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Enhancement of the Effect of Nitrogen Fertilization on Common Bean Plants Grown in Sandy Soils by Cobalt Application

Nadia Gad and M.R. Abdel-Moez and M.E. Fekry Ali

Plant Nutrition Dept., National Research Centre, Dokki, Egypt

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ABSTRACT

To evaluate the effective role and impact of cobalt soil applications as well as different nitrogen fertilizer levels on nodulation parameters, vegetative growth, seed yield, seed mineral contents and seed nutritional quality of common bean plants of the Nebraska cultivar. Two field experiments were conducted during the growing seasons of 2020 and 2021 at the Experimental and Production Station, National Research Centre, El-Noubaria region, Behera governorate, Egypt, under newly reclaimed sandy soil conditions and a drip irrigation system. The experiment design was a split-plot design with three replicates. The obtained results sharply indicated that applying cobalt at 10 ppm to the soil led to significant increases in all studied parameters of nodulation rate, vegetative growth, seed yield, seed mineral contents and seed nutritional quality of bean plants. The nitrogenase enzymes activity was closely related to the increase in nodule numbers/plant and fresh and dry weights of nodules on bean plant roots. Increasing the nitrogen fertilizer level from 25 to 100% of the recommended nitrogen dose caused gradual increases in all studied parameters of bean plants. Significant differences were found among different nitrogen application levels except for root fresh and dry weights. The interaction effect between cobalt soil applications and different nitrogen fertilization levels had significant differences on all studied characters of common bean plants. Experimental treatments of applying cobalt to the soil at 10 ppm combined with a complete dose of nitrogen 100% of the recommended dose gave the highest significant values of all measured parameters. The lowest values were obtained when cobalt was applied at 0 ppm along with a 25% nitrogen fertilizer level.

Keywords: *Phaseolus vulgaris,* Nitrogenases activity, Vegetative growth, Seed yield attributes, Seed mineral contents, Seed nutritional quality.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a member of the Fabaceae family and one of the most important leguminous vegetable crops grown in Egypt for its green pods or dry seeds. It is considered an important staple food, provides appreciable amounts of plant proteins, dietary fibers, carbohydrates, minerals and calories. Nutritionally, legumes come after cereals in the human diet (Kouris-Blazos and Belski, 2016). It is believed that legumes and pulses are cheap and rich sources that can play a significant role in combating hunger and malnutrition as well as ensuring food security, mainly in developing countries (FAO, 2016).

Cobalt is a beneficial element for the growth of higher plants, although there is no evidence of its direct role in plant regulatory and biochemical pathways. Cobalt is an important and necessary element, especially for legumes, due to its vital symbiotic role in the process of atmospheric nitrogen fixation by specific soil microorganisms (Evans and Kliwer, 1964; Hu *et al.* 2021). Cobalt is a central atom of vitamin B-12. Vitamin B-12 is valuable for human and animal nutrition. Unlike other heavy metals, cobalt is safe for human consumption and up to 8 ppm can be consumed on a daily basis without any health hazards (Young, 1983). With age, the cobalt element does not accumulate in the human body as happens with the other heavy metals (Smith, 1991). Bean plants should form symbiosis relation with its symbiotic partner *Rhizobium* as other legumes. However, this kind of symbiotic relationship had many obstacles and factors that were responsible for the failure of

Corresponding Author: Nadia Gad, Plant Nutrition Dept., National Research Centre, Dokki, Egypt.

nodulation formation. Cobalt and cobalamin are needed by *Rhizobia* and other nitrogen-fixing bacteria in order to convert atmospheric nitrogen (N_2) into ammonia (NH_3), which is then used by plants. Cobalt plays a vital role in interactions with iron (Fe), nickel (Ni), and zinc (Zn) in maintaining cellular homeostasis.

Cobalt application had the greatest effect on nodulation rate in bean plants, which finally reflected on total nitrogen accumulation in the plant shoots (Castro *et al.,* 1994). Cobalt is an essential component of cobalamin, which is required for the activation of several enzymes and co-enzymes, responsible for the formation of leghaemoglobin and is involved in biological atmospheric nitrogen fixation by nodules of leguminous plants (Mathur *et al.,* 2006). Also, Younis (2007) showed that cobalt soil application at 100 mg/kg increased nodule numbers and their mass in Indian bean plants. In the same regard, Nasef *et al.* (2008) found that cobalt application at 15 ppm showed significantly higher nodule numbers and weight, nodule N content, leghaemoglobin content, total biomass production and seed yield of peanut plants compared with untreated plants. Balachandar *et al.* (2003) pointed out that cobalt had a significant effect on cowpea root nodule parameters, i.e., total nodule number/plant and nodule fresh and dry weights, compared with the untreated plants. They also stated that cobalt could be used as a coenzyme, confirming the benificial role of cobalt in biological nitrogen fixation in legumes. Furthermore, all cobalt application levels significantly increased the nitrogenase enzyme activity which was paralleled and related to the increment of nodule numbers and their fresh and dry weights as well as the capacity and efficiency of nitrogen fixation by soybean roots compared with the control (Nadia Gad *et al.* (2013 and 2014). According to Abd El-Moez and Nadia Gad (2002) applied cobalt at 8 ppm increased nodule formation on cowpea roots, as a result atmospheric symbiotic nitrogen fixation by microorganisms was enhanced, which reflected on macronutrient contents particularly nitrogen content.

Cobalt application to the soil at 50 mg kg^{-1} was found to have significant positive effects on vegetative growth and nutrient absorption of blackgram plants relative to control plants. The highest values of mineral contents in blackgram seeds were also achieved (Jayakumar *et al.,* 2008). Applying cobalt to the soil at 12.5 ppm gave the maximum values of vegetative growth, seed yield, oil yield and its quality, mineral status contents and chemical components in canola plants compared to control plants. In addition, cobalt treatments progressively increased all determined parameters relative to untreated canola plants (Nadia Gad, 2010). Nadia Gad and Abd El-Moez (2011) showed that the addition of 6 ppm cobalt had a significant positive effect on broccoli growth, head yield quantity and quality, and mineral contents in heads, but beyond this rate, further application of cobalt had an adverse effect. In the same regard, cobalt soil application at 50 mg kg^{-1} as cobalt chloride resulted in the best values of root length, shoot length and number of nodules/plant in groundnut plants, whereas a higher rate of cobalt, caused a slight decrease in all these parameters (Vijayarengan, 2009). Basu and Bhadoria (2008) reported that soil application of cobalt at lower dose (0.21 kg ha^{-1}) with *Rhizobium* inoculation significantly enhanced the uptake of N (81.4 kg ha^{-1}), P (8.9 kg ha^{-1}), K (9.31 kg ha^{-1}) as well as Co (0.27 kg ha^{-1}) by the kernel of groundnut. Moreover, the maximum values of N, P and K uptake, vegetative growth parameters and yield of summer groundnut were attained by applying *Rhizobium* + phosphobacterium inoculation + cobalt at 0.21 kg ha^{-1} followed by *Rhizobium* + cobalt at 0.21 kg ha^{-1} (Basu *et al.,* 2006).

The objective of this study was to determine the effect of cobalt soil applications plus different nitrogen fertilizer levels (100, 75, 50 and 25% of the recommended nitrogen dose) on nodulation rate, vegetative growth, seed yield, seed mineral contents and seed nutritional quality of common bean plants of the Nebraska cultivar, grown under newly reclaimed sandy soil conditions.

2. Materials and Methods

2.1. Plant material

Two field experiments were conducted at the Experimental and Production Station, National Research Centre, El-Noubaria region, Behera governorate, Egypt, during the growing seasons of 2020 and 2021. The objective of the current study was to evaluate the effect of different nitrogen fertilizer levels (100, 75, 50 and 25% of the recommended dose of nitrogen) and cobalt as soil applications at rates of 0 and 10 ppm on nodulation rate, vegetative growth, seed yield, mineral contents and nutritional quality of common bean plants of the Nebraska cultivar.

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The experimental design was split-plot in three replicates, where nitrogen fertilizer levels (100, 75, 50 and 25%) were occupied in the main plots, whereas, cobalt applications (Zero, 10 ppm) were distributed in the sub-plots. The nitrogen fertilizer used was ammonium sulfate (20.6% N), and the calculated amounts of applied nitrogen levels were divided into two equal portions, one added with the experimental soil preparation and the other before the second irrigation. Moreover, cobalt (Co) in the form of cobalt sulfate heptahydrate ($CoSO_4.7H_2O$, MW 281.10) was used as a source of cobalt.

Common bean seeds of the Nebraska cultivar were obtained from the Legume Crop Research Department, Field Crop Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Bean seeds were sown on the first week of March in the two seasons of 2020 and 2021, in hills with 3 seeds per hill and 25 cm apart on both sides of 5 drip irrigated ridges with 3.5 m length and 0.6 m width. The net area of each experimental sub-plot was 10.5 m^2.

2.2. Experimental soil analysis

The physical and chemical characteristics of representative soil samples from the experimental site were determined before sowing and are presented in Table 1. Particle size distributions and some soil moisture constants were determined as described by Blackmore *et al.* (1987). Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen, and the availability of P, K, Fe, Zn, Mn and Cu were determined according to the procedures of Black *et al*. (1982). The soluble, available and total cobalt were analyzed according to the method described by Cottenie *et al.* (1982).

Table 1: Physical and chemical characteristics of the experimental soil site.

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 (mmolc L^{-1})				**Soluble anions (mmolc L^{-1})**		
pH	**EC**	**$CaCO_3$**	**OM**	**Ca^{++}**	**Mg^{++}**	**K^+**	**Na^+**	**HCO_3^-**	**Cl^-**	**$SO_4^=$**
8.49	1.74	34	2.0	3.5	1.9	.8	11.2	0.2	12.4	3.8
Cobalt Mg g^{-1}				**Total available mg 100 g^{-1} soil**			**Available micronutriments Mg g^{-1}**			
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

All agriculture management practices for common bean crop production under newly reclaimed sandy soil conditions, including regular irrigation, fertilization, pest and disease and weed control were followed as recommended by the Ministry of Agriculture and Land Reclamation (MOALR).

2.3. Experimental treatments

The current study included the following 6 experimental treatments in total:

Different nitrogen fertilizer application levels:

1. 100% of the recommended dose of nitrogen,
2. 75% of the recommended dose of nitrogen,
3. 50% of the recommended dose of nitrogen,
4. 25% of the recommended dose of nitrogen,

Cobalt soil application rates:

1. at a rate of 0 ppm,
2. at a rate of 10 ppm

2.4. Data recorded

2.4.1. Measurements of nodulation rate and nitrogenase activity

The nodule numbers per plant and nodule fresh and dry weights (g) were recorded on common bean root samples randomly collected from each experimental sub-plot after 50 days from the sowing date. In addition, the activity of the nitrogenase enzyme, which is produced by rhizobacteria and responsible for the reduction of atmospheric nitrogen (N_2) to ammonia (NH_3), was determined according to Hardy *et al*. (1968). Where common bean plants were gently uprooted, the root nodules were collected and placed in 500 ml serum bottles and then sealed with a suba-seal rubber septum. 10% of the gas phase was replaced with C_2H_2 (acetylene gas), then bottles were incubated in the dark at room temperature for 2 hrs. Thereafter, the production of C_2H_4 (ethylene gas) was measured by injecting one ml of the gas sample into a gas chromatography device (GC). Nitrogenase enzyme activity values were expressed as µmol C_2H_4/g/h.

2.4.2. Measurements of plant vegetative growth parameters

All common bean plant vegetative growth parameters, including plant height (cm), number of branches and leaves per plant, total leaf area (cm^2) as well as shoot and root fresh and dry weights (g), were measured after 80 days from the planting date by uprooting 10 randomly selected plant samples from each experimental sub-plot. Then samples were transferred to the laboratory to estimate the above mentioned parameters according to FAO (1980).

2.4.3. Measurements of plant yield attributes

At the harvesting stage, after 110-120 days from the sowing date, common bean yield attributes such as number of pods/plant, weight of pods/plant (g), weight of 100 seeds (g) and total seed yield (ton/fed.) were recorded according to Gabal *et al*. (1984).

2.4.4. Chemical constituents

During the harvesting stage, seed samples were randomly collected from 12 common bean plants from each experimental sub-plot and then dried common bean plant samples were threshed by hand to obtain the seeds. Afterwards, the seeds were winnowed manually to clean and remove any foreign matter and broken seeds. Seed samples were then dried for 48 hrs in an electrical oven at 70ºC. The dried seed samples were ground to a fine powder in a high-speed stainless steel mill to pass a 1 mm sieve and then subjected to various chemical analyses.

2.4.4.1. Mineral contents

A weight of 0.2 g of the previously ground dried bean seed samples was wet digested using a mixture of sulfuric acid (H_2SO_4 98%) with hydrogen peroxide (H_2O_2 30%) according to the method described by Wolf (1982). On a dry weight basis, the common bean seeds mineral contents were determined in acid digested solutions.

In acid digested solutions of bean seed samples, total nitrogen was determined using the Kjeldahl method, and phosphorus was also assayed using the modified colorimetric method (molybdenum blue) using a spectrophotometer (SPECT-RONIC 20D, Milton Roy Co. Ltd., Houston, USA) according to Cottenie *et al*. (1982). While potassium was measured using the flame photometer method (JENWAY, PFP-7, ELE Instrument Co. Ltd., Essex, UK). In addition, Fe, Zn, Mn, Cu and Co were analyzed using Atomic Absorption Spectroscopy (Analyst 200, Perkin Elmer, Inc., Massachusetts, USA), as described by Chapman and Pratt (1982).

2.4.4.2. Nutritional quality

The percentages of seed protein, seed total carbohydrate, seed total soluble sugars (TSS) and seed starch were determined in the above mentioned finely powdered of dried seed samples according to AOAC (1995).

2.5. Statistical analysis

The data of the two experimental seasons of 2020-2021 and 2021-2022 were collected, tabulated and evaluated for normality and homogeneity within and between replications and years. Both replications (three) and years (two) were considered random effects in the mathematical model, and then subjected to a combined analysis of the variance as described by McIntosh (1983). The data were subjected to a statistical analysis of variance procedure of two-way ANOVA using the statistical analysis software (Statistical Analysis System, SAS-Stat). The least significant differences test (LSD) was employed to separate and compare the significant differences among means of treatment at the 5% level of significance according to the procedures reported by Sendicor and Cochran (1980).

3. Result and Discussion

3.1. Nodulation rate and nitrogen fixation

The data listed in Table (2) clearly demonstrated that soil applied cobalt at a rate of 10 ppm had a significant positive effect on nodulation parameters of plant roots than untreated plants. Common bean plants illustrated that the higher values of nodule number/plant, nodule fresh and dry weights/plant as well as nitrogenase enzyme activity were obtained when grown in soil treated with cobalt at 10 ppm as shown in the combined data of both seasons.

Table 2: Common bean nodulation parameters as affected by cobalt soil applications under different nitrogen levels after 50 days from the sowing date (combined analysis of both seasons).

Treatments		Nodule number/plant	Nodule fresh weight/plant (g)	Nodule dry weight/plant (g)	N-ase activity µmol C_2H_2/g/h
Without cobalt		7.18	1.72	0.443	6.33
With cobalt		19.64	2.93	0.813	15.33
100% N		27.55	4.445	1.140	17.58
75% N		18.70	3.255	0.855	15.06
50% N		4.71	0.985	0.295	6.02
25% N		2.67	0.610	0.220	4.66
Without cobalt	**100%**	18.3	4.02	1.02	14.08
	75%	10.4	2.86	0.75	11.24
	50%	0.0	0.0	0.0	0.0
	25%	0.0	0.0	0.0	0.0
With cobalt	**100%**	36.8	4.87	1.26	21.08
	75%	27.0	3.65	0.96	18.87
	50%	9.42	1.97	0.59	12.03
	25%	5.33	1.22	0.44	9.32
LSD at 5%	**Co**	**2.16**	**0.03**	**0.21**	**1.12**
	N	**2.00**	**0.42**	**0.18**	**0.95**
	Co x N	**1.91**	**0.40**	**0.20**	**0.60**

Increasing the nitrogen fertilizer level from 25 to 100% of the recommended nitrogen dose, led significantly to a progressive increase in nodulation parameters. The highest significant values of nodulation parameters, i.e., nodule number/plant, nodule fresh and dry weights/plant and nitrogenase enzyme activity were achieved by fertilizing common bean plants with 100% of the recommended nitrogen dose. On the other hand, nitrogen treatment at 25% of the recommended nitrogen recorded the lowest values of the above mentioned traits.

Regarding, the interaction between cobalt soil applications and different nitrogen fertilization levels, there were significant differences effects detected on nodulation parameters. Treating common bean plants with cobalt had a significant effect on nodule formation at all nitrogen levels applied. It could be noted that the highest values of nodule number/plant, nodule fresh and dry weights/plant and nitrogenase enzyme activity were recorded when common bean plants received cobalt at 10 ppm combined with nitrogen fertilizer at 100% of the recommended dose. On the other hand, when common bean plants received cobalt at 0 ppm plus nitrogen fertilizer at 25% the lowest values were attained.

These findings agreed with those of Naidu (2000) and Basu and Bhadoria (2008) who reported a relationship between nitrogenase enzyme activity, nodulation rate and the capacity and efficiency of biological atmospheric nitrogen fixation. Nitrogenase enzymes that are produced by rhizobacteria, are responsible for the reduction of atmospheric nitrogen (N_2) into ammonia (NH_3) which is a key step in the process of biological nitrogen fixation (BNF).

In addition, Nasef *et al.* (2008) stated that applied cobalt to growth medium at 15 ppm showed significantly higher nodule numbers/plant, fresh and dry weights and nodule nitrogen content of peanut plants compared with untreated plants, these results were also confirmed by Nadia Gad *et al.* (2014) who reported that cobalt application increased nodule numbers and fresh and dry weights of soybean plants as well as nitrogenase enzyme activity under different nitrogen applied rates. Moreover, Balachandar *et al.* (2003) and Mathur *et al.* (2006) indicated that cobalt is an essential component of cobalamin, which is highly needed for the activation of several enzymes as co-enzymes and responsible for the formation of leghaemoglobin, involved in biological atmospheric nitrogen fixation by nodules of leguminous plants. Furthermore, cobalt application caused an increase in the nitrogenase enzyme activity which was paralleled and related to the increment of nodule numbers and their fresh and dry weights as well as the capacity and efficiency of nitrogen fixation (Nadia Gad *et al.,* 2013).

3.2. Vegetative growth parameters

Data shown in Table (3) revealed that all vegetative growth parameters of common bean plants, i.e., plant height, number of branches and leaves/plant, total leaf area and fresh and dry weights of both shoots and roots, were promoted in relation to cobalt application. Cobalt treatment as a soil application at 10 ppm resulted in higher significant values of all measured vegetative growth parameters of common bean plants than untreated plants, except for root fresh and dry weights.

Table 3: Common bean vegetative growth parameters as affected by cobalt soil applications under different nitrogen levels after 80 days from the sowing date (combined analysis of both seasons).

Treatments		Plant height (cm)	Number/plant		Leaf area (cm^2)	Fresh weight (g)		Dry weight (g)	
			Branches	Leaves		Shoot	Root	Shoot	Root
Without cobalt		41.10	5.44	17.13	1767.5	115.00	5.33	24.85	1.54
With cobalt		44.90	7.53	22.28	2068.3	121.25	5.81	26.93	1.75
100% N		51.65	9.615	25.95	2264.0	129.50	6.180	29.75	1.905
75% N		46.00	8.175	21.65	2088.0	123.00	5.945	27.30	1.800
50% N		40.15	4.415	16.90	1776.5	114.50	5.285	24.50	1.485
25% N		34.20	3.725	14.30	1543.0	105.50	4.875	22.00	1.370
Without cobalt	**100%**	49.3	8.21	22.6	2152	127	6.03	28.9	1.85
	75%	44.0	6.72	17.8	1896	120	5.8	26.4	1.74
	50%	38.6	3.80	14.6	1584	112	5.01	23.3	1.32
	25%	32.5	3.03	13.5	1438	101	4.48	20.8	1.23
With cobalt	**100%**	54.0	11.02	29.3	2376	132	6.33	30.6	1.96
	75%	48.0	9.63	25.5	2280	126	6.09	28.2	1.86
	50%	41.7	5.03	19.2	1969	117	5.56	25.7	1.65
	25%	35.9	4.42	15.1	1648	110	5.27	23.2	1.51
LSD at 5%	Co	**2.00**	**0.29**	**0.97**	**97.2**	**2.5**	**NS**	**0.95**	**NS**
	N	**2.17**	**0.36**	**1.12**	**121.1**	**3.1**	**NS**	**1.11**	**NS**
	Co x N	**1.96**	**0.66**	**0.66**	**85.7**	**2.3**	**NS**	**0.75**	**NS**

Concerning the nitrogen fertilization levels, it is clear that increasing the nitrogen fertilizer level up to 100% of the recommended nitrogen dose, caused significant increases in all determined vegetative growth parameters of common bean plants. The highest significant values of vegetative growth parameters were found when applying 100% of the recommended nitrogen dose. In contrast, nitrogen treatment at 25% recorded the lowest significant values. No significant differences were realized among different nitrogen application levels on root fresh and dry weights as shown in Table (3).

Regarding, the interaction between cobalt soil applications and different nitrogen fertilization levels, significant differences effects were detected on all studied vegetative growth parameters of common bean plants. Generally, cobalt supplementation improved all vegetative growth parameters of the common bean at different nitrogen fertilizer levels. It could be stated that the highest values of all measured parameters were recorded when common bean plants received cobalt at 10 ppm combined with 100% of the recommended nitrogen fertilizer dose. In contrast, when those common bean plants received cobalt at 0 ppm plus nitrogen fertilizer at 25% the lowest values were achieved. However, there were no significant differences effect among experimental treatments of cobalt applications combined with different nitrogen fertilization levels on root fresh and dry weights.

Similar results were reported by Abdul Juleel *et al.* (2009 a and b), who found that cobalt application induced favorable growth responses in legume plants. Also, the obtained results were confirmed by Nadia Gad (2006) who found superiority of the vegetative growth parameters of pea plants when nitrogen fertilizer treatment was combined with the application of cobalt. Application of cobalt at 1 kg/ha significantly increased plant height, number of branches per plant and dry matter in lentil plants relative to the control treatment (Sahay and Singh, 2012).

3.3. Yield attributes

Data presented in Table (4) clearly indicated that cobalt treatment had a significant favorable effect on common bean yield attributes such as pod number/plant, pod weight/plant, 100-seed weight and seed yield as ton/fed relative to untreated plants. The higher and lower significant values of the aforementioned yield characteristics were found with cobalt treatments of 10 and 0 ppm, respectively.

Table 4: Common bean yield parameters as affected by cobalt soil applications under different nitrogen levels after 120 days from the sowing date (combined analysis of both seasons).

Treatments		Pods number/plant	Pods weight/plant (g)	100 seeds weight (g)	Seeds yield (Ton/fed.)
Without cobalt		15.73	9.62	33.70	2.88
With cobalt		19.93	14.12	42.35	3.58
100% N		26.15	17.35	55.15	3.890
75% N		22.50	14.90	47.90	3.666
50% N		13.25	9.02	28.85	2.795
25% N		9.41	6.20	20.20	2.568
Without cobalt	**100%**	23.7	14.4	50.4	3.494
	75%	20.6	12.6	44.7	3.101
	50%	10.8	6.63	22.9	2.639
	25%	7.82	4.84	16.8	2.280
With cobalt	**100%**	28.6	20.3	59.9	4.286
	75%	24.4	17.2	51.1	4.231
	50%	15.7	11.4	34.8	2.950
	25%	11.0	7.56	23.6	2.856
LSD at 5%	**Co**	**0.95**	**0.95**	**2.9**	**0.144**
	N	**1.13**	**1.3**	**3.7**	**0.152**
	Co x N	**0.81**	**0.82**	**2.5**	**0.124**

Concerning the nitrogen fertilizer levels, it is obvious that with increasing the nitrogen fertilization level from 25 to 100% of the recommended nitrogen fertilizer dose, significant increases in all yield attributes of common bean plants occurred. The highest significant values of yield characteristics of common bean plants were attained by applying 100% of the recommended nitrogen fertilizer dose. On the other side, nitrogen treatment at 25% gave the lowest values.

As for the interaction between cobalt soil applications and different nitrogen fertilizer levels, there were significant differences effect detected on yield attributes of common bean plants. It could be pointed out that the highest significant values of all measured yield characteristics were recorded when bean plants grown in medium supplied with cobalt at 10 ppm plus 100% of the recommended nitrogen fertilizer dose. Conversely, when bean plants were grown in medium supplied with cobalt at 0 ppm plus nitrogen fertilizer at 25% the lowest values were achieved.

These results agreed with those obtained by Banerjee *et al*. (2005) who stated that cobalt application enhanced yield and quality in groundnut. Results also revealed that, as mentioned by Nadia Gad (2006) cobalt increased pea pod yields by about 26.5% with the application of 75% nitrogen as urea and by 29.3% with 75% nitrogen as ammonium nitrate compared with the control treatment (100% nitrogen only without cobalt). According to, Abdul Jaleel *et al*. (2009 a and b) addition of cobalt to the growth medium caused an increment in all yield parameters of maize plants, such as the number and weight of cobs per plant and seed yield per plant.

3.4. Chemical constituents

3.4.1. Mineral contents

The data presented in Table (5) illustrated that cobalt application had a significant synergetic effect on the mineral contents of N, P, K, Mn, Zn, Fe, Cu and Co in common bean seeds compared with untreated plants. Applying cobalt to the soil at 10 ppm gave higher significant values of mineral contents in common bean seeds relative to cobalt applied at 0 ppm.

Table 5: Mineral contents in common bean seeds as affected by cobalt soil applications under different nitrogen levels (combined analysis of both seasons).

Treatments		Macronutrients (%)			Micronutrients (ppm)				Cobalt (ppm)
		N	P	K	Mn	Zn	Cu	Fe	
Without cobalt		2.71	0.696	1.470	14.05	12.60	15.50	177.75	0.878
With cobalt		2.97	0.782	1.740	15.45	14.88	16.90	182.50	7.60
100% N		3.32	0.802	2.08	17.50	16.75	19.60	181.5	4.33
75% N		3.11	0.764	1.77	16.10	14.90	18.10	178.0	4.34
50% N		2.86	0.734	1.39	13.85	12.35	14.55	179.0	3.92
25% N		2.09	0.658	1.19	11.55	10.95	12.55	182.0	4.37
Without cobalt	100%	3.18	0.762	1.89	16.7	14.9	19.0	178	0.89
	75%	3.03	0.719	1.61	15.3	13.6	17.5	175	0.85
	50%	2.75	0.681	1.26	13.2	11.7	13.6	181	0.91
	25%	1.89	0.623	1.12	11.0	10.2	11.9	177	0.86
With cobalt	100%	3.46	0.841	2.27	18.3	18.6	20.2	185	7.77
	75%	3.18	0.809	1.93	16.9	16.2	18.7	181	7.82
	50%	2.96	0.787	1.51	14.5	13.0	15.5	177	6.92
	25%	2.28	0.692	1.25	12.1	11.7	13.2	187	7.88
LSD at 5%	Co	**0.15**	**0.029**	**0.22**	**0.19**	**0.29**	**0.95**	**0.3**	**0.5**
	N	**0.26**	**0.035**	**0.39**	**0.23**	**0.35**	**1.11**	**0.7**	**0.3**
	Co x N	**0.39**	**0.025**	**0.13**	**0.17**	**0.25**	**0.80**	**0.5**	**1.2**

The obtained results strongly revealed that increasing the application of nitrogen fertilizer levels up to 100% led to a progressive increase in all analyzed mineral contents in bean seeds. Significant differences were noticed among all nitrogen application levels on all analyzed mineral contents. Nitrogen application level of 100% gave the highest values of all determined mineral contents in bean seeds. Whereas the lowest values were achieved at nitrogen level of 25% of the recommended dose. An opposite trend was obtained with Fe and Co contents, where the highest values of both were found with nitrogen application level of 25% and the lowest values were recorded by nitrogen level of 100% without significant differences between them.

The interaction effect between cobalt soil applications and nitrogen fertilizer levels recorded significant differences on all mineral contents in bean seeds. Applying cobalt at 10 ppm with a complete dose of nitrogen (100%) had the best seed status of N, P, K, Mn, Zn and Cu in common bean. It is of interest to clarify that bean plants that were grown in medium received cobalt at 10 ppm combined with nitrogen level of 100% of the recommended nitrogen fertilizer dose recorded the highest significant values of all measured mineral contents in common bean seeds except for Fe and Co contents. On the other side, those bean plants grown in medium treated with cobalt at 0 ppm combined with nitrogen fertilizer at 25% gave the lowest values of all mineral contents in bean seeds except for Fe and Co contents. Under cobalt application at 10 ppm with the 25% dose of nitrogen the highest values of both Fe and Co were observed, whereas, the lowest values were attained with cobalt at 0 ppm and 75% nitrogen level.

These results go along with the finding of Nadia Gad *et al.* (2013) who stated that cobalt had a beneficial effect on the mineral status of cowpea seeds under all applied nitrogen levels compared with untreated plants. Also, Jayakumar *et al.* (2008) mentioned that when cobalt was applied to the soil, all mineral compositions of blackgram were increased when compared with untreated plants. The highest values of Mn, Zn, and Cu contents were reached with cobalt application at 50 mg/kg soil.

3.4.2. Nutritional quality

Data in Table (6) sharply showed that applying cobalt to the growth medium at 10 ppm significantly improved the percentages of total protein, total carbohydrate, total soluble sugars and starch in common bean seeds compared to cobalt treatment at 0 ppm.

Table 6: Nutritional quality of common bean seeds as affected by cobalt soil applications under different nitrogen levels (combined analysis of both seasons).

Treatments		Protein (%)	Total carbohydrate (%)	Total soluble sugars	Starch (%)
Without cobalt		16.85	50.70	5.76	3.19
With cobalt		18.58	51.80	6.54	4.47
100%N		20.55	54.3	7.40	4.38
75% N		19.40	53.0	6.43	3.71
50% N		17.85	50.0	5.57	3.89
25% N		13.05	47.8	5.21	3.35
Without cobalt	**100%**	19.5	53.6	6.78	3.65
	75%	18.9	52.2	5.85	3.22
	50%	17.2	49.7	5.34	3.11
	25%	11.8	47.3	5.08	2.77
With cobalt	**100%**	21.6	55.0	8.02	5.11
	75%	19.9	53.7	7.01	4.19
	50%	18.5	50.3	5.80	4.66
	25%	14.3	48.2	5.33	3.92
LSD at 5%	**Co**	**0.23**	**0.87**	**0.45**	**0.19**
	N	**0.29**	**0.95**	**0.33**	**0.26**
	Co x N	**0.19**	**0.79**	**0.39**	**0.15**

There were significant differences obtained among different nitrogen treatments on the percentages of total protein, total carbohydrate, total soluble sugars, and starch in common bean seeds. Applying a complete dose of nitrogen fertilizer (100% of the recommended dose) gave the superiority of all studied characters of nutritional quality in bean seeds, followed significantly by 75% of the nitrogen dose treatment.

Regarding the interaction effect between cobalt soil applications and different nitrogen fertilizer dose treatments, there were significant differences realized on all studied characters of nutritional quality in bean seeds. It is apparent that the highest values of all measured characters of nutritional quality in bean seeds were recorded when bean plants grown in medium supplied with cobalt at 10 ppm combined with 100% of the recommended nitrogen fertilizer dose. In contrast, when bean plants received cobalt at 0 ppm plus 25% nitrogen fertilizer dose, the lowest values were illustrated.

The obtained results are in agreement with Vijayaregan *et al.* (2009) who stated that cobalt applied to the soil at 50 mg/kg significantly affected the biochemicals of groundnut seeds, such as total proteins, total carbohydrates, total soluble sugars, starch, and amino acids under different nitrogen doses when compared with untreated plants. In the same regard, Nadia Gad *et al.* (2014) concluded that cobalt had a significantly positive effect on the chemical contents, i.e., proteins, total carbohydrates, as well as total soluble sugars in soybean seeds with all applied nitrogen doses when compared with control plants.

The obtained results could be explained due to that cobalt is a beneficial element for higher plant growth as well as essential and necessary for legume nodulation rate and biological symbiotic fixation of atmospheric nitrogen, which reflected on legume plant growth, yield and quality (Hu *et al.* 2021).

4. Conclusion

In the light of the illustrated results from the current study, it may be concluded that under newly reclaimed sandy soil conditions, applying cobalt to the growth medium at a rate of 10 ppm combined with a complete dose of nitrogen fertilizer at 100% of the recommended nitrogen dose to common bean plants, caused an evident enhancement in all measured characters of nodulation rates and nitrogenase enzyme activity, vegetative growth, seed yield attributes, seed mineral contents and seed nutritional quality.

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