Journal of Applied Sciences Research, 7(12): 1778-1784, 2011
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Physiological and chemical response of Lemongrass (*Cymbopogon citratus*) to cobalt B – Endogenous hormones, chemical and Nutrional contents

[1]Nadia Gad and [2]Eman E. Aziz[2]

[1]Plant Nutrition Department, National Research Centre, Cairo, Egypt.
[2]Medicinal and Aromatic plants Research Department, National Research Centre (12622), Dokki, Cairo, Egypt.

ABSTRACT

Two field experiments were conducted to evaluate the effect of cobalt on Lemongrass herbs quality. Lemon grass seedlings were sown in Nubaria Farm, National Research Centre - Behara Governorate - Delta Egypt. Cobalt was added in the form of cobalt sulphate in five concentrations i.e., 0.0, 7.5, 15.0, 22.5 and 30.0 ppm cobalt. All the plants received natural agricultural practices during the growth period. The obtained results indicated that all the previous cobalt treatments significantly increased (except Fe content) and the physiological parameters of Lemongrass herbs when compared with the control treatment. The addition of 22.5 ppm cobalt) had a significant promotive effect of the Lemongrass endogenous hormones(Auxins,Gebberllins and cytokinens) , herbs quality such as total soluble solids, total protein, total lipids, total phenols and the contents of N, P, K, Mn, Zn and Cu as compared with other concentrations. With increasing cobalt, abscisic acid increased while Fe decreased. Higher concentrations more than 22.5 ppm excreted adverse effect. Generally, the obtained results showed that cobalt has positive role on , herbs quality Of Lemongrass plants.

Key words: Cobalt, Lemongrass, hormones, Minerals content.

Introduction

Mineral elements which either stimulates growth or those essential only for certain plant species, or under special condition, are usually defined as beneficial elements. Cobalt is considered one of those elements, although there is no evidence cobalt bas any direct role in the metabolism of higher plants.

Cobalt is required in low levels for maintaining high yields of tomato (Renner *et al.,* 2003), squash (Atta - Aly, 1998), groundnut (Basu *et al.,* ,2006), parsley (Laila Helmy and Nadia Gad, 2002) sweet potato (Nadia Gad and Hala Kandil, 2008), canola (Nadia Gad, 2010), and broccoli (Nadia Gad and Abd - El-Moez 2011) and peppermint (Aziz *et al.,* 2011).

Cobalt is essential elements for the synthesis of vitamin B12, which is required for human and animal nutrition (young, 1983). Unlike other heavy metals, Cobalt is sever for human consumption and up to 8 ppm can be consumed on a daily basis without health hazard (young, 1983).

Cobalt is unequivocally essential for leguminous crops as it is required for nitrogen fixation by bacteria in root nodules (Witte *et al.,* 2002) and it even has beneficial effect on some no leguminous crops (Locke *et al.,* 2000)

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, vitaminA and betacarotene (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

Corresponding Author: Nadia Gad, Plant Nutrition Dept., National Research Centre, Dokki, Egypt.
E-mail: drnadiagad @ yahoo.com

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).

Laila Helmy and Nadia Gad (2002) showed that, cobalt at 25 mg/kg soil had a positive effect of parsley plant growth yield, minerals composition (N, P, K, Mg, Ca, Mn, Zn and Cu) as well as chlorophyll content, total soluble solids, L-Ascorbic acid. Also, cobalt significantly increased parsley leaves. The main aroma constituent of parsley leaves is 1, 3, 8-p-menthatriene which forms about (67%) compared with control. Nadia Gad (2005b), found that cobalt resulted in maximum growth of tomato plants compared the control, cobalt attributed to catalase and peroxidase activities. These enzymes are known to induce plant respiration, so superior resulting in successive consumption for products of photosynthesis and consequently in plant growth. Moreover cobalt gave a positive effect due to several induced effects in hormonal synthesis (Auxins and Giberellins) metabolic activity-cobalt hence increasing the catabolism rather than anabolism.

Nadia Gad *et al.,* (2006) stated that under Rass Seder location (South Sinai Governorate-Egypt), as a newly reclaimed soil. Cobalt had a significant promotive effect on olive yield, fruits oil, fruits macro (N, P and K) and micronutrients (Mn, Zn, and Cu) as well as fruits quality and endogenous hormones (Auxins, Gibberllins and Abscisic acid) especially with organic fertilizer. Eman, Aziz *et al.,* (2007) found that, cobalt at 20 mg/kg soil posses a synergistic effect on status of N, P, K, Mn and Zn in Roselle leaves and calyces. Also, cobalt increased Roselle anthocyanins and flavons content.

Nadia Gad and Hala Kandil (2009) Demonstrated that, all cobalt treatments (2.5, 5.0, 7.5, 10.0 and 12.5 ppm) significantly increased the growth, yield of root and menirals composition (except Fe content). Cobalt at 7.5 ppm caused the maxium growth, root yield, menirals composition and sugar yield of sugar beet compared to control and other cobalt rates. Cobalt at 7.5 ppm gave superior figures in chemical contents (%) such as total protein carbohydrates, vitamin "C", sucrose and glucose. Roots total acidity (%) percentage decreased as cobalt increased therefore improved the root quality.

Recently, Nadia Gad (2010) demonstrated that, under Nubaria location (Behara Governorate, Delta – Egypt) condition, applying cobalt is suitable concentration gave a significant increase in the seed yield of canola compared the control. Cobalt at 12.5 ppm gave a synergistic effect on canola growth, seed yield, oil yield and oil quality compared to untreated plants. All cobalt treatments increased the contents of macronutrients (N, p and K), micronutrients (Mn, Zn and Cu) as well as chemical constituents (total protein, total carbohydrates and total phenols) as compared to control plants.

More recently, Aziz, Eman *et al.,* (2011) Found that cobalt at 15 ppm gave the greatest fresh and dry herb yield, the highest essential oil yield as well as improve the status of macro (N,P and K) and micro (Mn,Zn,Cu) nutrient content of peppermint plants. The highest level of cobalt (30 ppm) increased the principal components of menthone (37.84%) and isomenthone (15.19%) while cobalt at 7.5 ppm recorded the highest content of L- (-)- menthol (28.54%) as compared with control.

The aim of the present experiments to study the role of cobalt in Lemongrass endogenous hormones, chemical constituents and nutritional status.

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 Endogenous hormones, chemical and Nutrional of *Cymbopogon citrates plant*.

Soil analysis:

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).

Plant material and experimental works:

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

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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.

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).

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.2008-2009, 2009-2010.

Measurement nutritional contents:

Macronutrients (N, P, K,) and micronutrients (Fe, Mn , Zn and Cu) of lemongrass as well as cobalt were determined according to Cottenie *et al*.,., (1982).

Measurement chemical constituents:

The concentration of the studied chemical contents percentages (total soluble solids - total carbohydrates, total protein and total lipids) in lemongrass were determined according to A.O.A.C (1970).

Determination of endogenous hormones:- Auxins (IAA), Gibberellins (GAS), cytokinins and abscisic acid in lemongrass were determined according to Shindy and Smith (1975)

Combine data of the two seasons was statistically analyzed according to Snedcor and Cochran (1982).

Results and Discussions

Endogenous hormones:

Data presented in Table (2) indicated that all cobalt concentrations increased lemon grass herb endogenous hormones such as Auxins, Gibberllins and Cytokinins. Cobalt at 22.5 ppm recorded the superior results of lemon grass phytohormones. Increasing cobalt levels up to 22.5 ppm significantly increased these hormones. However, higher cobalt level above 22.5 ppm was depressive and caused reduction effect. Plant hormones are natural products; they stimulate physiological response in plant growth. Different strategic are being employed to maximize plant growth. These results are in harmony with those obtained by Cassan *et al.*, (2001) who found that the effect of applied Auxins and Gibberillins might be through the activation of a specific enzyme, which participates RNA and protein synthesis. Results also agreement with those obtained by Nadia Gad (1989) who domenostated that low cobalt level had a significant promotive effect in tomatoes hormonal synthesis and metabolic activity. On the other hand, reduced the activity of enzymes such as catalase and peroxides and hence increasing the anabolism rather than catabolism. While the higher cobalt levels gave the adverse effect.

The presented data in Table (2) also indicate that Abscisic acid showed a gradual increase as cobalt concentrations increased. Abscisic acid prevented the stomatal opening and caused their closure in Lemongrass plants and reduced the transpiration rate under newly reclaimed soil conditions (El-Nubaria location). These results are agree with those obtained by Nadia Gad (1989 & 2005 a) who indicated that Abscisic acid prevented the stomatal opening and caused their closure in both tomato and squash leaves and reduced the transpiration rate.

Data in Table (2) clearly indicated that the content of endogenous hormones is second cut higher than first one.

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Table 2: Endogenous hormones of Lemongrasses as affected by cobalt Addition (mean of two seasons)

Cobalt Treatments (ppm)	Auxins	Gibberellins	Cytokine's	Abscises acid
(Ug/gm fresh tissue of herb)				
	First Cut			
Control	1.267	1.490	1.196	-
7.5	2.388	2.290	1.368	0.968
15.0	2.875	2.918	2.226	1.386
22.5	3.212	3.340	2.781	2.744
30.0	2.705	3.219	2.233	3.251
LSD 5%	0.173	0.619	0.169	0.385
Second cut				
Control	1.279	1.623	1.206	-
7.5	2.515	2.466	1.718	0.997
15.0	2.944	3.245	2.421	1.624
22.5	3.439	3.669	3.184	2.981
30.0	2.901	3.405	2.802	3.404
LSD 5%	0.330	0.150	0.489	0.518

Nutritional status:

Macronutrients (N, P and K):

Presented data in Table (3) revealed that all cobalt doses (7.5, 15.0, 22.5 and 30.0 ppm) significantly increased the content of N, P and K in Lemon grass herb as compared with control. Cobalt at 22.5 ppm gave the highest values of N, P and K. This means that increasing cobalt levels above 22.5 ppm in plant media gave the adverse effect. These results are in harmony with those obtained by Nadia Gad (2010) who found that cobalt at 12.5 ppm gave a synergistic effect in the status of N, P and K of canola plants compared with control. Increasing cobalt concentration in plant media more than 12.5ppm gave the adverse effect.

Table 3: Nutritional Status of Lemongrass as affected by cobalt addition.

Cobalt Treatments(ppm)	Macronutrients (%)			Micronutrients (ppm)				Cobalt (ppm)
	N	P	K	Mn	Zn	Cu	Fe	
First Cut								
Control	1.45	0.36	1.37	28.6	13.2	18.0	242	2.1
7.5	1.66	0.39	1.44	37.3	16.8	19.9	231	5.2
15.0	1.82	0.44	1.59	40.4	21.4	24.6	208	7.8
22.5	1.98	0.56	1.68	46.5	28.5	27.4	189	10.3
30.0	1.86	0.47	1.57	39.3	26.6	24.5	167	13.8
LSD 5%	0.26	0.03	0.06	0.36	0.46	0.20	0.08	2.3
Second Cut								
Control	1.48	0.28	1.25	27.1	19.4	18.8	252	2.3
7.5	1.59	0.33	1.33	34.2	22.6	20.3	244	5.5
15.0	1.78	0.36	1.40	39.5	25.8	23.9	221	7.8
22.5	1.96	0.39	1.62	43.3	28.6	26.6	197	10.1
30.0	1.75	0.31	1.51	38.2	25.4	23.4	174	13.2
13.2LSD 5%	0.24	0.02	0.05	0.34	0.42	0.60	1.22	2.2

Confirm these results Basu *et al.,* (2006) who stated that application of low cobalt levels significantly increased N, P and K content in groundnut plants as compared with control and the higher cobalt levels had a reeducation.

Micronutrients (Mn, Zn and Cu):-

Results presented in Table (3) show the effect of cobalt on micronutrients in Lemongrass herb. Data revealed that, all cobalt concentrations had a significant promotive effect for better status of Mn, Zn and Cu in herb of Lemongrass compared with the control. Cobalt at 22.5 ppm gave the highest values of these elements. Increasing cobalt addition in plant media more than 22.5 ppm reduced the promotive effect.

These results are in agreement with those obtained by Laila Helmy and Nadia Gad (2002) who showed that cobalt at 25 kg-1 soil gave the better status of Mn, Zu and Cu of barsely herbs-increasing cobalt level had adverse effect.

Iron Content:

Data in Table (3) clearly indicated that, increasing cobalt levels in plant media resulted in a progressive depression effect of iron content in Lemongrass herbs. These data are in harmony with those obtained by Bisht

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(1991); Nadia Gad and Nagwa, Hassan (2011) who found certain antagonistic relationships between the two elements (Co,Fe), and revealed that the relative response of Fe to the control indicated continuous of those elements. They also added that the hazardous effect of cobalt being severely in wilting appearance and reduction for net photosynsis processes.

Cobalt Content:

Presented results in Table (3) showed that, increasing cobalt doses in plant media increased cobalt content in Lemongrass herbs for two harvests compared with the control.

These results are in good agreement with Nadia Gad *et al.*, (2011) who stated that increasing cobalt concentration in plant media increased cobalt content in vaba been plants for two seasons

Chemical constituents:

The concentration of the studies chemical contents (percentages of total soluble solids, total protein and total lipids) as well as total phenols (mg/g dry weight) in Limongrass as affected by different levels of cobalt are given in Table (4), Data clearly indicated that the highest values of all chemical parameters were obtained by using the level of 22.5 ppm cobalt for two harvests. All cobalt levels had a significant positive effect compared with control. Results also revealed that the relative calculate values as percentage from control. It is evident that cobalt rate at 22.5 ppm increased the content of total soluble solids (15.9%) total carbohydrates (32.4%), total protein (38.7%) while total phenols were increased (42.0%)respectively, in first harvest . In second harvest cobalt at 22.5 ppm increased the content of total soluble solids (19.1%), total carbohydrates (32.9%) m total protein (32.4 %) while total phenols were increased up to 124 %.

These results are in harmony with those obtained by Nadia Gad and Hala Kandil (2008) who stated that cobalt at 10 ppm had a favorable effect on some chemical contents such as total soluble solids , mono sugars , protein , starch , careteniods and vitamin C (L- Ascorbic acid) in sweet potato roots .

Data also revealed that increasing cobalt above 22.5 ppm decreased all the mentioned parameters as compared with their corresponding values by using cobalt level at 22.5 ppm exception total phenols which increased as cobalt level increased.

Increasing cobalt addition in plant media from 7.5 to 30.0 ppm increased the percentage of total phenols content 4.21 and 4.38 mg/g dry weight for first and second harvests respectively.

Table 4: Chemical constituents of lemon grass as effected By cobalt addition. (Mean of two seasons)

Cobalt treatments (ppm)	Total soluble solids	Total Carbohydrates	Total protein	Total lipids	Total phenols (mg/g Dry weight)
(%) First Cut					
Control	13.86	16.56	8.36	15.08	1.65
7.5	14.23	17.68	9.73	15.91	2.86
15.0	15.62	19.81	10.42	16.78	3.57
22.5	16.07	21.93	11.54	18.00	4.00
30.0	15.19	18.76	10.48	17.42	4.21
LSD 5%	0.35	0.43	0.06	0.79	0.08
Second Cut					
Control	14.21	16.31	9.24	16.21	1.81
7.5	14.46	17.24	9.92	16.88	2.47
15.0	15.72	19.76	11.11	17.52	3.26
22.5	16.92	21.68	12.23	18.38	4.06
30.0	15.49	18.39	11.04	17.72	4.38
LSD 5%	0.35	0.43	0.06	0.79	0.08

In this concern, Nadia Gad (2010) revealed that increasing cobalt up to 12.5 ppm increase all chemical parameters of canola seeds such as oil (37.4 %), protein (22.4 %), total carbohydrates (37.4 %) total phenols (58.5 %). Increasing cobalt more than 12.5 ppm decreased all chemical parameters exception total phenols which increased as cobalt level increased. Shahidi and Nazk (1989) showed that extraction of phenolic compounds use as natural antioxidants would present new opportunities for Canola industry. Pratt and Hudson (1990) stated that sources of natural antioxidants are primarily plant phenolics that may occur in all parts of the plant.

Conclusion:

Cobalt content of lemongrass herbs 8.0 and 8.2 in first and second harvest respectively with the highest level of cobalt addition in plant media (30.0 ppm). Young (1983) reported that the daily cobalt requirement for

human nutrition could reach 8 ppm drinking water without health hazard. Cobalt is promising element in the newly reclaimed soils and it is considered a beneficial element for higher plants.

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