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Response of Cowpea (Vigna Anguiculata) to Cobalt Nutrition

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Abstract: Two field experiments were carried out to study cowpea physiological and chemical response to cobalt nutrition. Experiments were conducted at Research and production Statation, National Research Centre, El-Nobaria, Beheara Governorate, Delta, Egypt under drip irrigation system. Cobalt added once i.e. 0.0, 4, 6, 8, 10 and 12 ppm.

The Obtained Results Indicate That:

- Cobalt concentrations significantly improve all growth and yield quantity and quality compared with control.
- Cobalt at 8 ppm gave the highest figures of all nodulation, growth and yield parameters as well as minerals composition and chemical constituents.
- Increasing cobalt in plant media reduced iron content; there are antagonistic relationships between two elements (Fe &Co).
- Increasing cobalt concentration more than 8 ppm excerted the adverse promotive effect.

Key words: Cowpea · Cobalt · Nitrogen fixation · Growth · Yield

ITRODUCTION

Cowpea is an annual legume, Important to the live food of millions of people as a vegetable. Cowpea seeds are a nutritions component in the human diet as well as a nutritious livestock feed. The protein in cowpea seeds is rich in lysine and tryptophan amino acids compared to cereal seeds. Therefore, cowpea seed is valued as a nutritional supplement to cereals and an extender of animal proteins.

Cobalt is considered to be a beneficial element for higher plants in spite of the absence of evidence for direct role in their metabolism. This is true in spite of essentiality for photosynthetic activities of lower plants such as euglena gracilis; it was frequently reported to be localized in various sub-cellular fractions as in chloroplasts [1].For legumes, cobalt is an essential element for certain microorganisms particularly those fixing atmospheric nitrogen, its deficiency seems to depress the efficiency of N_2 fixation. The Co-enzyme cobalamin has cobalt (III) as a metal component, chelated to four nitrogen atoms at the center of a prophyrin structure simslar to that of iron in hemin. In rhizobium species, three enzymes are known to be cobalamin dependent with cobalt induced changes in their activities are primarily responsible for the relationship to nodulation and nitrogen fixation in legumes. These enzymes are: methionine synthase, ribonucleatidereductase and methylmalonyl Co-enzyme Amutase [2].

Riley and Dilworth [3] showed that the importance of cobalt to the growth and development of leguminasea plants was mainly due to the effect of cobalt on the activity and population of both atmospheric nitrogen micro-organisms of *Azotobacter* and *Nitrobacter*. Nadia Gad [4] added that the endogenous auxins and gibberellines in plants grown in nutrients solution culture having 0.25 ppm were significantly increased and enhanced the growth and yields of tomato plants compared with the control. Smith [5] showed that Cobalt is an essential element for the synthesis of vitamin B12

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which is required for animals and human nutrition. Singh *et al*. [6] pointed that cobalt at the rate of 2.0mg/ kg soil gave the highest fresh and dry weights of *Phasselus vulgaris* compared with the control. Jana *et al*. [7] showed that cobalt at 1.0 ppm increased growth yield of groundnut compared with the control. Vinay *et al*. [8] added that both fresh and dry weights as well as seeds yield of clusterbean increased with application of 2.0 mg Co and 40 mg phosphorus / kg soil.Nasef *et al*. [9] stated that cobalt gave significantly higher biomass production and seeds yield of cowpea compared with untreated plants.In pot experiments, [10 and 11] demonstrated that cobalt at 50 mg/kg soils increased growth parameters such as plant hight, root length, shoots and roots dry weight along with yield parameters such as seedling vigor, number of pods per plant in green gram (*Vigna radiate L.*) and maize (*Zea mays L.*) plants. Dilworth *et al*. [12] stated that the specific activities of cobalt deficient nodules remained very low even when nitrogenase did develop. Their large mass of nodules allowed cobalt deficient plants to reach 20-50% of the normal activity / plant, but specific activities were only 5-13 % of peak activities in cobalt treated. Nodule bacteroid content and leghemoglobin were linerly related to cobalamin content. It is suggested that there may be a function in N_2 fixation in legume nodules for a non-cobalamin form of cobalt. Yoshida [13] found that cobalt at 1.0 mg /L gave maximum vitamin B_{12} production in the sap of kidney bean (*phaseolus vulgaris*) and lupine (*lupinus spp.*). Concentration of B_{12} active substances was higher in roots than shoots, particularly in nodulated plants. In all legumes tested, most of B_{12} active substances were present in the nodules. It was also observed that, the increase in nitrogenase activity was parallel and related to the increase in nodules number and efficiency. Well known, the nitrogenase is complex enzyme enables fixation of atmospheric nitrogen [14].

Balai *et al*. [15] added that cobalt recorded the maximum nodulation in groundnut roots. Nadia Gad [16] pointed that cobalt at 8 ppm increased total nodules number and dry weight, number and weight of effective nodules and root dry weight in pea (*pisumsativum L*) plants. Balai and Majumder [17] found that cobalt increased number and weight of cowpea nodules as well as the content of leghemglobin content of root nodules. Halakandi l[18] added that cobalt significantly increased nodules formation in faba bean roots. younis [19] showed that cobalt at 100 mg/kg soil increased nodules number and their mass in *(Lablab purpureus)* plants. Recently, [20 and 21] showed that cobalt at 50 mg/kg soil increased growth parameters such as plant hight, root length, total leaf area, shoots and roots dry weights and pods yield of groundnuts and soybean compared with the control. Morerecently, [22] showed that is an essential element for certain microorganisms, particularly those fixing atmospheric nitrogen in particular, for nodules formation, its deficiency seems to depress the efficiency of N fixation processes in faba bean.

MATERIALS AND METHODS

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 [23]. 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.,* [24]. Determination of soluble, available and total cobalt was determined according to method described by Cottenie *et al.,* [25]. Some physical and chemical properties of Nubaria soil are shown in Table 1.

Experimental Works: A Preliminary pot experiment was conduct at Wire house of National Research Centre to define cobalt concentrations range which gave growth and yieldresponse. Seedlings of cowpea (at the third true leaves) irrigated once with cobalt concentrations: 0.0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ppm. According to the preliminary experiment results, the concentration range of cobalt which gave the cowpea response growth and yield were 0.0, 4, 8, 12and 16ppm. Cobalt at 8 ppm gave the best growth and yield parameters of cowpeas.

Tow field experiments conducted in the Production and Research Station, Notional Research Centre, EI-Nubaria under drip irrigation system, during the two successive seasons of 2011 and 2012. Experiments were carried out to evaluate cowpea physiological response to cobalt nutrition. The sandy loam soil with plot area consists of five ridges, 3.5 meter in length and 60 cm width (10.5m^2= 1/400 fed). Calcium super phosphate (15.5 %) at the rate of 150 kg P_2O_5/fed, chicken manure (Table 2) at the rate of 15 m^3/fed and potassium sulphate (48% k_2O) at the rate of 100 kg /fed were added during soil preparation. Seeds of cowpea (*vigna*unguiculata var. karim-7). was inoculated prior to sowing with a specific strain of rhizobium (Rhizobium melitota.). Seeds were sown on April, 2011and 2012 summer seasons.

The seedlings (at the third true leaves) were irrigated once with six concentrations of cobalt (0.0, 4, 8, 12, 16 and 20 ppm) as a cobalt sulphate form.

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 ($dS\ m^{-1}$)	$CaCO_3$%	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		Availablemicronutriments			
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).

Table 2: Some properties of chicken manure

					Available nutrients (%)		DTPA-extractable (ppm)			
O.M (%)	Total N(%)	C/N ratio	pH (1:25)	EC dsm^{-1}	P	K	Fe	Mn	Zn	Cu
36.0	2.96	7.07	6.40	8.85	0.72	0.93	566	36.8	28.2	34.7

All required agricultural managements for plants growth and production were carried out as recommended by Ministry of Agriculture.

Measurements of Vegetative Growth: After 80 days from sowing, all growth parameters of cowpea plants such as plant height, root length, number of branches and leaves as well shoot and root fresh and dry weights were recorded according to FAO [26].

Nodulation and Nitrogenase Activity: Nodules number and weight were recorded after 50 days from sowing. Nitrogenase activity was determined according to Hardy [27]. Cowpea plants were gently uprooted then the root nodules were placed in 500 ml serum bottles and were sealed with suba-seal rubbers and 10 % of the gas phase was replaced by C_2H_2 then bottles were incubated in dark at room temperature for 2hr. production of C_2H_4 was measured by injecting one ml gas sample into (GC). Nitrogenase activity values were recorded as µmol C_2H_4 /g/h.

Measurements of Plant Yield: After 120 days from sowing respectively, cowpea yield parameters such as pods number/plant, weight of pods/plant, weight of seeds/plant, 100 seeds weight, total pods yield (kg/fed), total pods yield (Ton/fed) were recorded according to Gabal *et al*. [28].

Nutritional Status: For chemical analysis, seeds sampled either from the intact plant for each treatment of both seasons was oven dried at 70°C for 48 hr ground and kept to chemical determinations. For extraction a weight of 0.2 g finely powdered dry sample and digested using a mixture of sulfuric acid (H_2SO_4) with hydrogen peroxide (H_2O_2) according to the method described by Cottenie *et al* . [25].

Chemical Constituents: The percent of total carbohydrates, total soluble sugars, total soluble solids, of cowpea seeds were determined according to A.O.A.C [29].

Statistical Analysis: All data were subjected to statistical analysis according to procedure outlined by SAS, [30] computer program and means were compared by LSD method according to Snedecor and Cochran [31].

RESULTS AND DISCUSSION

Vegetative Growth: Data presented in Table 3, outline the response of growth parameters to cobalt. It is clear that cobalt promotes all growth parameters such as plant height, root length, number of branches and leaves as well as leaf area index in both 2011 and 2012 seasons. Cobalt at 12 ppm enhanced all growth parameters of cowepea plants. Increasing cobalt above 12 ppm reduce the promotive effect. These results are in harmony with [32] who found that favorable growth responses associated with low cobalt level attributed to low catalase and peroxidase enzymes activity. These enzymes are known to induce plant respiration possibly resulting in successive consumption for products of photosynthesis and subsequently reduction plant growth. Similar results are reported by Abdul Juleel *et al*. [11] who added that cobalt at 50 mg/kg soil increased all growth parameters, pigment contentsantioxidant enzymes like catalase, peroxidase and polyphenol oxidase in greengram plants compared with the control. Data in the Table 3, revealed that cobalt application had a promotive effect on both fresh and dry matter content of cowpea plants compared with the control at intervals of 80 days from sowing. These results are agreed with those of [33] who found the stimulation effect of cobalt on plant metabolism and endogenous plant hormones. Cobalt significantly increased the concentration of endogenous auxins and gibberellins, which increase the growth compared with control.

Nodulation Parameters: Data presented in Table4 reveal that cobalt had a significant synergistic effect on cowpea root nodules parameters such as total nodules number / plant, fresh and dry weights of nodules compared with the untreated plants. Cobalt at 12 ppm gave the highest nodules parameters of cowpearoots after 50 days from sowing for the two seasons. Increasing cobalt level above 12 ppm reduces the promotive effect. These results are in harmony with those obtained by Yadav and Khanna [2]. Who stated that cobalt application improves the nodules number per plant and increased the formation of leg hemoglobin required for nitrogen fixation. Similar results are reported by Basu *et al*. [35] who found that cobalt increased number and dry weights of nodules per plant as well as leghemoblobin content in cowpea roots especially with phosphobacterium.

Data in Table 4 also showed that cobalt addition significantly increased the activity of nitrogenase of cowpea root nodules after 50 days from sowing for both two seasons compared with the untreated plants. Cobalt at 12 ppm recorded the maximum nitrogenase activity. Increasing cobalt more than 12 ppm decrease

Table 3: Cowpea growth parameters as affected by cobalt levels after 80 days from sowing (mean of two seasons).

		Number/Plant				Dry weight (9)	
Cobalt treatments (ppm)	Plant hight (cm)	Branches	Leaves	Leaf area (cm^2)	Root length (cm)	Shoot	Root
Control	42.2	7.4	25.3	1465	17.2	31.8	3.14
4	44.5	8.6	26.8	1685	18.7	33.5	3.77
8	47.6	9.2	29.4	1829	19.5	36.6	4.21
12	46.0	9.0	28. 0	1785	19.2	35.2	4.03
16	43.8	8.4	27.5	1660	18.0	33.7	3.87
LSD 5%	1.6	0.2	0.3	44	00.4	0.2	0.18

Table 4: Cowpea nodulation parameters as affected by cobalt levels after 50 days from sowing (mean of two seasons).

Cobalt treatments (ppm)	Nodules No. Per plant nodule	Nodules fresh Weight/plant (g)	Nodules Weight/plant (g)	N-ase activity µmol C_2H_2/g/h
Control	53.4	5.82	1.84	22.6
4	74.8	6.69	2.37	23.5
8	89.9	7.86	2.83	26.2
12	82.5	6.44	2.40	27.0
16	76.8	6.82	2.26	25.9
20	72.6	6.78	2.17	24.4
LSD 5%	2.2	0.4	0.3	0.7

Table 5: Cowpea yield parameters as affected by cobalt levels after 120 days from sowing (mean of two seasons).

Cobalt treatment (ppm)	Pods No.Per plant (pod)	100 seeds Weight (g)	Pods Weight/plant (g)	Seeds yield Kg/fed
Control	15.0	9.3	12.2	996
4	19.6	12.7	16.5	1318
8	24.0	15.8	20.9	1353
12	20.9	13.5	17.7	1340
16	19.0	12.8	16.8	1329
20	17.8	10.7	14.3	1108
LSD 5%	0.6	1.2	0.3	11

Table 6: Minerals composition in Cowpea seeds as affected by cobalt levels (mean of two seasons).

	Macronutrients (%)			Macronutrients (ppm)				
Cobalt treatments (ppm)	N	P	K	Mn	Zn	Cu	Fe	Cobalt (ppm)
Control	1.6	0.21	1.30	16.5	11.2	15.0	51.5	0.89
4	1.9	0.23	1.36	17.2	12.7	16.3	49.9	1.03
8	2.3	0.28	1.43	19.5	14.3	17.5	49.3	2.12
12	2.2	0.25	1.40	18.3	14.0	17.2	48.8	3.36
16	2.0	0.24	1.39	18.0	13.6	16.9	46.9	4.69
20	1.8	0.24	1.37	17.7	13.6	16.6	46.5	5.08
LSD 5%	0.2	0.2	0.2	0.5	0.3	0.3	0.4	0.8

the favorable effect.These results are agree with those reported by Basu *et al*. [35] those stated that cobalt improved nodules of groundnut, also they suggested that with cobalt treatment nodules bacteroid content and leghemoglobin were linearly related to cobalamin content. According to Sarada and Polasa [36], cobalt is an essential element for growth of *rhizobium*, the specific bacteria involved in legume nodulation and fixation of atmospheric nitrogen into amino acids and proteins in legumes. Finally, cobalt is an essential element for legumes due to bacteria on root nodules and to synthesize vitamin B_{12} which required for the microorganisms fixing nitrogen in nodules and nitrogenase activity in cowpea.

Pods Yield Characteristics: Table 5 exhibit the effect of cobalt on cowpea yield parameters. Cobalt had a significant promotive effect on number and weight of pods / plant, weight of seeds / plant and total pods yield. Cobalt at 12 ppm in plant media increased total pods yield (ardab/fed =75kg) about 34.5%. These results are agree with those obtained by Nadia Gad [16] who found that cobalt at 8 ppm increased pods yield of peas. These data are in harmony with those obtained by Balachandar *et al*. [37] who pointed out that cobalt is a necessary element to legumes, in particular, for nodule formation and nitrogen fixation.

Nitrogen, P and K Content: Obtained data (Table 6) clearly indicate that cobalt treatment had a maximum content of N, P and K in cowpeaseeds comparison with untreated plants. The results reveal, as expected and as mentioned by Abd El-Moezand Nadia Gad [34] that cobalt at 8 ppm increased macronutrients (N, P and K) content in both shoots and roots of cowpea plants under different levels of nitrogen.

Manganese, Zn, Cuand Co Content: Data in Table 6, indicate that cobalt had significant highest values of Mn, Zn,Cu and Co of cowpea seeds compared with the untreated plants. These results are in harmony with those reported by Jayakumar *et al*. [38] who found that all minerals composition of blackgram were increased with cobalt at 50 mg/kg soil, when compared with the control.

Iron Content: The present data in Table6 indicate that cobalt significantly decrease iron content in cowpea seeds compared with the untreated plants. These results arein harmony with those obtained by Bisht [39] and Blaylock *et al*. [40] that, cobalt addition in plant media showed progressive depression effect on iron status in tomatoes and soyabean plants. They added that certain antagonistic relationships between cobalt and iron.

Table 7: Chemical content of cowpea seeds as affected by cobalt levels (mean of twoseasons).

Cobalt treatment (ppm)	Protein %	Total Soluble Solids %	Total Carbohydrates %	Total Soluble Sugar%
Control	10.0	34.6	21.2	9.68
4	11.9	34.9	23.8	10.5
8	14.4	36.2	24.5	11.9
12	13.8	35.8	24.0	11.4
16	2.5	35.1	23.6	10.8
20	11.3	35.1	23.1	10.2
LSD 5%	0.6	0.3	0.5	0.3

Cobalt Content: Data in Table (6) reveal that increasing cobalt levels in plant media increased cobalt contentment in cowpea seeds compared with control treatment. These results clearly indicated that cobalt content goes along with the concentration of cobalt added. The obtained results are in good agreement with those obtained by NadiaGad *et al.* [22]. El-kobbia and osman [41] pointed that there was evidence that when plant roots absorb water, soil solution containing cobalt moves from the non-rhizosphere soil towards roots by mass flow.

Chemical Constituents: The amount of protein, total carbohydrate, total soluble sugars and total soluble solids percentages in cowpea seeds as affected by cobalt are given in Table 7. Results indicate that all the mentioned parameters were significantly increased by cobalt nutrition. Cobalt 12 ppm increased all chemical contents as a quality of cowpeaseeds. Increasing cobalt above 12 ppm results the depression promotive effect.

These results are in harmony with those obtained by Nadia Gad [42] revealed that cobalt addition in plant media increased protein, total soluble solids, total carbohydratesand total soluble sugars in groundnut seeds.

CONCLUSION

Cobalt is a promising element in the newly reclaimed soils. It is used to reduce the harmfull effect of high temperature, drought and salinity in these soils. Cobalt is an essential element in both human and animal nutrition. The daily cobalt requirements for human nutrition could reach 8 ppm depending on cobalt levels in the local supply of drinking water without health hazard.

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