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Response of Faba Bean (*Vicia faba* L.) to Cobalt Amendments and Nitrogen Fertilization

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ABSTRACT

Two field experiments were conducted in the winter growing season in 2008 and 2009 on faba bean (*Vicia faba* L. var. 'Nubaria 3') at Nubaria Agricultural Station, National Research Center to evaluate the plant response to cobalt amendments at different rates of N fertilization. Prior to that, a preliminary greenhouse pot experiment was conducted in 3 replicates on the same plant and NRC farm soil within the year of 2007 to define the more promotive concentration of cobalt between 10 concentrations, started from 2, 4, 8, 12, 16, and 20 mg/L cobalt. The plants left to grow until 60 days-old and then removed for measurement of some plant parameters and chemical analysis. The amendment of cobalt to the soil improved the growth parameters of the faba bean plants inoculated with *Rhizobium leguminosarum* var. *vicia* bacteria compared to the cobalt untreated plants (control). Values of plant height, leaves number and area, shoot dry weight, root dry weight as well as nodules number and weight, seed yield, nitrogenase activity and plant mineral content were gradually increased by the successive increase in cobalt dose from 0 to 12 ppm. At the highest dose of cobalt (16 ppm), these values tended to decrease. The data clearly exhibited also that all yield measurements at all N doses were scanty and uneconomically in absence of cobalt amendment, particularly at the low doses of N fertilization. It could be concluded that cobalt 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_2 fixation process.

Keywords: heavy metal, legumes, nitrogen fixation, nodulation, nitrogen amendment

INTRODUCTION

Legumes play an important role in human nutrition, science they are rich source of protein, calories, certain minerals and vitamins. In African diets, legumes are the major contributors of protein and calories for economic and cultural reasons (Tgungula and Garjila 2006). *Vicia faba* L.) is widely used in the Mediterranean region as source of protein in both human and animal nutrition. Faba bean is the most important seed legume in Egypt. It is produced on more than 50% of the total area under food legumes (Doughty and Walker 1982). In spite of the rapid increase in population, the rise in animal protein prices and reclamation of new agricultural areas, the area under faba bean annual decreased. This is mostly due to competition from other winter crops, mainly wheat and clover (Salem *et al*. 1983). Symbiotic N_2 fixation by legumes is important as a practical means of improving the yield and quality of these crops. It depends on the interaction between the root nodule bacteria (*Rhizobium* spp.) and the host plant. This interaction can be affected with many factors including, in particular nitrogen and micronutrients fertilization and inoculation process which may affect the occurrence, growth and survival of the rhizobia, modify nodule formation and influence the function of the formed nodules (Vincent 1965). In addition many workers found that legumes depend upon symbiotic N_2 fixation seems to require cobalt (Co) in trace amounts for the proper functioning of the nodules (Abdel-Ghaffar 1982). In Co-deficient plants, nodulation proceeds normally, but the nodules are inactive more numerous, their color is yellow instead of the normal pink color and they do not fix nitrogen. Co is involved in symbiotic N_2 fixation via vitamin B12 with leghaemoglobin production as a transfer agent in N_2 fixation process within the nodules. Raggio and Raggio (1982) reviewed that addition of Co to nodulated *Alfalfa* increased the yield while inoculation alone or Co addition does not. They also added that the addition of 5×10^{-6} M Co to the soil affected nodule size and leghaemoglobin level in lupin and *Glycine max* plants. Bergersen (1971) mentioned that nodulated legumes have a unique requirement of Co. Other several investigators have shown that Co is essential for the symbiotic association in legumes, in general, but no response to the element could be demonstrated when adequate mineral nitrogen was supplied (Lowe and Evans 1961; Nicholas *et al*. 1962). Co is essential for growth of rhizobia, the specific bacteria involved in legume nodulation and fixation of atmospheric nitrogen into amino acids and protein. Vitamin B12 which contains Co is synthesized by rhizobia and circulated in leghaemoglobin which is directly related to N_2 fixation. Thus a deficiency in Co is shown in reduced vitamin B12 production and lower N_2 fixation process (Evans and Kilwar 1964; Young 1983). Smith (1991) demonstrated that Co is an essential element for the synthesis of vitamin B12 that required for animals and human nutrition. Troitskaya *et al*. (1989) stated that Co accumulation was directly proportional to vitamin B12 content and the reduction of B12 biosynthesis in soybean root-nodules was due to low Co accumulation. In soil deficient-Co, soybean accumulated 3.5-5.0 times less Co in the root-nodules than other legumes (*Lupinus digitatus*, *Phaseslus vulgaris*, *Sesamum indicum* and *Vicia faba* L.). Occonner (1992) found that soybean grown without Co was severely retarded and exhibited severe nitrogen deficiency leading to death of about 25% of untreated growing plants. He added that amendment of few grams of Co per acre can resolve deficiency symptoms in 10-20 days. Co deficiency has been detected in some type of soil in some countries and can be corrected by application of Co-sulphate between 0.03-0.15

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kg/ha (Tisdale *et al*. 1982). In a pot experiment using sandy loam soil, Badawy and El-Tagoury (1977) showed that, Co amendment to faba bean plants with the rate of 5 mg Co-sulphate/pot significantly increased plant and nodule dry weight and leaf N-content more than Co-seed treatment before sowing. Co also improves many plant characteristics and physiological processes including stem and colcoptiles elongation, leaf disc expansion and opening of hypocotyls (Ibrahim *et al*. 1989). Yashida (1998) reported that, the addition of Co at 10 mg/L in plant media slightly increased vitamin B12 production, number and weight of root nodules in lupine plants (*Lupinus digitatus*) compared to untreated control. Basu *et al*. (2006) showed that Co at rate of 0.21 kg/ha increased plant height and leghaemoglobin content of groundnut plants. Nasef *et al*. (2008) stated that Co at 0.16 mg/kg seed-dressing resulted in significantly increase in nodule number and weight, nodule N-content, leghaemoglobin content, total biomass production and seed yield compared to untreated groundnut plants. Through her work on peas plants, Gad (2006) pointed out that soil amended with Co sulphate at rate of 8 µg/L improved nodulation process, increased effective nodules, both fresh and dry weight of shoots, roots, pods quality and quantity. Co also increased chemical contents i.e. total soluble solids (TSS), protein as well as macro- and micro-nutrients content of pea seeds. More recently, Jayakumar *et al*. (2009) and Vijayarengan *et al*. (2009) showed that Co application at the rate of 50 mg/kg soil had a beneficial effect on biochemical contents i.e. sugar, protein and amino acids of groundnut seeds (*Arachis hypogaea*). Co also being increased nodule number, plant growth parameters as long photosynthetic pigments chlorophyll *a* and *b* content in leafs of groundnut plants compared to untreated control plants.

The present work aims to evaluate the role of Co on faba bean growth parameters and the interaction between Co and different doses of N fertilization.

MATERIALS AND METHODS

Two field experiments were conducted in the winter growing season (October) in 2008 and 2009 on faba bean (*Vicia faba* L.) at Nubaria Agricultural Station, National Research Center to evaluate the plant response to Co amendments at different rates of N fertilization. Prior to that, a preliminary greenhouse pot experiment was conducted in 3 replicates on the same plant and NRC farm soil within the year of 2007 to define the more enhancing concentration of Co between 10 concentrations: 2, 4, 8, 12, 16, and 20 µg/l of Co. The plants left to grow until 60 days-old and then removed for measurement of some plant parameters (fresh and dry weight of shoots, roots, leaves and nodules) and mineral contents (N, P, K, Mn, Zn, Cu, Fe, Co). The obtained data exhibited that, the more promising Co concentrations were 4, 8, 12 and 16 µg/l. These four concentrations were used as treatments at design of the following experiment grown in 2008.

Field experiment 1

Seeds of faba bean (*Vicia faba* L.) var. 'Giza'kindly obtained from Agronomy Department, Ministry of Agriculture, Egypt were inoculated with selective strain of *Rhizobium leguminosarum* var. *vicia* prior to sown on October 2008 under drip irrigation system. Three plots with an area of 10.5 m^2 (1/400 fed (1 feddan = 4200 m^2)) were used as replicates for each treatment. Mineral fertilizers were applied as recommended doses. Phosphorus was added as superphosphate (15.5% P_2O_5) during soil preparation. After 21 days of sowing, ammonium sulphate (20.5% N) as a source of N was applied, whereas potassium as K_2SO_4 (48% K_2O) was added just before full-blooming. Faba bean seedlings (at third truly leaf) were irrigated once with Co sulphate solution at the concentration of 4, 8, 12, and 16 µg/l. Untreated 3 plots were used as control treatment. All agricultural practices were done when needed.

Field experiment 2

This experiment was done to evaluate the interaction between the highly enhancing dose of Co (12 µg/l) and different doses of N fertilization and its effect on faba bean production aiming to minimize the rate of N fertilization. Depending upon rhizobial inoculation of seeds and application of Co as a enhancing agent, ratios of 100, 75, 50 and 25% of N recommended dose (120 kg/fed) in the form of $(NH_4)_2$ SO_4 were used to fulfill the N requirements of growing plants. The experiment was carried out on the winter season (October) of 2009 with the same agricultural practices followed in the 1st field experiment.

Soil analysis

Physical and chemical properties of the soil were carried out. Particle size distribution along with soil moisture content, were determined up to Blackmore *et al.* (1972). Soil organic matter, CaCO3, EC (electric conductivity), pH, cations and anions were analyzed according to Black *et al*. (1982). Macro- and micronutrients as well as total Co were assessed in *aqua rejia* extract. Soluble and available Co (DTPA extractable) all were estimated as mentioned by Cottenie *et al*. (1982).

Plant samples were taken at flowering stage 50 days after sowing for determination of growth parameters, whereas the plants were harvested at maturity stage after 110 days of sowing for evaluation of yield parameters according to FAO (1980).

Nitrogenase activity of plant nodules was estimated according to FAO (1980). All data were subjected to statistical analysis according to procedure outlined by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The physical analysis exhibited that soil texture is sandy loam, since sand is formed the larger value (82.6%). While silt and clay represent together 17.4% of the total constituents of soil (**Table 1**). Thereby, the soil water constants are matched with the soil properties, field capacity is 14.4% which is low. The organic matter content, soluble cations, soluble anions, available macro-and micro- nutrients proved that the soil is poor and infertile, with low mineral content. Co forms, soluble, available and total were found in fair values.

As clearly observed from the data, the amendment of Co to the soil improved the growth parameters of the faba bean plants inoculated with *Rhizobium* bacteria compared to the Co untreated plants (control). Values of plant height, shoot dry weigh and root dry weight were gradually increased markedly by the successive increase in Co dose from 0 to 12 µg/l (**Table 2**). At the highest dose of Co (16

Table 1 Physical and chemical analyses of soil.

	%		
Sand	**Silt**	**Clay**	**Texture**
82.6	14.6	2.8	Sandy water
	Soil water constant % weight basis		
saturation	**Field capacity**	**Wilting point**	**Available water**
32.0	14.4	3.9	10.5
	chemical		
pH 1:2.5	**EC dsm^{-1}**	**$CaCO_3$ %**	**Organic matter %**
8.0	1.0	7.2	0.2
	Soluble cations (meq/L)		
Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**
9.0	1.4	3.3	0.3
	Soluble anions (meq/L)		
CO_3^{--}	**HCO_3^{-}**	**Cl^{-}**	**SO_4^{--}**
	1.18	0.60	12.15
	Macronutrients		
Total-N	**Available P**	**Available K**	**Mg/100 g soil**
15.1	13.0		21.0
	Micronutrients available		
Fe	**Mn**	**Zn**	**Cu**
4.47	2.61	1.44	4.00
	Cobalt mg/L		
Soluble	**Available**	**Total**	
0.30	1.77	6.28	

Table 2 Faba bean parameters as affected by cobalt amendments.

Cobalt amendments ppm	Shoot DW g/plant	Root DW g/plant	Plant height cm	Leaves		Nodules		N-ase activity
				No. plants	Leaf index cm^2	No. plants	Dry weight g	µ mol $_{C2H4}$/g/h
0.0	13.7	1.93	96.2	15.0	482.0	80.0	2.97	16.7
4.0	19.2	2.44	107.4	21.0	676.0	126.0	3.75	17.5
8.0	25.6	3.94	119.9	30.0	957.0	190.0	4.61	16.6
12.0	32.0	5.09	125.3	38.0	1226.0	230.0	4.98	20.4
16.0	27.6	4.87	121.8	36	1151.0	201.0	4.72	17.8
LSD	0.07	18.24	0.13	0.02	2.01	4.97	0.04	0.01

Table 3 Mineral contents of *faba bean* plant as affected by cobalt amendments.

Mineral contents / Co-amend-ments mg/L	Shoot								Roots							
	Macronutrients %			Micronutrients ppm					Macronutrients %			Micronutrients ppm				
	N	P	K	Mn	Zn	Cu	Fe	Co	N	P	K	Mn	Zn	Cu	Fe	Co
0.0	0.87	0.30	0.31	30.7	19.4	25.4	109.0	0.88	0.38	0.07	0.09	53.6	47.3	32.5	169.0	2.30
4.0	1.45	0.34	0.35	32.9	22.6	28.6	106.0	1.19	0.56	0.09	0.12	59.3	50.8	38.7	164.0	3.25
8.0	1.98	0.37	0.38	35.1	25.2	31.5	102.0	1.65	0.84	0.12	0.17	63.0	54.2	41.3	160.0	4.60
12.0	2.36	0.40	0.42	38.0	29.5	34.8	98.3	2.39	1.36	0.16	0.20	67.9	58.7	44.6	156.0	5.57
16.0	2.23	0.38	0.39	40.5	31.2	37.1	94.6	4.62	0.97	0.13	0.19	69.0	60.5	47.0	152.0	7.01
LSD	0.03	0.01	0.005	0.06	0.06	0.1	0.7	0.4	0.01	0.01	0.01	0.2	0.5	0.3	1.04	0.3

Table 4 Mineral content and seed index of faba bean as affected by cobalt amendments.

Cobalt amendments ppm	Macronutrients %			Micronutrients ppm				
	N	P	K	Mn	Zn	Cu	Fe	Co
0.0	0.98	0.30	0.21	21.3	13.9	24.0	28.2	0.55
4.0	1.28	0.32	0.32	24.0	15.4	26.5	45.0	0.87
8.0	1.67	0.47	0.27	26.7	18.5	29.1	42.3	1.19
12.0	2.30	0.54	0.30	29.6	21.3	32.7	39.8	1.86
16.0	1.80	0.51	0.29	31.5	23.5	34.3	37.5	2.07
LSD	0.22	0.02	0.2	0.13	1.6	1.8	2.1	0.05
Cobalt amendments ppm	**TSS ppm**	**Protein content %**	**Pod/plant**	**Seeds/plant**	**Weight 100-seed/g**	**Seed yield /plant g**	**Seed yield /plant g**	
0.0	10.3	6.13	10	28	77.5	17.79	427	
4.0	10.8	8.00	12	33	80.3	22.95	551	
8.0	11.1	10.40	13	39	86.5	27.75	666	
12.0	11.9	14.4	14	45	91.6	32.05	769	
16.0	11.6	11.80	13	40	87.3	28.77	691	
LSD	0.3	1.13	0.01	0.8	0.67	1.21	12.8	

TSS: Total soluble solids

µg/l), these values tended to decrease. The flourish effect included also leaves number and area per plant as well as nodule number and dry weight. It is likely that the increase in leaves number and area accelerate the photosynthesis process and force the plants to build up more of biomass which is reflected on the dry matter of plants. In addition the enhancing happened in the nodule formation process resulted in increasing the efficiency of *Rhizobium* bacteria to perform with N_2 fixation at high capacity for producing healthy plants. The same findings were obtained by Walser *et al*. (1996) who stated that Co application (2.7 kg Co/ha) increased tomato (*Lycopersicon esculentum*) leaf numbers as well as surface of chloroplast/unit leaf area, chlorophyll content, leaf area index and rate of photosynthesis. On the other hand, Angelov *et al*. (1993) demonstrated that excessive Co reduced chlorophyll synthesis and rate of photosynthesis. Similar data were also obtained by other authors who found that Co stimulated the growth and development of plants, nodules and increased the N accumulated in leguminous plants (Danilova and Demkima 1967; Airinel 1987; Joshi *et al*. 1987; Chetti *et al*. 1995; Naidu 2000).

It was also observed that, the increase in nitrogenase activity was parallel and related to the increase in nodules number and efficiency (Dalton and Mortenson 1972). As well known, the nitrogenase is a complex enzyme which enables fixation of atmospheric nitrogen. In nitrogen-fixing bacteria, the enzyme nitrogenase derives the reaction of atmospheric di-nitrogen fixation in presence of ATP. Watson *et al*. (2001) reported that, in bacteria the requirement for Co is primarily due to its presence in vitamin. B12 which plays an important role in methionine biosynthesis. The increase in N-ase activity reached its maximum when Co dose of 12 µg/l is used. It is apparently from the data that this dose of Co (12 µg/l) in case of faba bean plants is the marginal concentration for all estimated values.

Macro- and micronutrients are major constitutes of all living materials as known. It was observed that the increase in amended Co dose followed by a steady increase in plant contents of macro-nutrients (NPK) and micro-nutrients (Mn, Zn, Cu) and consequently Co content, except in case of Fe content, since it was gradually decreased by the increase in added Co (**Table 3**). The reduction rate of Fe was more or less proportioned with amended concentration of Co. This finding was also stated by Bisht (1991) on cowpea; Blaylock *et al*. (1993) on tomato and soy bean; and Kandil and Gad (2009) on tomato who mentioned that, there is an antagonistic effect between Fe and Co on the absorption sites of plant roots using different concentration of Co ranged between 5-12 µg/l. It was also observed that Co accumulated in root tissues higher than in shoot ones. The same conclusion mentioned by Pettersson (1976) and Atta *et al*. (1991), but not very much being know about the mechanism of uptake and translocation of all heavy metal in general. The beneficial effect of Co on the increase happened in the total mineral content could be deduced to the positive role of Co in water movement and its tendency towards the rhizosphere area near the plant root zone and consequently, the enhancement occurred in the mineral uptake by the growing plants, particularly in case of water stress as mentioned by El-Kobbia and Osman (1987). Almost, the most reliable Co dose was that one contains 12 µg/l, since it realized the highest values of macro- and micro-nutrients in both shoots and roots. These data are relevant with the finding of Yadav and Khanna (1988); Raj and Rao (1996) who

Table 5 Faba bean yield as affected by nitrogen fertilization and cobalt amendments.

N-dose* %	Pods/plant no.		Seeds/plant no.		Weight 100-seed (g)		Weight of seed g/plant		Seed yield/fed (kg)	
	-Co	+Co	-Co	+Co	-Co	+Co	-Co	+Co	-Co	+Co
25	6	9	17	29	49.0	59.6	10.7	20.5	257	492
50	7	10	20	32	56.1	64.2	12.6	22.4	302	538
75	8	12	23	33	65.3	83.7	15.0	28.2	360	677
100	10	14	28	39	79.5	91.6	17.8	32.1	427	770
LSD	0.63	0.52	0.91	0.78	1.05	3.56	1.11	1.02	20.59	25.82

found that the combined treatment of *Rhizobium* + Co had increased the mineral uptake by legumes.

The present data showed that the seeds obtained from Co untreated soil (control) contained the lower values of macro- and micronutrients, although it was inoculated with *Rhizobium*. While those obtained from the Co treated soil in combination with *Rhizobium* inoculation contained the higher values of both nutrients (**Table 4**). The increase in macro- and micronutrients were more pronounced, particularly at the use of the larger dose of Co amendments up to concentration of 12 µg/l. It is important to mention that the use of the highest concentration of Co, 16 µg/l, resulted in negative effect on the macro-nutrients percentage as well as Fe. These data are in agreement with those obtained by Gad and Atta (2006). The increase in N due to the increase in Co dose was more pronounced than those obtained by P and K. This increase in N% reached its peak (2.35-fold the control treatment) at 12 µg/l Co. could be reduced to the stimulating effect of the interaction between the successful rhizobial inoculation and Co amendment which finally led to accumulation of nitrogen in form of protein through N_2 fixation process. These data are in harmony with the findings of David and Good-Sell (2002) who reported that nitrogen is needed by all living organisms to build protein and nucleic acids. Atmospheric N is very common on the earth, as it comprises just over 75% of the molecules in air, however, it is very stable and difficult to break apart into individual nitrogen atoms. N-fixing bacteria have the ability to convert N gas into ammonia, which is easily combined with other radicals to form the building block of proteins and nucleic acids. Meanwhile, the relation between Fe and Co in seed is little different than that in shoots and roots. The middle concentrations (8, 12 µg/l) of Co led to corresponding increases in Fe content in the seed. Fe content of seeds in the highest Co amendment was comparatively low but still larger than the Co untreated treatment (control). As mentioned before, this phenomenon may be due to the antagonism between the two elements. Although, the gradual increase in Co content of the faba bean seeds due to the usable amendments of Co, but they still safe for animal and human nutrition with no hazardous effect. Young (1983) stated that Co seeds content less than 8 µg/l is not harmful for human beings.

Regarding to the yield parameters (TSS, protein content, pods and seed yield), the mutual combination between rhizobial inoculation and the gradually increasing doses of Co resulted in accelerating the physiological mechanism of faba bean plant in particular protein content which reached the maximum value (14.40%, equal to 2.33-fold the Co free treatment) at the Co dose 12 µg/l.

Although, the seed yield obtained from the different treatments was relatively low (mean yield between 1.25: 1.50 t/fed) but the yield gradually increased significantly by increasing Co dose. This favorable effect could be deduced to the success of nodule formation process, as a result of Co amendments, which led to more N_2 fixation and consequently more protein accumulation and finally increasable seed yield. Similar results were obtained by Gad (2006b) with peas and Vijayarengan *et al*. (2009) with groundnut.

It could be recommend the concentration of 12 µg/l to be apply to faba bean plant for improving growth and yield parameters.

The stimulated obtained results due to the interaction between rhizobial inoculation and Co amendments, particularly in case of using Co concentration of 12 µg/l, which was resulted in maximizing all plant growth parameters, seed quality and quantity. This results led to design the present experiment using that beneficial Co dose with descending doses of the initial N dose (100 kg per feddan (4200 m^2) of ammonium nitrate 33.5% N). The data clearly exhibited that all yield measurements at all N doses were scanty and uneconomically in absence of Co amendment, particularly at the low doses of N fertilization (**Table 5**). Use the lower dose of N fertilization (25%) was not the good choice, since, all the measurable yield values were minimized even in presence of Co, so the 50 and 75% N doses. Gradually significant increases in values were observed with increasing N dose in presence or absence of Co dose as shown in **Table 5**.

These increases gradually ascending to reach maximum at the highest applied N dose (100% of normal N fertilization) in presence of Co amendment (12 µg/l). It is an evident on the importance of Co as a limiting factor for yield production of faba bean plants in infertile soils. Similar observations were mentioned by other workers (Yadav and Khanna 1988 on different legume plants, Gad (2006) on pea plant using concentration of 8 µg/l Co). It could be concluded that the interaction between Co and mild N fertilization may enriched faba bean yield due to their mutual effect on N_2 fixation process.

REFERENCES

Abdel–Ghaffar AS (1982) Nodulation problems and response to inoculation. *Proceeding of the first OAU/STRS International African Conference on Biofertilizers*, Cairo, Egypt 22-26 March, 1982

Airinel A (1987) Effect of cobalt on some sugar beet variaties. *Fiziologiya Rastenii* **19**, 87-92

Angelov M, Tsonev T, Dobrinova K, Velikova V, Stoynaova T (1993) Changes in some photosynthetic parameters in pea plants after treatment with cobalt. *Phytosynthetica* **28**, 289-295

Atta Aly MA, Gad N, Kobbia TM (1991) Effect of cobalt on tomato plant growth and mineral content. *Annual Agricultural Science Ain Shams University* **36**, 617-624

Badawy MA, Tagory NI (1977) Effect of cobalt on nodulation, growth and yield on faba bean. *Crop Research Histar* **2 (1)**, 116-119

Basu M, Bhadoria PBS, Mahapatra SC (2006) Influence of microbial culture in combination with micronutrient in improving the groundnut productivity under alluvial soil of India. *Acta Agricultural Slovenica* **87**, 435-444

Bergersen FJ (1971) Biochemistry of symbiotic nitrogen fixation in legumes. *Annual Review of Plant Physiology* **22**,121-140

Bisht JC (1991) Interrelation between mineral plant tissues iron and cobalt. *Pesquisa Agropecuaria Brasileira* **16**, 739-746

Black CA, Evans DD, Ensminger LE, White GL, Clark FE (1982) *Methods of Soil Analysis* (Part 2), Agron Inc., Madison, Wisconsin

Blackmore AD, Davis TD, Jolly VD, Walser RH (1972) *Methods of Chemical Analysis of Soils*, New Zealand Soil Bureau, 10 pp

Blaylock AD, Davis TD, Jolly VD, Walser RH (1993) Influence of cobalt and iron on photosynthesis, chlorophyll and nutrient in regreening chlorotic tomatoes and soybeans. *Journal of Plant Nutrition* **8**, 823-838

Chetti MB, Antony E, Mummigatti UV, Dodamani MB (1995) Role of nitrogen and *Rhizobium* on nitrogen utilization efficiency and productivity potential in groundnut genotypes. *Farming Systems* **11 (1-2)**, 209-216

Cottenie A, Verloo M.,Kiekens L, Velgh G, Camerlynech R (1982) *Chemical Analysis of Plants and Soils*, Laboratory of Analytical Agrochemical, State University, Ghent, Belgium, 63 pp

Dalton H, Mortenson LE (1972) Di-nitrogen (N_2) fixation (with a biochemical emphasis). *Bacteriology Reviews* **36**, 231-260

Danilova TA, Demkima EN (1967) Role of cobalt in nitrogen accumulation by leguminous plants. *Dokl Akademia Nauk USSR* **172**, 487-490

David ID, Good-Sell (2002) The energy requirements for nitrogen fixation in nodulated legumes. *Advances in Legume Sciences* **2**, 105-116

Doughty J, Walker A (1982) Legumes in Human Nutrition. Food and Nutrition paper 20, FAO, Rome, pp 152

El-Kobbia T, Osman A (1987) Salinity and cobalt interaction in tomato plants. *Soil Science Rural Sociology* **47**, 103-115

Evans HJ, Kliewer M (1964) Vitamin B compounds in relation to the requirements of cobalt for higher plants and nitrogen fixing organisms. *Annals of the New York Academy of Science* **112 (2)**, 732-755

FAO (1980) *Soil and Plant Testing as a Basis of Fertilizer Recommendations. Soil Bulletin* 3812, FAO, Rome, **55**, pp 15-19

Gad N (2006) Increasing the efficiency of nitrogen fertilization through cobalt application to pea plant. *Research Journal of Agriculture and Biological Sciences* **2 (6)**, 433-442

Gad N, Atta-Aly MA (2006) Effect of cobalt on the formation growth and development of adventitious roots in tomato and cucumber cuttings. *Journal of Applied Science Research* **2 (7)**, 423-429

Ibrahim A, El-Abd Sci SO, El-Beltagy AS (1989) A possible role of cobalt in salt tolerance of plant. *Egyptian Journal of Soil Science* **Special Issue**, 359-371

Jayakumar K, Abdul Jaleel C, Zhang C-X, Azooz MM (2009) Cobalt-induced variations in growth and pigment composition of *Arachis hypogaea* L. *Academic Journal of Plant Sciences* **2**, 74-77

Joshi PK, Bhatt DM, Kulkarni JH (1987) Groundnut root nodulation as affected by micronutrients application and *Rhizobium* inoculation. *International Journal of Tropical Agriculture* **5 (3-4)**, 199-202

Kandil H, Gad N (2009) Effect of inorganic and organic fertilizers on growth and production of broccoli (*Brassica oleracea* L.). *Journal of Agricultural Science, Mansoura University* **34 (11)**, 10771-10779

Lowe RH, Evans HJ (1961) Cobalt requirement for the growth of rhizobia. *Proceeding of Natural Academy Sciences USA* **83**, 210-211

Naidu PH (2000) Response of bunch varieties of groundnut to *Rhizobium* inoculation. *Legume Research* **23 (2)**, 130-132

Nasef MA, Abd El-Hamid AM, Salem HM, Abd El-Hamid AF (2008) Efficiency of applied rates and methods of cobalt on growth, yield and elemental composition of peanut plant growth on a sandy soil. *Annals of Agriculture Science, Moshtohor* **42 (2)**, 851-860

Nicholas DJ, Kobayashi M, Wilson PW (1962) Cobalt requirement for inorganic nitrogen metabolism in microorganisms. *Microbiology* **48**, 1537-1542

Occonner MB (1992) Role of cobalt in soil and plant nutrition. *New Zealand Journal of Agricultural Science (Wellington)* **18**, 119-122

Pettersson O (1976) Heavy metal ion uptake by plants from nutrient solutions with metal ion, plant species and growth period variations. *Plant and Soil* **45**, 445-459

Raggio M, Raggio N (1982) Root nodules. *Annual Review of Plant Physiology* **13**, 104-128

Raj AK, Rao DSRM (1996) Effect of *Rhizobium* inoculation, nitrogen and phosphorus application on yield and yield attributes of groundnut. *Legume Research* **19 (3-4)**, 151-154

Salem SA, Caeser K, Bartels M (1983) Field studies on the irrigation level and cultivation in faba bean (*Vicia faba* L.) in Northern Egypt. *Zeitschrift für Acker- und Pflanzenbau* **152**, 325-335

Smith RM (1991) Trace elements in human and animal nutrition. *Micronutrient News and Information* **9**, 11

Snedecor GW, Cochran WG (1980) *Statistical Methods* (7th Edn), Iowa State University Press Ames Iowa, USA

Tgungula D, Garjila Y (2006) Effect of Mo application on growth and yield of cowpea in Yla, Nigeria. *American-Eurasian Journal Agriculture and Environmental Science* **1 (2)**, 96-101

Tisdale SL, Nelson WL, Beaton JD (1982) *Soil Fertility and Fertilizers* (4th Edn), Macmillan Publishing Co., New York, 752 pp

Troitskaya GN (1989) The effect of growth conditions on B12 content in root nodules of soybean plants. *Fiziologiya Rastenii* **30**, 158-164

Vijayarengan P, Abdul Jaleel C, Zhang C-X, Jayakumar K, Azooz MM (2009) Biochemical variations in groundnut under cobalt applications. *Global Journal of Molecular Sciences* **4 (1)**, 19-22

Vincent JM (1965) Environmental factors in the fixation of nitrogen by legumes, In: Bartholomew WV, Clarck FF (Eds) *Soil Nitrogen*, American Society of Agronomy, Madison, Wisconsin, USA, pp 384-435

Walser RH, Jolley VD, Davis TD (1996) Effect of cobalt application on structural organization of photosynthetic apparatus of tomato leaves. *Journal Plant Nutrition* **19**, 358-363

Watson RJ, Hets R, Martin T, Savard M (2001) *Sinorhizobium meliloti* cells biotin and either cobalt or methionine for growth. *Applied and Environmental Microbiology* **67 (8)**, 3767-3770

Yadav DV, Khanna SS (1988) Role of cobalt in nitrogen fixation: A review. *Agricultural Review* **9 (4)**, 180-182

Yashida S (1998) *Rhizobium* production of vitamin B12 active substances and their localization in some leguminous plants. *Japanese Journal of Soil Science and Plant Nutrition* **69**, 435-444

Young RS (1983) Recent advances on cobalt in human nutrition. *Micronutrients News and Information* **3**, 2-5