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Role of Cobalt on Cowpea Growth and Yield under Different levels of Nitrogen

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Abstract: Two field experiments were carried out to evaluate role and importance of cobalt under different levels of nitrogen on nodules efficiency, growth, yield, minerals composition and chemical characteristics of cowpea plants. The experiments were conducted at Research and Production Station, National Research Centre, El-NobariaBeheara Governorate, Delta- Egypt, under drip irrigation system during 2011 and 2012 seasons.

The obtained results are summarized in the following:

- Cobalt significantly increased nitrogenous activity which was parallel and related to the increased nodules numbers, weights of cowpeaand its efficiency especially with both 100 and 75% nitrogen fertilizer.
- Cobalt gave the highest figures of all growth and yield parameters of cowpea with 100% N followed by 75% N compared with the untreated plants.
- Growth and yield parameters of cowpea treated with cobalt and 50% N was not significant while cobalt with 25% N gave the lowest figures.
- Cobalt increased the efficiency of nitrogen fertilization, amendment reduce the recommended dose of about by 25% and resulted in superior cowpea pods and seeds yield about by 25.6 % and 18.84 with75% N respectively, compared with 100 N without cobalt.
- Cobalt significantly increased the content of N, P, K, Mn and Zn as well as chemical contents especially with 100% and 75% nitrogen levels.

Finally, the addition of Cobalt to the soil, save 25% nitrogen fertilizer and could be reduced the nitrogen environmental pollution and induced the agricultural cost for more money of farmers.

Key words: Cobalt and nitrogen · Cowpeayield-Nodulation · Nitrogen fixation

INTRODUCTION

Cowpea is an annual legume. Important to the live food of millions of people as a vegetable. Cowpea seeds are a nutrition 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 an essential element for the synthesis of vitamin B12 which is required for human and animal nutrition [1, 2]. Cobalt is an essential element for legumes due to its essentiality for the micro-organisms fixing atmospheric nitrogen [3, 1].Yoshida[4] reported that the addition of cobalt at 1.0 mg/ L in plant media increased vitamin B12 production as well as all growth parameters and pods yield of *lupinesspp*. Due *et al*. [5] showed that cobalt at 8 ppm had a greatest growth parameters, pods and seeds yield in cowpea. Abdel Moez andNadia Gad [6] pointed that cobalt at 8 ppm increased fresh and dry weights of both shoots and roots in cowpea plants. Cobalt saved about 75% of the added inorganic fertilizer (N, P and K) and 33% of the adder organic cotton compost. Sowicki[7] and Basu *et al*. [8] reported that cobalt at 0.21 k/ha increased plant hight, number of branches and leaves, leaf area index, dry weight of shoots as well as pods yield of groundnut. Balai *et al*. [9] and

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Banerjee *et al*. [10] found that cobalt recorded the maximum leaf area index, dry matter accumulation in aerial parts of the plants, root dry weight, plant height as well as pods yield in both cowpea and groundnuts compared with the control. Nadia Gad [11] pointed that cobalt at 8 ppm had a greatest fresh and dry weights of both shoots and roots as well as pods yield quantity and quality of peas compared with the control and other cobalt levels. Basu *et al* [12] showed that cobalt at 0.21 kg/ha proved groundnut growth as dry matter content and the percent in pods yield compared with the control. Hala Kandil [13] showed that cobalt significantly increased faba bean growth and yield (pods and seeds).

Cobalt was directly proportional to vitamin B12 content which plays an important role in enhancement fixation of atmospheric nitrogen. Cobalt is essential for growth rhizobia, the specific bacteria involved in legume nodulation and nitrogen fixation into amino acids and protein. In nitrogen-fixing bacteria, the nitrogenase enzyme drives the reaction of atmospheric dinitrogen fixation in presence of ATP [14]. Basu *et al* [8] added cobalt at 0.21 kg/ha which increased number and dry weight of nodules per plant as well as leghemoglobin content in peanut roots especially with *phosphobacterium*than *rhizobium* treatment. Banerjee and Sounda [10] and Balai *et al* [9] demonstrated that cobalt significantly improvement total nodules number and dry weight, number and weight of effective nodules and root dry weight in both groundnut and cowpea.

Balai *et al* [9] added that cobalt recorded the maximum nodulation in groundnut roots. Nadia Gad [15] 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 [9] found that cobalt increased number and weight of cowpea nodules as well as the content of leghemglobin content of root nodules. Halakandil [13] added that cobalt significantly increased nodules formation in faba bean roots. Yagdin [16] showed that cobalt at 100 mg/kg soil increased nodules number and their mass in *(Lablab purpureus)* plants. Later on, Jayakumar *et al* [17, 18] found that cobalt had beneficial effect on nodules number and their dry weight in both soybean and peanut roots.

Recently, Nadia Gad [19] stated that the amendment of cobalt at 8 ppm to the soil improved the growth parameters, nodulation rate,nitrogenase activity, buds and seed yield as well as minerals content in groundnuts. Also, all yield measurement at all N doses were scanty and uneconomically in absence of cobalt amendment, particularly at the low doses of N fertilization

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 [20]. Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen and available P, K, Fe, Mn, Zn, Cu were run according to [21]. Determination of soluble, available and total cobalt was determined according to method described by Cottenie *et al.* [22]. 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 yield response. 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, 12 and 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 with different levels of nitrogen. 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 compost (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, 2011 and 2012 summer seasons.

Ammonium sulphate $(NH_4)_2SO_4$ (20.5% N) at the rate of 325 kg/fed was basic amount (100% N) as control. The levels of ammonium sulphate treatments were calculated to be corresponding to 25, 50 and 75% of the control. The seedlings (at the third true leaf) were irrigated once with cobalt at the rate of 8 ppmas according to Ref. [6].

Table 1: Some physical and chemical properties of Nubaria soil

Physical properties							
Particle size distribution %				Soil moisture constant %			
Sand	Silt	Clay	Soil texture Sandy loam	Saturation	FC	WP	AW
	25.6	3.6		32	19.2	6.1	13.1

Chemical properties											
				Soluble cations ($meq^{-1}L$)				Soluble anions ($meq^{-1}L$)			
pH 01:02.5	EC ($dS\ m^{-1}$)	$CaCO^3$%	OM %	Ca^{++}	Mg^{++}	K^+	Na^+	HCO_3^-	CO_3	Cl^-	$SO_4^=$
8.49	1.74	3.4	0.2	0.8	0.5	1.6	1.8	0.3	-	1.9	0.5

Cobalt ppm			Total Available mg 100 g^{-1} soil			Availablemicronutriments 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

Table 2: Chemical properties of chicken manure.

O.M (%)	Total N (%)	C/N ratio	pH (1:25)	EC dsm^{-1}	Available nutrients (%) P	K	Fe	DTPA-extractable (ppm) Mn	Zn	Cu
36	2.96	7.07	6.4	8.85	0.72	0.93	566	36.8	28.2	34.7

Soil pH and EC were measured in 1:10 soil water suspension

A Number of 8 Treatments Were Concluded:-

- 1-$(NH_4)_2SO_4$ 100% recommended dose as control
- 2-$(NH_4)_2SO_4$ 75 %
- 3-$(NH_4)_2SO_4$ 50 %
- 4-$(NH_4)_2SO_4$ 25 %
- 5-$(NH_4)_2SO_4$ 100% + Co at 8ppm
- 6-$(NH_4)_2SO_4$ 75% + Co at 8ppm
- 7-$(NH_4)_2SO_4$ 50% + Co at 8ppm
- 8-$(NH_4)_2SO_4$ 25% + Co at 8ppm

All required agricultural managements for plants growth and production were carried out as recommended by Ministry of Agriculture. The experimental design was a piece ofsplinteronce with three replications for each treatment

Nodulation and Nitrogenase Activity: Nodules number and weight were recorded after 50 days from sowing-Nitrogenase activity was determined according to Hardy [23]. 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 Vegetative Growth: After 80 days from sowing, all growth parameters of both 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 [24].

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 (Ardab/fed) were recorded according to [25].

Chemical Constituents: The percent of total carbohydrates, total soluble sugars, total soluble solids, of seeds were determined according to [26].

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* [22].

Statistical Analysis: All data were subjected to statistical analysis according to procedure outlined by SAS[27] computer program and means were compared by LSD method according to[28].

RESULTS AND DISCUSSION

Nodulation Parameters: The obtained data in Table 3 indicate that cobalt had a significant positive effect on cowpea root nodules parameters under different nitrogen levels compared with the untreated plants. Cobalt recorded the maximum cowpea nodules parameters i.e. number of total nodules / plant, fresh and dry weights of nodules with 100% nitrogen followed by 75% nitrogen. Reducing the level of nitrogen up to 50% nitrogen was not significant while with 25% nitrogen cobalt gave the lowest nodulation after 50 days from sowing in the two seasons. These results were agree with those obtained by Balai Majumdar [9] and Nadia Gad [15], they pointed that cobalt significantly improved total nodules number and dry weight, number and weight of effective nodules and root dry weights in both cowpea and pea plants compared with the control. Similar results are reported by Balachandar *et al* [29] who found that the presence of cobalamin Co-enzym in the nodules of several legumes confirmed the role of cobalt in nitrogen fixation. Thus a deficiency in cobalt is shown in reduced vitamin B_{12} production and lower nitrogen fixation. The obtained data in Table.3 reflected that cobalt can play a vital role in increasing nitrogenase enzyme activity with all nitrogen levels of cowpea root nodulation after 50 day from sowing in the two seasons, compared with the untreated plants. Cobalt recorded the best rate of the nitrogenase enzyme activity with 100% followed by 75% nitrogen compared with the control (100% nitrogen alone). With the rate of 50% nitrogen, cobalt was noninsignificantly while, with 25% nitrogen gave the lowest ones. These results are in harmony with [30] showed that the co-enzyme cobalamin has cobalt (III) as a metal component, cheleated to four nitrogen atoms at the center of a prophyrin structure similar to that of iron in hemin. In rhizobium species, enzymes dependent with cobalt induced their activities are primarily responsible for the relationship to nodulation and nitrogen fixation in legumes. Similar results reported by Riley and Dilwarth[31] who demonstrated that cobalt is required for the growth and population of micro-organisms responsible of nitrogen fixation. In spite of the mentioned requirements for cobalt, plant species remarkable differences to excess cobalt.

Finally, root nodules parameters and nitrogenous enzyme activity in nodules of cowpea was significant influenced by cobalt [8].

Cobalt is essential for growth of the *rhizobium*, the specific bacteria involved in legume nodulation and fixation of atmospheric nitrogen into amino acids and proteins in legumes.Vitamin B_{12} production and lower nitrogen fixation.

Vegative Growth: Cowpea growth parameters as affected by cobalt and nitrogen levels after 80, days from sowing are given in Table. 4. Data indicate that cobalt at 8ppm had a significant promotiveeffect on all growth parameters of cowpea after 80 days from sowingunder different levels of nitrogen compared with untreated plants. It is clear that cobalt enhance all growth parameters such as plant height, root length, number of branches and leaves of cowpea in both seasons especially with 100% and 75% nitrogen compared with the control (100% nitrogen alone). As nitrogen level was reduced, the promotive effect significantly reduced. Cobalt treatment with 25% nitrogen gave the lowest growth parameters of cowpea for two studied seasons.

Confirm, these results [19]showed that cobalt at 8 ppm significantly increased all groundnut growth parameters such as plant height, number of branches and leaves per plant, leave area as well as shoot and root biomass.

These observations are consistent with previous reports obtained by Nadia Gad [32] who found that cobalt being with positive effect due to several induced effects in hormonal synthesis and metabolic activity, while it's reduce the activity of some enzymes such as peroxidase and catalase in tomato plants and hence increasing the anabolism rather than catabolism. Data in Table 4 also indicated that cobalt significantly increased fresh and dry weights in cowpea shoots especially with 100% and 75% nitrogen in two studied seasons compared with the control. These results were agree with those obtained by Hanson *et al*[33] who found that cobalt is considered to be a beneficial element for higher plants and is a kind of trace element and heavy metal found in soil. Milletti[34] added that supplementing nutrient solution with cobalt improved the growth of tomato plants and enhanced both flowering and fruiting.

Yield Parameters: Obtained results in Table 5 showedthat cobalt had a significantly promotive effect on all yield parameters such as number of pods / plant,

Table 3: Cowpea nodulation parameters as affected by cobalt under different nitrogen levels after 50 days from sowing (mean of two seasons).

Nitrogen treatments %	Nodules No. Per plant (nodule)	Nodules fresh Weight (g)	Nodules dry Weight/plant (g)	N-ase activity µmol C_2H_2/g/h
Without cobalt				
100	52.9	5.8	1.73	24.5
75	51.5	5.68	1.7	23
50	48.4	5.59	1.56	21.8
25	46.5	5.19	1.51	20.3
With cobalt(8ppm)				
100	88.5	7.88	1.98	26.9
75	83	7.52	1.91	25.2
50	79.6	7.01	1.83	24.5
25	73.1	6.48	1.7	23.3
LSD 5%	1.4	0.9	0.05	0.3

Table 4: Cowpea growth parameters as affected by cobalt under different nitrogen levels after 80 days from sowing (mean of two seasons).

Nitrogen treatments %	Plant height (cm)	Number/Plant		Leaf area (cm^2)	Root length (cm)	Dry weight (9)	
		Branches	Leaves			Shoot	Root
Without cobalt							
100	41.9	7.1	25.6	1461	17	31.5	3.13
75	39.8	6.8	21.8	1246	15.7	29.6	2.87
50	36.2	5.7	20.1	1157	14.4	27.1	2.69
25	34	5.2	17.9	1032	12.5	25	2.45
With cobalt(8ppm)							
100	46.9	9.8	29.3	1828	19.3	36.5	4.18
75	42.3	9.1	26.7	1699	17.7	33.1	3.77
50	40	8	24.3	1496	16.2	31.2	3.53
25	37.7	6.5	21	1224	14	29.3	3.34
LSD 5%	2.2	0.5	0.8	0.88	0.4	0.3	0.2

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

Nitrogen treatment %	Pods No. Per plant (pod)	100 seeds Weight (g)	Pods Weight/plant (g)	Seeds yield Ardab /fed
Without cobalt				
100	15.9	9.53	14	968
75	14	8.42	13.7	855
50	12.9	7.78	10.8	788
25	10.2	6.18	9.92	624
With cobalt(8ppm)				
100	23.8	15.6	20.3	1356
75	22.5	14.8	18.9	1284
50	20.7	13.1	17	1186
25	17	12.2	15.9	1067
LSD 5%	1.8	0.3	0.9	67

weight of pods / plant, weight of seeds/ plant and weight of 100 seeds of cowpea. Cobalt increased all cowpea yield parameters under different nitrogen levels in two studiedseason's comparison with untreated plants. These results were agree with those of [35] who pointed that, in loamy sand soil yields of soyabean and cowpea were highest at 60 days of growth with cobalt addition in soyabean and at 30 and 60 days of growth with cobalt in cowpea. Cobalt gave the highest growth and yield parameters. The quadratic and square root functions predicted significant relationships among yields of those legumes at different stages. Nadia Gad[36] added that the endogenous ouxins and gibberellines in tomato plants grown in nutrients solution culture having 0.25 ppm cobalt were significantly increased. These hormones enhancement in growth and yield of tomatoes compared with the control. According to Due *etal*[5], cobalt at 8 ppm had a greatest growth parameters, pods and seeds yield in cowpea compared with the untreated plants. Data in Tables 5 also revealed that cobalt gave the maximum yield parameters with 100% nitrogen followed by 75% nitrogen. On the other hand, cobalt with 50% nitrogen has no significant effect and gave the lowest figures with 25% nitrogen in the two seasons. Such as pattern may be attributed, according to Due *etal*[5], they found that cobalt recorded the maximum leaf area index, dry matter accumulation in plant shoots and roots as well as pods yield in both cowpea and groundnut compared with the control. Data in Table (5) showed also, the relative calculated values of cowpea yield as percentage from control. It is evident that cobalt increased cowpea pods yield about 62.5 % and 60.0 % respectively in both studied seasons. These results reveal, as mentioned by Nadia Gad[15] who found that cobalt increased pea pods yield about 26.2% with 75% nitrogen as urea and by 29.3 % with 75% nitrogen as ammonium nitrate compared with the control (100% nitrogen alone). According to,AbdulJaleel*etal*[37 and 38] cobalt addition in soil increased all growth parameters along with yield parameters such as seedling vigour, number and weight of pods and seeds yield / plant in greengram (*Vigna radiate L.*) and maize (*Zeamaiz L.*) plants.

Nutritional Status: Data presented in Table 6 show the beneficial effect of cobalt on mineral composition (N, P, K, Mn, Zn and Cu) of cowpea seeds in both two seasons. Data alsoindicate that, with increasing cobalt level in plant media, cobalt in seeds increase. Data reflected the superiority nutritional statusof cobalt with 100% nitrogen and the favorable effect of cobalt with 75% nitrogen. Cobalt with 50% N was non-significant and with 25% nitrogen gave the lowest nutritional status. These results are in good agreement with those found by Jana *et al* [39] who stated that cobalt had a positive effect for better status of all mineral in groundnut seeds compared with the control. Basu*et al* [8] added that cobalt gave a higher oil content and superior nutrients uptake by groundnut seeds compared with the control. According to Jayakumar *et al* [40], all minerals content of blackgram were increased with cobalt at 50 mg/kg soil when compared with the control. On the other hand, these results are agree with [41] and [42], who stated that, cobalt addition in plant media resulted in proportion significant reduction in iron content. This indicates, again, the competition between cobalt and iron content in soybean and canola shoots and seeds.

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

N treatments %	Macronutrients (%)			Micronutrients (ppm)				
	N	P	K	Mn	Zn	Cu	Fe	Cobalt (ppm)
Without cobalt								
100	1.55	0.27	1.29	16.2	11.6	14.8	51.3	0.87
75	1.49	0.22	1.24	15.6	11	14.2	49.5	0.85
50	1.36	0.19	1.18	15	10.5	15.8	48.6	0.82
25	1.19	0.12	1.13	14.6	10	13	46.3	0.79
With cobalt(8ppm)								
100	2.35	0.37	1.41	19.2	14.7	17.6	48.5	3.11
75	2.11	0.33	1.36	18.7	13.3	17.1	48.3	3.01
50	1.97	0.23	1.3	18	11.9	16.8	37.1	2.87
25	1.85	0.16	1.25	16.9	11.2	16.1	56.7	2.69
LSD 5%	0.12	0.4	0.5	0.4	0.5	0.6	0.3	0.2

Table 7: Chemical content of cowpea seeds as affected by cobalt under different nitrogen levels (mean of two seasons).

Nitrogen treatment %	Protein %	Total Carbohydrates %	Total Soluble Sugars %	Total Soluble Solids %
Without cobalt				
100	9.69	20.9	9.65	33.8
75	9.31	20.2	9.59	30.1
50	8.5	19.8	9.47	28.6
25	7.44	18.6	9.11	26.5
With cobalt (8ppm)				
100	14.7	24	11.8	36
75	13.2	22.8	11.2	34.8
50	12.3	21.3	10.8	33
25	11.6	20.6	10.4	31.3
LSD 5%	0.7	0.4	0.36	0.8

Confirm, these results,[43] who stated that cobalt at 12 ppm different doses of N fertilization and its effect on Faba bean production aiming to minimize the rate of N fertilization.Depending upon rhizobiall inoculation of seeds and application of cobalt asenhancing agent, ratios of 100, 75 and 25% N recommended dose.

Chemical Constituents:The amount of protein, total carbohydrate, total soluble sugars and total soluble solids in cowpea seeds as affected by cobalt and different nitrogen levels are given in Table 7. Results indicate that all the mentioned parameters were significantly increased by cobalt nutrition with all nitrogen levels. Cobalt increased all chemical contents as a quality of cowpea seeds.

These results are in harmony with those obtained by Nadia Gad [19] Revealed that cobalt addition in plant media increased protein, total soluble sugars and carbohydrates, total soluble solids in groundnut seeds.

CONCLUSION

Our results showed that the addition of cobalt led to increase in cowpea growth as well as the quantity and quality of their yield at the usual amount of fertilizer. The result showed also that the addition of cobalt with 75% of the recommended amount of nitrogen fertilizer led to good results and often convergent with the results of full fertilization. These results lead to the conclusion that the addition of cobalt saved about 25% of recommended nitrogen fertilizer dose and enhanced cowpea yield quantity and quality. Therefore, it could be reduced the agricultural cost for more money to farmers.

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