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The Effective Role of Cobalt in Increasing the Nitrogen Absorption Efficiency in Lentil Crop

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ABSTRACT

A field experiments was carried out to evaluate the effective role and importance of cobalt nutrient treatments (0 and 10 ppm as soil applications) on parameters of nodulation rate, vegetative growth, seed yield, mineral contents and nutritional quality of lentil plants of Giza 9 cultivar grown under different nitrogen fertilization rates (100, 75, 50 and 25% of the recommended dose of nitrogen). The experiment was conducted at the Experimental and Production Station, National Research Centre, El-Noubaria region, Behera governorate, Egypt, under newly reclaimed sandy soil conditions and drip irrigation system during season of 2020 and 2021. A split-plot design with three replicates was used. The obtained results summarized that cobalt treatment at 10 ppm as soil application resulted in significant increases in all studied parameters of nodulation rate, vegetative growth, seed yield, mineral contents and nutritional quality of lentil plants. The nitrogenase enzymes activity was paralleled with and related to the increase of nodule numbers per plant and fresh and dry weights of nodules on lentil roots. The obtained results strongly revealed that increasing nitrogen fertilizer levels from 25 to 100% led to progressive increases in all analyzed traits. Significant differences were found among different nitrogen application levels. The interaction effect between cobalt applications and different nitrogen fertilization levels recorded significant differences on all studied characters in lentil plants. Experimental treatments of cobalt at 10 ppm combined with nitrogen at 100% of the recommended level gave the highest values of all measured parameters. While the lowest values were illustrated when cobalt at 0 ppm plus nitrogen fertilizer at 25% were applied. As a beneficial element, cobalt has the ability to increase nitrogen efficiency and save almost 25% of nitrogen fertilizer application.

Keywords: *Lens culinaris,* Nitrogenases activity, Vegetative growth, Seed yield, Root nodules, Mineral contents, Seed nutritional profiles.

1. Introduction

Lentil (*Lens culinaris),* is an annual edible legume of the pea family Fabaceae. It is among the crops that have been mentioned in the Holy Quran. Thus, it is recognized as one of the oldest leguminous crops among the five pulse crops in the cropping pattern of Egyptian agriculture, coming after the faba bean.

Legumes play an important role in human diets because they are cheap and rich sources of plant proteins, carbohydrates, calories, certain minerals and vitamins. Legumes are the major source of proteins and calories for a large sector of the inhabitants in rural areas, for economic and cultural reasons (Tgungula and Garjila, 2006).

Cobalt is a beneficial element for higher plants, although there is no evidence of its direct role in plant regulatory and biochemical processes. Cobalt is an important element, especially for legumes due to its vital biological role in the process of atmospheric nitrogen fixation by microorganisms (Evans and Kliwer, 1964). 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). Cobalt application to the soil at 50 mg kg^{-1} was found to have significant positive effects on blackgram plants

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vegetative growth and nutrient absorption compared with control plants. The highest values of Mn, Zn, and Cu in blackgram were recorded with cobalt application at 50 mg kg^{-1} soil (Jayakumar *et al.,* 2008). In addition, Nadia Gad and Abdel-Moez (2015) reported that cobalt application levels significantly increased the yield traits of fenugreek plants compared with untreated plants. Also, Nadia Gad *et al.* (2015) mentioned that applying cobalt at a rate of 17.5 ppm had a significant enhancing effect on okra yield quantity and quality compared to untreated plants. According to Asisan *et al.* (2018) cobalt has both a beneficial advantage and a detrimental disadvantage effect on plants. Cobalt consequently promotes better plant growth, i.e., plant height, and root length, as well as inhibiting root growth by retarding cell division and hindering the uptake and translocation of nutrients and water. Vijayarengan (2014) demonstrated that cobalt application at a rate of 50 mg kg^{-1} as cobalt chloride resulted in a greater significant root length (25.10 cm), shoot length (42.42 cm), and number of nodules (63.25), in cowpea plants, whereas at a higher rate of cobalt, all of these parameters decreased. Nadia Gad (2012) revealed that cobalt application at 8 ppm recorded the highest nodule numbers per plant and fresh and dry weights of nodules. In addition, the increment in the nodule formation process may be due to an increase in the efficiency of rhizobium bacteria to perform biological N fixation at a high capacity to produce healthy plants. That means cobalt might play a vital role in increasing the nitrogenase enzyme activity of groundnut root nodulation as compared with untreated plants. The increase in nitrogenase activity was paralleled by and related to the increase in nodule numbers and its efficiency. Nadia Gad *et al.* (2014) showed that cobalt application at 12 ppm significantly increased nitrogenase activity, which was paralleled and related to the increase of root nodule numbers and weights as well as mineral contents in soybean seeds, particularly with applied rates of 100 and 75% nitrogen fertilizer. They also found that cobalt application to the soil at 12 ppm saved 75% of applied nitrogen fertilizer compared with untreated plants.

Cobalt increased cytoplasmic pressure and leaf resistance to dehydration consequently, it decreased the wilting coefficient of the plants, and their drought stress tolerance. On the other hand, increasing cobalt levels in the soil beyond 12 mg kg^{-1} resulted in a significant reduction in the contents of these nutritive elements. The reduction seemed to be positively related to the excess concentration of cobalt. Howell and Skoog (1955) found that cobalt also promoted many developmental processes, including stem coleoptile elongation, the opening of hypocotyle hooks, leaf expansion and bud initiation and development.

The current study was carried out to evaluate the effects of cobalt soil applications and 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 lentil plants Giza 9 cultivar, grown under newly reclaimed sandy soil conditions.

2. Materials and Methods

A field experiment was performed at the Experimental and Production Station, National Research Centre, El-Noubaria region, Behera governorate, Egypt, during the growing season of 2020/2021. This study was carried out to determine the most suitable nitrogen fertilization level and cobalt rate for lentil plants of Giza 9 cultivar.

The experiment was laid out in a split-plot design with three replicates. Nitrogen fertilization levels of 100, 75, 50 and 25% of the recommended dose of nitrogen, were arranged in the main plots, while cobalt soil application rates (0 and 10 ppm) were distributed within the sub-plots. The source of nitrogen fertilizer was ammonium sulfate (20.6% N). While cobalt (Co) in the form of cobalt sulfate heptahydrate ($CoSO_4.7H_2O$, MW 281.10) was used as a source of cobalt. Lentil seeds cultivar (Giza 9) obtained from the Legume Crop Research Department, Field Crop Research Institute, Agricultural Research Center (ARC), Giza, Egypt, were sown on March 1st, 2020 and 2021, respectively, in hills with 2 seeds per hill and 20 cm apart at 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.

The physical and chemical properties of the experimental soil 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).

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

Table 1: Physical and chemical properties 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	

2.1. Data recorded

Samples of lentil roots from each experimental sub-plot were randomly collected after 50 days from the sowing date to determine the nodule numbers per plant and nodule fresh and dry weights (g). Furthermore, enzymes that are produced by rhizobacteria and responsible for the reduction of atmospheric nitrogen (N_2) to ammonia (NH_3), nitrogenases activity as µmol C_2H_4/g/h was determined according to Hardy *et al*. (1968).

After 80 days from the sowing date, vegetative growth parameters of lentil plants, such as plant height (cm), number of branches and leaves per plant, leaf area (cm^2) as well as shoot and root fresh and dry weights, were recorded according to FAO (1980).

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

During the harvesting stage, seed samples were randomly collected from 12 lentil plants from each experimental sub-plot, and then dried lentil plant samples were threshed by hand to obtain the seeds. Afterwards, seeds were 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.

A weight of 0.2 g of the previously ground dry 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) to determine mineral contents,

In acid digested solutions of 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 determined using Atomic Absorption Spectroscopy (Analyst 200, Perkin Elmer, Inc., Massachusetts, USA), as described by Chapman and Pratt (1982).

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

2.2. Statistical analysis

All data sets 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 5% level of probability according to the procedures reported by Sendicor and Cochran (1980).

3. Results and Discussion

3.1. Nodulation parameters and nitrogen fixation

The presented data in Table (2) obviously showed that cobalt treatment had a significant stimulative effect on lentil nodulation parameters, i.e., nodule numbers/plant, nodule fresh and dry weights as well as nitrogenase enzyme activity. Cobalt soil treatment resulted in higher significant values of nodulation parameters in treated plants than in untreated plants.

The highest values for nodulation parameters were shown with 100% of the recommended nitrogen dose, followed by 75% and 50% of the recommended nitrogen dose in descending order. However, 25% of the recommended nitrogen gave the lowest significant values of the above-mentioned traits.

Table 2: Lentil nodulation parameters as affected by cobalt soil applications under different nitrogen levels after 50 days from the sowing date (Mean of two seasons).

Treatments		Nodules No. per plant	Nodules fresh weight/plant (g)	Nodules dry weight/plant (g)	N-ase activity µmol C_2H_2/g/h
Without cobalt		18.04	4.60	1.814	14.75
With cobalt		26.43	6.11	2.463	18.55
100% N		33.90	7.53	2.995	18.50
75% N		26.40	6.14	2.605	17.15
50% N		17.70	4.53	1.710	15.90
25% N		10.93	3.22	1.243	15.05
Without cobalt	**100%**	26.9	6.77	2.65	16.6
	75%	21.2	5.23	2.42	15.2
	50%	14.7	3.82	1.31	14.0
	25%	9.36	2.56	0.875	13.2
With cobalt	**100%**	40.9	8.28	3.34	20.4
	75%	31.6	7.05	2.79	19.1
	50%	20.7	5.24	2.11	17.8
	25%	12.5	3.87	1.61	16.9
LSD at 5%	**Co**	**5.6**	**1.40**	**0.50**	**1.3**
	N	**6.4**	**1.25**	**0.23**	**0.8**
	Co x N	**3.14**	**1.31**	**0.74**	**0.3**

In terms of the interaction effect, there were significant differences detected among the studied factors on nodulation parameters. Generally, lentil plants that received cobalt at 10 ppm and 100% nitrogen recorded the highest significant values of the nodulation parameters, 40.9, 8.28, 3.34 and 20.4, respectively. In contrast, the lowest values, 9.36, 2.56, 0.875 and 13.2, respectively, were attained by those lentil plants treated with cobalt at 0 ppm and 25% nitrogen.

These results are in agreement with those obtained by Basu and Bhadoria (2008) who stated that cobalt improved the nodulation rate on groundnut roots. Cobalt treatment significantly increased nodule banter content and leghemoglobin production, which were linearly related to cobalamin. According to Sarada and Polasa (1992) cobalt is an essential element for the growth of rhizobium, the specific bacteria involved in legume nodulation and the symbiotic fixation of atmospheric nitrogen into amino acids and proteins in legumes. Nitrogenase enzymes that are produced by rhizobacteria are responsible for the reduction of nitrogen (N_2) to ammonia (NH_3) which is a key step in the process of biological nitrogen fixation (BNF).

3.2. Vegetative growth parameters

Data shown in Table (3) revealed that cobalt treatment at 10 ppm as soil application gave significantly higher values of vegetative growth parameters of lentil plants, i.e., plant height, number of branches and leaves/plant, total leaf area and fresh and dry weights of shoots and roots than untreated plants.

There were significant differences effects realized among different nitrogen application levels. The highest values of all studied parameters of plant vegetative growth were found with application of 100% of the recommended nitrogen dose. While, the lowest values of the aforementioned traits were obtained with 25% of the recommended nitrogen.

Table 3: Lentil vegetative growth parameters as affected by cobalt soil applications under different nitrogen levels after 80 days from the sowing date (Mean of two seasons).

Treatments		Plant height (cm)	Number/Plant		Total leaf area (cm^2)	Fresh weight (g)		Dry weight(g)	
			Branches	Leaves		Shoots	Roots	Shoots	Roots
Without cobalt		22.83	3.85	56.15	1305.75	10.41	3.16	2.78	0.88
With cobalt		28.48	4.40	60.40	1406.25	11.10	3.69	3.00	1.02
100% N		34.15	4.90	67.4	1480.5	12.95	3.975	3.440	1.060
75% N		29.05	4.40	62.4	1378.5	11.65	3.755	3.135	0.995
50% N		24.10	3.85	55.5	1325.0	10.10	3.165	2.700	0.910
25% N		15.30	3.35	47.8	1240.0	8.33	2.795	2.275	0.840
Without cobalt	**100%**	31.5	4.6	65.2	1434	12.3	3.77	3.26	0.97
	75%	26.4	4.2	60.6	1338	11.5	3.70	3.05	0.91
	50%	20.8	3.6	53.0	1264	9.89	2.64	2.64	0.86
	25%	12.6	3.0	45.8	1187	7.96	2.51	2.17	0.79
With cobalt	**100%**	36.8	5.2	69.6	1527	13.6	4.18	3.62	1.15
	75%	31.7	4.6	64.2	1419	11.8	3.81	3.22	1.08
	50%	27.4	4.1	58.0	1386	10.3	3.69	2.76	0.96
	25%	18.0	3.7	49.8	1293	8.69	3.08	2.38	0.89
LSD at 5%	**Co**	**1.2**	**0.4**	**5.4**	**33**	**0.8**	**0.37**	**0.32**	**0.05**
	N	**2.2**	**0.6**	**4.5**	**73**	**2.4**	**0.07**	**0.21**	**0.06**
	Co x N	**2.64**	**0.7**	**4.0**	**15**	**1.7**	**0.11**	**0.30**	**0.11**

As for the interaction effect between cobalt soil applications and different nitrogen application levels, there were significant differences on measured vegetative growth parameters of lentil plants. Cobalt treatment at 10 ppm plus nitrogen application at 100% of the recommended dose gave the highest significant values for all determined parameters of vegetative growth of lentil plants. While cobalt at 0 ppm combined with the application of 25% nitrogen recorded the lowest values.

The achieved results are in harmony with those obtained by Atta Aly *et al*. (1991) who stated that low cobalt application was associated with favorable growth responses of tomato plants. They also added that there was a significant increase in endogenous phytohormones, auxins and gibberellins and a reduction in the activities of catalase and peroxidase enzymes, as a result, tomato plant growth was enhanced. These results were also confirmed by Sahay and Singh (2012) who stated that cobalt application at 1 kg/ha significantly increased plant height, number of branches per plant and dry matter of lentil plants relative to the control treatment.

3.3. Yield characteristics

Data in Table (4) showed that cobalt soil application had positive significant effects on all lentil yield characteristics such as pod number/plant, pod weight/plant, seed weight/plant and seed yield compared to untreated lentil plants. The higher and lower significant values of the above mentioned yield characteristics were obtained by cobalt treatments of 10 and 0 ppm, respectively.

Concerning the nitrogen application levels, increasing the nitrogen applications from 25 to 100% of the recommended nitrogen dose, led to significant increases in all lentil yield characteristics. The highest significant values of yield characteristics of lentil plants were achieved by applying 100% of the recommended nitrogen dose. On the other hand, nitrogen treatment at 25% of the recommended nitrogen recorded the lowest values.

Table 4: Lentil yield characteristics as affected by cobalt soil applications and different nitrogen levels after 120 days from the sowing date (Mean of two seasons).

Treatments		Pods No. per plant	Pods weight/plant (g)	Seed weight/plant (g)	Seeds yield Ton/fed.
Without cobalt		31.18	6.64	1.73	2.11
With cobalt		38.60	8.20	2.11	2.68
	100% N	44.00	9.22	2.305	2.732
	75% N	36.60	7.97	2.175	2.589
	50% N	32.30	6.76	1.855	2.272
	25% N	26.65	5.72	1.335	1.991
Without cobalt	**100%**	39.8	8.46	2.03	2.378
	75%	32.6	6.96	1.96	2.309
	50%	29.0	6.17	1.68	2.107
	25%	23.3	4.96	1.25	1.649
With cobalt	**100%**	48.2	9.98	2.58	3.086
	75%	40.6	8.97	2.39	2.868
	50%	35.6	7.35	2.03	2.436
	25%	30.0	6.48	1.42	2.333
LSD at 5%	**Co**	**5.0**	**0.87**	**0.19**	**0.103**
	N	**3.6**	**0.79**	**0.07**	**0.202**
	Co x N	**7.3**	**0.31**	**0.17**	**0.784**

Regarding, the interaction between cobalt soil applications and different nitrogen levels, significant differences effects were detected on yield characteristics of lentil plants. It could be pointed out that the highest values of all measured yield characteristics were recorded when lentil plants received cobalt at 10 ppm combined with 100% of the recommended nitrogen fertilizer. In contrast, when lentil plants received cobalt at 0 ppm plus nitrogen fertilizer at 25%, the lowest values were achieved. It is of interest to note that experimental treatment with cobalt at 10 ppm combined with 75% nitrogen increased lentil seed yield by about 20.6% compared with no cobalt application plus 100% nitrogen.

The addition of cobalt to the plant growth medium saved about 25% of the recommended nitrogen fertilizer dose. These results agreed with those obtained by Banerjee *et al*. (2005) who stated that cobalt recorded the maximum dry matter accumulation in plant shoots and roots, as well as enhanced yield 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 without cobalt). According to, Abdul Jaleel *et al*. (2009 a and b) addition of cobalt to the soil caused an increment in all growth and yield parameters of maize plants, such as seedling vigor, number and weight of pods and seed yield per plant.

3.4. Mineral contents

The data presented in Table (5) showed that cobalt had a significant synergetic effect on macronutrients (N, P, and K) and micronutrient contents (Mn, Zn, Fe, Cu and Co) in lentil seeds compared with untreated plants. Applying cobalt to the soil at 10 ppm resulted in significant increases in the mineral contents (N, P, K, Mn, Zn, Cu, Fe and Co) of lentil seeds relative to cobalt at 0 ppm.

The obtained results strongly revealed that increasing nitrogen fertilizer levels from 25 to 100% resulted in a progressive increase in all analyzed mineral contents in lentil seeds. Significant differences were found among different nitrogen application levels. Nitrogen application level of 100% gave the highest values of all determined mineral contents in lentil seeds. Whereas the lowest values were achieved at nitrogen level of 25% of the recommended dose.

Table 5: Mineral contents in lentil seeds as affected by cobalt soil applications under different nitrogen levels (Mean of two seasons).

Treatments		Macronutrients (%)			Micronutrients (ppm)				Cobalt (ppm)
		N	P	K	Mn	Zn	Cu	Fe	
Without cobalt		1.890	0.498	1.158	12.63	10.35	8.24	147.75	0.620
With cobalt		2.778	0.497	1.273	13.75	10.59	8.42	143.50	5.56
100% N		3.02	0.528	1.485	16.55	12.95	10.27	153.00	3.24
75% N		2.65	0.518	1.275	13.75	10.55	9.10	148.50	3.15
50% N		1.94	0.483	1.135	11.85	9.87	7.85	143.00	3.05
25% N		1.73	0.462	0.966	10.60	8.52	6.10	138.00	2.93
Without cobalt	**100%**	2.33	0.522	1.36	16.2	12.7	10.11	156	0.78
	75%	1.96	0.516	1.23	13.0	10.4	9.01	151	0.69
	50%	1.79	0.485	1.08	11.3	9.81	7.79	145	0.53
	25%	1.48	0.468	0.961	10.0	8.49	6.04	139	0.48
With cobalt	**100%**	3.71	0.533	1.61	16.9	13.2	10.42	150	5.69
	75%	3.33	0.520	1.32	14.5	10.7	9.19	146	5.61
	50%	2.09	0.480	1.19	12.4	9.92	7.91	141	5.57
	25%	1.98	0.456	0.971	11.2	8.54	6.15	137	5.38
LSD at 5%	**Co**	**0.91**	**0.024**	**0.22**	**1.2**	**1.38**	**1.76**	**0.04**	**0.19**
	N	**0.19**	**0.017**	**0.15**	**1.3**	**1.32**	**1.75**	**0.04**	**0.05**
	Co x N	**0.50**	**0.012**	**0.011**	**1.3**	**0.05**	**1.11**	**0.02**	**4.10**

The interaction effect between cobalt soil applications and nitrogen fertilizer levels recorded significant differences on all mineral contents in lentil seeds. It is of interest to clarify that lentil plants that received cobalt at 10 ppm combined with nitrogen fertilizer level of 100% recorded the highest significant values of all measured micro and macro-elements in lentil seeds except for Fe content. Conversely, those lentil plants treated with cobalt at 0 ppm combined with nitrogen fertilizer at 25% recorded the lowest values of all mineral contents in lentil seeds except for P and Fe contents.

Jayakumar *et al*. (2008) found that all mineral compositions of blackgram were increased by cobalt addition to the soil compared with untreated plants. The highest values of Mn, Zn, and Cu were reached with cobalt application at 50 mg/kg soil. To confirm these results, Asisan *et al*. (2018) showed that cobalt has both a beneficial advantage and a detrimental disadvantage effect on plants. Cobalt consequently led to better plant growth, plant height, root length and crop yield, but inhibited root growth by retarding cell division, hindering the uptake and translocation of nutrients and water.

3.5. Nutritional components

Data in Table (6) showed that cobalt soil application increased total protein, total carbohydrate, total soluble sugars, and vitamin A contents in lentil seeds under different nitrogen levels compared with untreated plants. Chemical constituents of lentil seeds were significantly improved by using cobalt at 10 ppm when compared with seeds of untreated plants which received cobalt at 0 ppm.

There were significant differences among different nitrogen treatments in total protein, total carbohydrate, total soluble sugars, and vitamin A contents in lentil seeds. Nitrogen treatment at 100% of the recommended dose recorded the superiority of all studied parameters of nutritional components in lentil seeds, followed by 75% nitrogen treatment with a significant difference between them on total protein only.

Table 6: The nutritional components of lentil seeds as affected by cobalt soil applications under different nitrogen levels (Mean of two seasons).

Treatments		Protein (%)	Total Carbohydrate	Total soluble Sugars	Vitamin A M/100g
Without cobalt		11.86	17.80	1.13	4.35
With cobalt		18.63	19.50	1.16	4.88
	100% N	18.90	19.60	1.20	5.00
	75% N	16.60	19.25	1.18	4.75
	50% N	14.65	18.45	1.13	4.50
	25% N	10.83	17.30	1.08	4.20
Without cobalt	**100%**	14.6	19.0	1.18	4.8
	75%	12.4	18.6	1.16	4.5
	50%	11.2	17.7	1.11	4.2
	25%	9.25	15.9	1.06	3.9
With cobalt	**100%**	23.2	20.2	1.21	5.2
	75%	20.8	19.9	1.19	5.0
	50%	18.1	19.2	1.14	4.8
	25%	12.4	18.7	1.09	4.5
LSD at 5%	**Co**	**5.7**	**0.5**	**0.5**	**0.3**
	N	**1.77**	**1.8**	**1.05**	**0.3**
	Co x N	**3.15**	**2.8**	**0.03**	**0.6**

The interaction between cobalt soil applications and different nitrogen fertilizer treatments had significant differences effect on all studied characters of chemical constituents in lentil seeds. It is evident that the highest values of all measured nutritional components in lentil seeds were recorded when lentil plants received cobalt at 10 ppm combined with 100% of the recommended nitrogen fertilizer. In contrast, when lentil plants received cobalt at 0 ppm plus nitrogen fertilizer at 25%, the lowest values were illustrated.

The achieved results could be explained on the basis of the results concluded by Hu *et al*. (2021) who pointed out that cobalt is a beneficial element for the growth of higher plants, but it's essential and necessary for legume nodulation and the fixation of atmospheric nitrogen, which in turn reflected on legume plant growth and reproduction. Furthermore, Nadia Gad *et al*. (2013) added that the cobalt treatment significantly increased the chemical contents of soybean seeds compared with the control treatment. The attained results are confirmed by Vijayaregan *et al*. (2009) who stated that cobalt applied at 50 mg/kg soil had a beneficial effect on biochemicals of groundnut seeds like total proteins, total carbohydrates, total soluble sugars, starch, and amino acids when compared with untreated plants.

4. Conclusion

Based on the results of this study, it is possible to conclude that the experimental treatment of cobalt applied to the soil at 10 ppm plus nitrogen fertilizer level of 100% caused an apparent enhancement of all determined characters of nodulation rates and nitrogenase enzyme activity, vegetative growth, seed yield, mineral contents and chemical constituents relative to the experimental treatment of cobalt at 0 ppm plus nitrogen fertilizer level of 25% of the recommended dose of lentil plants grown under newly reclaimed sandy soil conditions.

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