi1 *et al.,* (2009) reported that the main components of *C. citratus* oil are neral, geranial and citronellol represented about 80 % of the essential oil . More over the essential oil of lemongrass is characterized by a high content of citral (>45%) (Khanuja *et al.,* 2005) as well as the quality is generally determined by its content of citral (Negrelle and Gomes, 2007).

The highest the 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 compared with control and this effect was companied with decreasing the relative content of limonene and citronellol.

Table 4: Essential oil composition of *Cymbopogon citratus* as influenced with cobalt nutrition.

Essential oil constituents	Cobalt Concentration (ppm)				
	Control	7.5	15.0	22.5	30.0
Limonene	6.87	4.24	5.63	4.74	5.36
1,8 cineol	0.91	1.13	0.76	0.88	0.79
α terpinolene	0.20	0.38	0.53	-	0.33
Terpeniolene	0.35	0.58	0.61	0.26	0.61
Linalool	1.31	1.13	1.01	0.36	1.16
Citronellal	0.83	0.47	0.75	1.08	0.39
Neral	35.54	34.33	33.65	34.29	36.17
Geranial	19.58	27.83	29.04	29.24	29.26
Citronellol	31.25	22.02	24.50	23.26	23.11
Geranyl acetate	0.21	0.35	0.23	-	-
Neryl acetate	1.50	1.82	1.66	1.66	1.38
Caryophyllen oxid	0.34	0.92	0.46	0.69	0.67
Total Identified	98.89	95.2	98.83	96.46	99.23

Conclusion:

Cobalt at 30 ppm have possible role in indigenous medicinal diuretic plants (*Cymbopogon citrates*) and recorded the relatively high level of neral (36.17%) and geranial (29.26 %), which represented about 65.43% of lemongrass oil and its quality is generally determined by the content of citral (neral and geranial isomers).

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