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Influence of Cobalt on Soybean Growth and Production under Different Levels of Nitrogen

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Abstract

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

The obtained results are summarized in the following:

Cobalt significantly increased nitrogenase activity which was parallel and related to the increased nodules numbers, weights and its efficiency especially with both 100 and 75% nitrogen fertilizer.

Cobalt gave the highest figures of all growth and yield parameters of Soybean with 100% N followed by 75% N compared with the untreated plants.

Growth and yield parameters of Soybean 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 seeds and oil yields by about 20.8% and 17. 4 respectively, with75% N, 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 75% nitrogen and could be reduced.

Key-Words: Cobalt and nitrogen - Soybean yield-Nodulation –Nitrogen fixation

Introduction

Soybean (***Glycine max L. Merril***) is an important crop as food and feed, it contains high nutritional values and has steady increase global demand for its vegetable oil and protein. It has high seed protein content 30-40% and about 18-22% seed oil content. return per unit area and difficulties in marketing chanells,

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In Egypt, Soybean acreage has declined during the last 20 years from about 100.000 fad in 1991 to about 20.000 fad in 2009 due to, increased production costs, reduced net thus, it faces strong competition from the other summer crops. Accordingly, the total production becomes insufficient for consumption. Therefore, it is necessary to introduce the crop to new land regions, reduce production costs and increase productivity per unit area in order to increase Soybean total production at national level.

In Egypt there is no doubt that increasing production of vegetable oils become a dire need under the low local production of 150.000 ton (in 2010), while the consumption reach 820.000 ton. In Egypt the area devoted to sunflower is so limited. Therefore, increasing the area of oil seed crops is an important target to reduce the gap between production and consumption of edible oils.

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/ littre in plant media increased vitamin B_{12} production as well as all growth parameters and pods yield of *lupines spp*. Due *et al*. [5] showed that cobalt at 8 ppm had a greatest growth parameters, pods and seeds yield in cowpea. Abdel Moez and Nadia 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 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 hight 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*. [8] 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 [12] showed that cobalt significantly increased faba bean growth and yield (pods and seeds

Cobalt was directly proportional to vitamin B_{12} 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 [13]. 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 [14] 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 (*pisum sativum 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. Hala Kandil [12]added that cobalt significantly increased nodules formation in faba bean roots. Younis [17] showed that cobalt at 100 mg/kg soil increased nodules number and their mass in *(Lablab purpureus)* plants. Later on, Jayakumar *et al* [18, 19]found that cobalt had beneficial effect on nodules number and their dry weight in both soybean and peanut roots.

Recently, Nadia Gad *et al* [20] stated that the amendment of cobalt at 12 ppm to the soil improved the growth parameters, nodulation rate, nitrogenous activity seed yield and minerals content in faba bean. 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.

Material 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** [21]. 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.,*** [22]. Determination of soluble, available and total cobalt was determined according to method described by **Cottenie *et al.,*** [23]. 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 70.2	**Silt** 25.6	**Clay** 3.6	**Soil texture Sandy loam**			**Saturation** 32.0		**FC** 19.2	**WP** 6.1	**AW** 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 micronutriments**				
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)

Experimental works: 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 physiological of Soybean 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 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 Soybean (*Glycine max L. Merril var. Giza 21)*. Was inoculated prior to sowing with a specific strain of rhizobium (*Rhizobium Jabonicum*). Seeds were sown on April, 2011 and 2012 summer seasons.

Table (2): Chemical properties of chicken manure

O.M (%)	Total N (%)	C/N ratio	pH (1:25)	EC dsm^{-1}	Available nutrients (%)		DTPA-extractable (ppm)			
					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

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

Ammonium sulphate NH_4So_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 12ppm.

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 12ppm
6. $(NH_4)_2SO_4$75% + Co at 12ppm
7. $(NH_4)_2SO_4$ 50% + Co at 12ppm
8. $(NH_4)_2SO_4$ 25% + Co at 12ppm

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

Measurements of vegetative growth:- After 80 days from sowing, all growth parameters of Soybean 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** [24].

Nodulation and nitrogenous activity:-Nodules number and weight were recorded after 50 days from sowing. Nitrogenous activity was determined according to **Hardy** [25]. Soybean 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). Nitrogenous activity values were recorded as µmol C_2H_4 /g/h.

Measurements of plant yield:- After 120 days from sowing respectively, Soybean yield parameters such as pods number/plant, weight of seeds/plant, 100 seeds weight, total seeds yield (kg/fed), and oil yield (Kg/fed) and oil yield (Kg/fed) were recorded according to **Gabal *et al*** [26].

Nutritional status:- For chemical analysis, seeds sampled either from the intact plant for each treatmen 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*** [23].

Chemical constituents: - The percent of total soluble solids, proteins, total carbohydrates and oil of seeds were determined according to **A.O.A.C** [27].

Statistical analysis: - All data were subjected to statistical analysis according to procedure outlined by (**SAS** [28]) computer program and means were compared by LSD method according to **Snedecor and Cochran** [29].

Results and Discussion

Nodulation parameters: The obtained data in Tables (3) indicated that cobalt at 12 ppm had a significant positive effect on Soybean root nodules parameters under different nitrogen levels compared with the untreated plants. Cobalt recorded the maximum Soybean 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 *et al*** [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 Soybean and pea plants compared with the control. Similar results are reported by **Balachandar *et al*** [30] 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 Soybean root nodulation after 50 day from sowing in the two seasons, compared with the untreated plants.

Table (3): Soybean 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	128.6	11.41	3.56	17.8
75	124.0	11.15	3.33	16.5
50	113.2	10.15	3.08	15.7
25	108.6	9.76	2.96	15.1
With cobalt (12ppm)				
100	162.3	15.78	4.90	20.9
75	159.9	15.30	4.52	19.0
50	133.5	14.87	4.45	17.9
25	124.5	14.34	3.87	17.0
LSD 5%	**4.0**	**0.5**	**0.16**	**0.35**

Root nodules parameters and nitrogenous enzyme activity in nodules of Soybean was significant influenced by cobalt [8].

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 insignificantly while; with 25% nitrogen gave the lowest once. These results are in harmony with **Epstein** [31] who 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** [32] who demonstrated that cobalt is required for the growth and population of micro-organisms responsible of nitrogen fixation. In spit of the mentioned requirements for cobalt, plant species remarkable differences to excess cobalt.

Vegetive Growth: - Soybean growth parameters as affected by cobalt and nitrogen levels after 80 days, from sowing are given in Table (4) Data indicated that cobalt at 12ppm had a significant promotive affect on all growth parameters of Soybean compared with untreated plants. It is clear that cobalt enhanced all growth parameters such as plant height, root length, number of branches and leaves leaf area of Soybean in both seasons especially with 100% and 75% nitrogen compared with the control (100% nitrogen alone). As nitrogen level was reduced, all growth parameters were significantly reduced. Cobalt treatment with 25% nitrogen gave the lowest growth parameters of Soybean in two seasons. These observations are consistent with previous reports obtained by **Nadia Gad** [33] 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 catabolism rather than anabolism. Data in Table (4) also indicated that cobalt significantly increased dry weights of both shoots and roots in Soybean especially with 100% and 75% nitrogen two seasons compared with the control.

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

Nitrogen treatments %	Plant hight (cm)	Number/Plant		Leaf area (cm^2)	Root length (cm)	Dry weight (9)	
		Branches	Leaves			Shoot	Root
Without cobalt							
100	85.8	8.7	35.3	1919	18.32	31.22	3.94
75	83.4	8.1	34.0	1826	18.09	30.67	3.29
50	82.8	7.8	32.8	1713	17.89	27.89	2.90
25	80.9	7.3	31.6	1688	17.19	20.66	2.56
With cobalt (12ppm)							
100	`88.8	9.8	38.1	2218	20.12	35.88	5.08
75	`87.1	9.0	36.8	2033	19.76	34.93	4.93
50	85.7	8.6	35.5	1969	18.21	32.32	4.32
25	83.4	7.4	32.6	1927	17.69	30.94	3.94
LSD 5%	0.4	0.6	1.2	35	0.23	0.55	0.34

These results were agree with those obtained by **Hanson *et al*** [34] 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** [35] added that supplementing nutrient solution with cobalt improved the growth of tomato plants and enhanced both flowering and fruiting.

Yield parameters: Obtained results in Tables (5) showed that cobalt had a significantly promotive effect on all yield parameters such as number of pods / plant, weight of seeds/ plant, weight of 100 seeds, seeds and oil yield/fed of Soybean. Cobalt increased all Soybean yield parameters under different nitrogen levels comparison with untreated plants. These results were agree with those of [38]. They 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 Soybean.

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

Nitrogen treatments %	Pods No. Per plant (pod)	seeds Weight/plant (g)	100 seeds Weight (g)	Seeds yield Kg/fed	oil %	Oil yield Kg/fed
Without cobalt						
100	**27.5**	**38.3**	**25.0**	**1648**	**18.30**	**321.58**
75	**27.1**	**37.8**	**24.8**	**1645**	**18.12**	**298.07**
50	**25.8**	**34.0**	**22.6**	**1578**	**17.11**	**269.99**
25	**24.2**	**32.2**	**21.9**	**1536**	**16.81**	**248.20**
With cobalt (12ppm)						
100	**33.7**	**44.0**	**31.0**	**1871**	**19.61**	**390.44**
75	**33.1**	**42.5**	**28.9**	**1835**	**19.19**	**352.14**
50	**31.9**	**40.3**	**26.3**	**1782**	**18.90**	**336.80**
25	**28.5**	**38.1**	**24.7**	**1727**	**18.33**	**316.56**
LSD 5%	**1.7**	**2.2**	**1.9**	**55**	**0.29**	**15.30**

Cobalt gave the highest yield parameters. The quadratic and square root functions predicted significant relationships among yields of those legumes at different stages [39]. According to **Due *et al*** [5], cobalt at 8 ppm had a greatest growth parameters, pods and seeds yield in Soybean compared with the untreated plants. Data in Table (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 *et al*** [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 Soybean yield as percentage from control. It is evident that cobalt increased soybean seeds yields and oil about 20.8 and 17.4 % respectively. These results reveal, as mentioned by **Nadia Gad** [31] 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). Confirm, **Abdul Jaleel *et al*** [40, 41]who found that 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 green gram (*Vigna radiate L.*) and maize (*Zea maiz L.*) plants.

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 B12 which contains cobalt is synthesized by the *rhizobium* and circulated in hemoglobin. The hemoglobin content in nodules directly to nitrogen fixation. Thus a deficiency Vitamin B_{12} production and lower nitrogen fixation [15].

Nutritional status: Data presented in Table (6) showed the positive effect of cobalt on mineral composition (N, P, K, Mn, Zn, Cu and Fe) of Soybean seeds. Data reflected the superiority of 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*** [42] who stated that cobalt had a promotion effect for better status of all mineral in both groundnut seeds compared with the control. **Basu *et al*** [15] added that cobalt gave a higher oil content and superior nutrients uptake by groundnut seeds compared with the control. According to **Jayakumar *et al*** [43], all minerals content of blackgram were increased with cobalt at 50 mg/kg soil when compared with the control.

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

Nitrogen treatments %	Macronutrients (%)			Macronutrients (ppm)				Cobalt (ppm)
	N	P	K	Mn	Zn	Cu	Fe	
Without cobalt								
100	3.81	0.189	1.91	**2.04**	26.6	24.7	54.5	0.68
75	3.36	0.184	1.85	**1.96**	24.9	23.1	54.3	0.65
50	2.76	0.179	1.81	**1.90**	23.5	21.8	53.6	0.61
25	2.19	1.174	1.77	**1.83**	22.8	20.5	53.1	0.61
With cobalt (12ppm)								
100	4.09	0.197	2.04	35.8	30.0	28.2	53.8	7.32
75	3.90	0.191	1.96	34.2	28.3	27.5	54.3	6.65
50	3.35	0.187	1.90	32.9	26.8	26.8	54.6	6.31
25	2.86	0.182	1.83	31.0	25.2	26.2	55.1	6.26
LSD 5%	**0.10**	**0.5**	**0.4**	**0.7**	**0.6**	**0.6**	**0.4**	**0.4**

Confirm **Nadia Gad *et al*** [44]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 as enhancing agent, ratios of 100, 75, and 25% N recommended dose.

Chemical Constituents:-The amount of protein, total carbohydrate, total soluble sugars, total soluble solids and oil percentages in Soybean 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 Soybean seeds.

These results are in harmony with those obtained by **Nadia Gad and Nagwa Hassan** [45] Revealed that cobalt addition in plant media increased protein, total soluble solids, total carbohydrates in sweet potato roots.

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

Nitrogen treatments %	Proteins	Total Soluble Solids	Total Carbohydrates	Oil
		%		
		Without cobalt		
100	23.81	16.85	15.3	**18.60**
75	21.00	16.29	15.0	**18.36**
50	17.25	16.13	13.8	**17.91**
25	13.69	15.37	12.9	**17.55**
		With cobalt (12ppm)		
100	25.56	17.90	17.2	**21.92**
75	24.38	17.33	16.0	**21.56**
50	20.94	16.86	15.2	**20.78**
25	17.88	16.29	13.7	**20.44**
LSD 5%	**0.63**	**0.16**	**0.9**	**0.34**

Conclusion

Cobalt is a necessary element to legumes. For Soybean, Cobalt at 12 ppm enhance nodule formation, nitrogen fixation, growth, seeds and oil yield of soybean seeds.

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 nitrogenous activity in Soybean.

The nitrogen environmental pollution and induced the agricultural cost for more money of farmers.

Cobalt saved about 75% of the recommended nitrogen fertilizer dose.

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