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Effect of *Rhizobium* inoculant, Nitrogen Starter and Cobalt on Stimulation of Nodulation, N Fixation and Performance of Faba Bean (*Vicia faba* L.) Grown under Salinity Stress

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ABSTRACT

In the modern agriculture systems, there is a general tendency to reduce the amounts of mineral nitrogen inputs and maximize their use efficiency to minimize environmental pollution and its hazards on public human health. It is well known that cobalt is a necessary trace element to leguminous crops and bacterial communities responsible for biological nitrogen fixation (BNF). So, two field trials were performed in a split-split-plot design with three replicates during two-successive winter seasons of 2019/2020 and 2020/2021 as the sake of studying the influence of *Rhizobium* inoculant, nitrogen application rates [100, 75 and 50% of nitrogen recommended dose (NRD) as starter, equivalent to 20, 15 and 10kg N fed^{-1}, respectively] and cobalt supplementation (seeds soaking and foliar application as combined method) at different concentrations of cobalt (0.0, 8.0 and 12.0 mg cobalt L^{-1}) on nodulation, nitrogenase activity and performance of faba bean plants grown on saline clay soil. Findings of this study showed that plants inoculated prior to sowing with the strain of *Rhizobium* at rate of 10 g inoculant per 1.0 kg seeds under N fertilization at rate of 15 kg N fed^{-1} with cobalt treatment at rates of 8.0 and 12.0 mg cobalt L^{-1} realized better results than that fertilized with ammonium sulphate at rate of 20 kg N fed^{-1} without both *Rhizobium* inoculation and cobalt treatment. Generally, cobalt treatments improved nodulation, nitrogenase activity and stimulated nodulation parameters, thus enhancing all studied growth criteria, green yield and its components of faba bean plants grown under salinity stress.

Keywords: Nodulation, nitrogenase activity, legumes, cobalt, inoculation.

INTRODUCTION

Due to the fact that most of Egyptian soils are poor in available nitrogen forms (NH_4 and NO_3), the chemical N-fertilizers are so essential for plant growth. However, the continuous application of synthetic nitrogen fertilizers causes several environmental and health noxious associated with surface and groundwater pollution by No_3 leaching form soil matrix into water table (Fixen and West 2002).Therefore, reducing the amount of synthetic nitrogen fertilizers and maximizing their efficacy will be a great challenge in agricultural management of the future research (Seadh, 2014). Biological fixation of atmospheric nitrogen by symbiotic bacteria such as rhizobia-legumes symbiosis is one of the promising technologies and the alternative solutions that play a key-role in reducing the consumption of synthetic nitrogen compounds, raising soil fertility, declining the cost of agriculture production and eliminating the potential N contamination (Herridge *et al.*, 2008).also, nitrogen contamination possess hazard effect on human(eutrophication), especially children (blow baby syndrome) (Mudu *et al.*, 2014) .

Cobalt is a necessary trace element to legumes *(e.g.* beans, alfalfa and clove) for nodules formation and symbiotic BNF. It is a component of a number of enzymes and enhances the drought resistance of seeds (Sherif *et al.*, 2017). Furthermore, Co and other micronutrients are necessary for the synthesis of vitamin B_{12} required for hemoglobin formation in nodules and the optimalN_2 fixation. Cobalt had been considered as a beneficial element for plant nutrition; however, it has been recently categorized as an essential element for plant nutrition by the Official Journal of the European Union, REGULATION (EU) 2019/ 1009.

Faba bean (*Vicia faba* L.) belongs to *Leguminosae*, which play an essential role in human nutrition since leguminous crops are rich sources of certain minerals, protein and calories (Gad *et al.*, 2011). Faba bean is one of the oldest leguminous crops grown in Egypt, where it was cultivated for food and feed purposes and known as "broad bean" or "horse bean" among many other local names (Mady, 2009). Also, it is one of the most efficient nitrogen-fixing legumes, where it can obtain all of its N requirements through BNF (Youseif *et al.,* 2017). Qados and Moftah, (2015) reported that the flowering stage of faba bean is critical and sensitive to salinity stress, where salinity reduces the yield of faba bean and they used silicon and nano-silicon to mitigate salinity hazard.

Therefore, the objective of the current study is to reduce chemical nitrogen fertilizers in faba bean production and maximize the role of rhizobia-legumes symbiosis in N fixation by using cobalt under salinity conditions.

MATERIALS AND METHODS

1.Experimental setup.

Two field trials were performed at Tag El-Ezz Experimental Farm, Agricultural Research Station,Temi El-Amdid District, El-Dakahlia Governorate, Egypt (31°31' 47.64" N latitude and 30°56' 12.88" E longitude) during the

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two successive winter seasons of 2019/2020 and 2020/2021.The work aimed at evaluating the effect of the strain of *Rhizobium leguminosarum* as main plots [once in the presence of strains inoculants (at rate of 10 g inoculant per 1.0 kg seeds) and other in the absence of *Rhizobium* addition], three rates of nitrogen starter using ammonium sulphate (20.5% N) as sub plots [100, 75 and 50% of NRD, equivalent to 20, 15 and 10 kg N fed^{-1}, respectively] and cobalt element supplementation (seeds soaking and foliar application as combined method) at different concentrations of cobalt sulphate solution (36%Co) as sub-sub plots [0.0, 8.0 and 12.0 mg cobalt L^{-1}] on reducing the noxious influence of salinity stress and faba bean productivity. The execution of the trial was done in a split-split plot design with three replicates. The sub-sub plot size was 10.5 m^2 (3.0 m × 3.5 m). Seeds of faba bean (*Vicia faba* L. Giza 716, early cultivar) were obtained from Food Legumes Department, Field Crop Research Institute, ARC, Giza, Egypt. The recommended seed rate (40 kg fed^{-1}) was sown on 14th and 15th of October in the first and second season, respectively. Cobalt element was applied in two ways as a combined method (seeds soaking and foliar spraying together), where seeds were soaked in different cobalt concentrations (0.0, 8.0 and 12.0 mg cobalt L^{-1}) for 10 hours before sowing and the plants were sprayed twice (after 35 and 50 days from sowing) with the same cobalt solutions by hand sprayer until saturation point (the volume of sprayed solution for each treatment was 120 ml) . The *Rhizobium* inoculant was obtained from the bio-fertilizer production unit of Soil, Water and Environment Research Institute, ARC, Giza, Egypt. The seeds were inoculated with *Rhizobium* (seeds were coated with inoculums before sowing using 40% Arabic gum as a sticker for plots that received Rhizobium inoculant) then were sown directly in hills by hand at the rate of 3 seeds $hill^{-1}$ on shoulder bed and in the 1/3 top of row ridge under surface irrigation system. All plots received calcium superphosphate (15% P_2O_5) at rate of 200 kg fed^{-1}during soil preparation. Effective nitrogen dose as ammonium sulphate (20.5% N) was applied at the above-mentioned rates in one dose after 15 days from sowing before the first irrigation, while potassium sulfate (48 % K_2O) was applied at flowering stage (65 days from sowing) at rate of 50 kg K_2O fed^{-1}. Controlling broomrapes (*Orobanche* spp.) was done using a fungicide obtained from Fac. Agric., Mans. Univ., Egypt. Other recommended agriculture practices for faba bean were done according to the traditional recommendations by Egyptian Ministry of Agriculture. Some of characteristics of experimental soil are presented in Table 1, where soil sample (0-30 cm depth) was taken and analyzed according to Buurman *et al.*, (1996) before cultivation. Soil cobalt was extracted by iodine nitrotetrazole chloride and determined using spectrophotometric method as described by Dospatliev *et al.*, (2010). Soil taxonomy was performed according to Soil Survey Staff (2010).

2. Vegetative and chemical measurements at flowering stage

At the period of 65 days after sowing (flowering stage), a random sample of five faba bean plants was taken from each sub-sub plot to estimate the following traits:

- Vegetative growth criteria including plant height, leaf area index as well as fresh and dry weights were measured. Leaf area index was determined according to Watson (1952) as the following formula:

LAI = unit leaf area per plant/unit ground area occupied by plant.

- Nodulation traits: Nitrogenase activity was measured according to Hardy *et al.*, (1968) and other nodulation parameters *i.e*. No. of nodules $plant^{-1}$, fresh and dry weights of nodules were measured.
- Chemical constituents: Total chlorophyll content was determined according to Sadasivam and Manickam, (1996). Digestion of faba bean leaves was done with a mixture of (H_2SO_4) and ($HClO_4$) for nitrogen but for cobalt ,the leaves sample was digested using nitric acid, H_2O_2 and hydrofluoric acid according to Gotteni *et al*., 1982. Total N (%) in leaves of faba bean plants was determined using Micro kjeldahl apparatus as described by (Jones *et al*., 1991). Cobalt in leaves was determined using ultrasonic slurry sampling electrothermal atomic absorption spectrometry as mentioned by Takuwa *et al.*, (1997).

3. Soil and plant measurements at harvesting stage.

The following measurements were done at harvesting stage (90 days from sowing).

- Pods yield measurements: Pod weight, No. of pods $plant^{-1}$, green yield.
- Nitrogen and cobalt contents in seeds were determined as formerly mentioned in leaves.
- Bioconstituents of seeds: Total carbohydrates and protein content in seeds were determined according to A.O.A.C (1995), where crude protein percentage was calculated by multiplication of N % in seeds by 6.25.
- Soil measurements: soil sample (0-30 cm depth) from each plot was taken to assess the influence of studied treatments on available nutrients content (N, P, K), where samples were analyzed according to Buurman *et al.*, (1996).

4. Flowers and pods measurement.

- Number of flowers $plant^{-1}$: Start of counting was at 60 days of plant age with 4 days intervals until 90 days. It was counted in samples consisted of five plants.
- Pod setting: Start of counting was at 75 days of plant age with 4 days intervals until 90 days.

5. Statistical Analysis.

It was done according to Gomez and Gomez, 1984, using CoStat (Version 6.303, CoHort, USA, 1998–2004)].

Table 1. Some experimental soil characteristics (combined analysis of the two seasons).

Particle size distribution , %				Soil moisture constants,%		
C. sand	**F. sand**	**Silt**	**Clay**	**FC**	**SP**	**WP**
6.30	9.23	36.78	47.69	44.6	89.20	22.3
Textural class is Clay				Chemical properties		
Soluble cations and anions, m molc L^{-1}				EC, dSm^{-1}	pH	O.M,%
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	6.67	7.9	1.20
20.67	17.8	22.24	6.03	The soil was classified as (Vertisols, Torrerts, Haplotorrerts, Typichaplotorrert, Clayey Hyperthermic, Sniectic).		
Soluble anions, mmol l^{-1}						
CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}			
N.D.	1.78	39.98	24.98			
Available element, mg kg^{-1}						
N	P	K	Co			
40.3	7.96	202.3	0.0025			

- **pH was determined in soil suspension (1: 5).**
- **Soil Electrical Conductivity (EC) and soluble ions were determined in saturated soil paste extract.**

N.D. means not detected

RESULTS AND DISCUSSION

Results

1.Nodulation and Nitrogen Fixation at flowering stage

Data of Table 2 show that using *Rhizobium* inoculant significantly increased nitrogenase activity (µmol C_2H_4/g/h) and other nodulation parameters *i.e*. No. of nodules plant^{-1}as well as fresh and dry weight of nodules (g plant^{-1}) compared to cultivation without inoculation.

Data of the same Table show that the highest values of nodulation traits and nitrogenase activity were recorded when faba bean plants received ammonium salphate (starter) at rate of 20 kg N fed^{-1} followed by 15 kg N fed^{-1}, while the nitrogen starter with 10 kg N fed^{-1} came in the third order.

Also, the statistical analysis show highly significant differences among the different concentrations of cobalt studied, where the values of nodulation traits and nitrogenase activity increased as the concentration of cobalt in the solution increased. On other words, the sequence of cobalt treatments were recorded as (12.0 mg Co L^{-1}) > (8.0 mg CoL^{-1}) > (0.0 mg CoL^{-1}).

Regarding the interaction influence, data of Table 2 showed that the combination of *Rhizobium* inoculant (applied or not), N starter rates and cobalt concentrations was significant. In this regard, faba bean plants inoculated with *Rhizobium* and fertilized with ammonium sulphate at rate of 20 kg N fed^{-1} and treated with cobalt at rate of 12.0 mg L^{-1} recorded the highest values of nodulation traits and nitrogenase activity, while the lowest values were realized when faba bean plants were fertilized with 10 kg N fed^{-1} without inoculation and cobalt supplementation. On the other hand, faba bean plants inoculated prior to sowing with *Rhizobium* under N fertilization at rate of 15 kg N fed^{-1} with cobalt treatment at rate of 8.0 and 12.0 mg cobalt L^{-1} gave nodulation performance and nitrogenase activity better than that fertilized with ammonium sulphate at rate of 20 kg N fed^{-1} without both *Rhizobium* inoculation and cobalt supplementation.

Fig 1 confirmed that increasing nitrogenase activity as a result of using cobalt sulphate solution at different rates caused marked increases in No. of nodules plant^{-1}. Generally, it can be stated that cobalt treatments improved nodulation, nitrogenase activity and stimulated nodulation parameters *i.e*. No. of nodules plant^{-1}, fresh and dry weights of nodules.

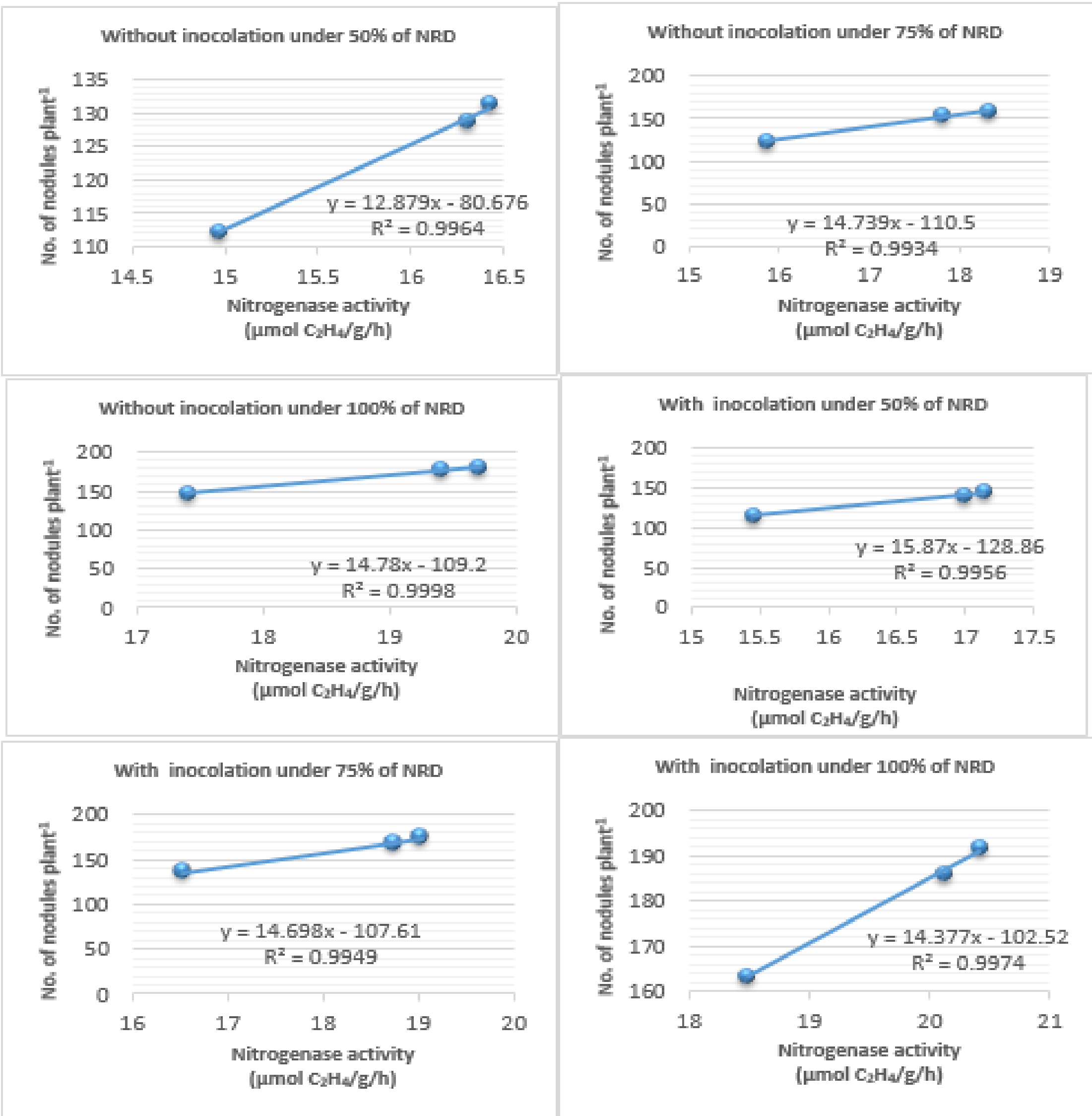

Fig1. Correlation between Nitrogenase activity (µmol C_2H_4/g/h) and No. of nodules plant^{-1} (as an average for both seasons).

Table 2. Effect of *Rhizobium* inoculant, nitrogen starter, cobalt element and their interactions on nodulation parameters of faba bean plant at 65 days from sowing during seasons of 2019/2020 and 2020/2021.

Characters / Treatments			No. of nodules plant^{-1}		Fresh weight of nodules (g plant^{-1})		Dry weight of nodules (g plant^{-1})		Nitrogenase activity (µmol C_2H_4/g/h)	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Inoculation										
Without inoculation			145.11^{b}	147.11^{b}	11.45^{b}	11.70^{b}	3.53^{b}	3.60^{b}	17.15^{b}	17.57^{b}
With inoculation			156.56^{a}	158.41^{a}	12.16^{a}	12.42^{a}	3.80^{a}	3.88^{a}	17.94^{a}	18.28^{a}
LSD at 5%			2.35	1.79	0.06	0.07	0.03	0.05	0.02	0.07
Nitrogen fertilization										
10 kg N fed^{-1}			127.72^{c}	129.72^{c}	10.43^{c}	10.65^{c}	3.13^{c}	3.19^{c}	16.03^{c}	16.39^{c}
15 kg N fed^{-1}			149.87^{b}	151.33^{b}	11.74^{b}	11.99^{b}	3.63^{b}	3.70^{b}	17.43^{b}	17.78^{b}
20 kg N fed^{-1}			173.67^{a}	175.94^{a}	13.17^{a}	13.46^{a}	4.20^{a}	4.29^{a}	19.05^{a}	19.46^{a}
LSD at 5%			1.56	2.30	0.03	0.01	0.04	0.04	0.31	0.02
Cobalt levels										
0.0 mg Co L^{-1}			132.22^{c}	134.06^{c}	10.72^{c}	10.96^{c}	3.23^{c}	3.29^{c}	16.28^{c}	16.63^{c}
8.0 mg Co L^{-1}			158.28^{b}	160.56^{b}	12.26^{b}	12.53^{b}	3.85^{b}	3.93^{b}	18.10^{b}	18.54^{b}
12.0 mg Co L^{-1}			162.00^{a}	163.67^{a}	12.44^{a}	12.69^{a}	3.92^{a}	4.00^{a}	18.25^{a}	18.60^{a}
LSD at 5%			1.67	2.20	0.03	0.04	0.03	0.04	0.11	0.05
Interaction										
Without inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	111.67^{l}	112.67^{m}	9.50^{r}	9.70^{r}	2.76^{o}	2.82^{o}	14.76^{o}	15.18^{o}
		8.0 mg Co L^{-1}	127.33^{j}	130.00^{k}	10.30^{o}	10.54^{o}	3.10^{m}	3.16^{m}	16.07^{l}	16.54^{l}
		12.0 mg Co L^{-1}	130.33^{ij}	132.33^{jk}	10.53^{n}	10.78^{n}	3.18^{m}	3.26^{l}	16.22^{kl}	16.61^{kl}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	122.33^{k}	123.67^{l}	10.04^{p}	10.23^{p}	2.95^{n}	3.00^{n}	15.70^{m}	16.03^{m}
		8.0 mg Co L^{-1}	152.67^{f}	155.33^{f}	11.91^{i}	12.16^{l}	3.72^{h}	3.79^{h}	17.65^{h}	18.01^{h}
		12.0 mg Co L^{-1}	157.33^{e}	159.00^{f}	12.18^{h}	12.47^{h}	3.82^{g}	3.90^{g}	18.06^{g}	18.58^{g}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	147.33^{g}	148.67^{g}	11.68^{j}	11.97^{j}	3.60^{i}	3.68^{i}	17.20^{i}	17.60^{i}
		8.0 mg Co L^{-1}	176.00^{c}	178.67^{c}	13.34^{d}	13.60^{d}	4.25^{d}	4.36^{cd}	19.17^{d}	19.64^{d}
		12.0 mg Co L^{-1}	181.00^{b}	183.67^{bc}	13.58^{c}	13.87^{c}	4.36^{c}	4.44^{c}	19.47^{c}	19.95^{c}
With inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	115.67^{l}	117.33^{m}	9.79^{q}	10.04^{q}	2.83^{o}	2.87^{o}	15.29^{n}	15.62^{n}
		8.0 mg Co L^{-1}	138.67^{h}	140.67^{hi}	11.07^{l}	11.29^{l}	3.41^{k}	3.46^{k}	16.82^{j}	17.15^{ij}
		12.0 mg Co L^{-1}	142.67^{h}	145.33^{gh}	11.39^{k}	11.57^{k}	3.50^{j}	3.56^{j}	17.02^{ij}	17.25^{j}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	134.00^{i}	137.33^{ij}	10.82^{m}	11.04^{m}	3.28^{l}	3.32^{l}	16.40^{k}	16.68^{k}
		8.0 mg Co L^{-1}	165.67^{d}	167.67^{de}	12.82^{f}	13.08^{f}	4.04^{e}	4.12^{e}	18.56^{f}	18.97^{f}
		12.0 mg Co L^{-1}	174.67^{c}	172.67^{d}	13.11^{e}	13.42^{e}	4.17^{d}	4.27^{d}	18.85^{e}	19.24^{e}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	162.33^{d}	164.67^{e}	12.48^{g}	12.78^{g}	3.94^{f}	4.03^{f}	18.31^{fg}	18.68^{g}
		8.0 mg Co L^{-1}	184.67^{b}	187.33^{ab}	13.83^{b}	14.20^{b}	4.48^{b}	4.56^{b}	19.90^{b}	20.35^{b}
		12.0 mg Co L^{-1}	190.67^{a}	192.67^{a}	14.12^{a}	14.33^{a}	4.58^{a}	4.68^{a}	20.27^{a}	20.56^{a}
LSD at 5%			4.10	5.38	0.08	0.10	0.08	0.09	0.27	0.13

2.Performance at different Periods from Sowing.

Data in Tables 3, 4, 5 and 6 show the effect of inoculation ,N fertilization, cobalt supplementation and their interactions on vegetative growth criteria and chemical constituents in leaves at flowering stage (65 days after sowing) as well as pods yield measurements at harvesting stage (90 days after sowing). In addition, No. of flowers plant^{-1} and pod setting (%) of faba bean plants were recorded.

Using biofertilizer with faba bean seeds before sowing significantly affected all the aforementioned traits, where the highest values were realized with *Rhizobium* inoculant, while the corresponding non-inoculated plants recorded the lowest values.

Regarding nitrogen fertilization, the soil addition of ammonium sulphate at rate of 20 kg N fed^{-1} was a superior treatment followed by 15 and 10 kg N fed^{-1}, respectively for all above-mentioned traits.

Concerning the effect of cobalt supplementation, data of the same Tables reveal that the values of all aforementioned traits significantly increased as the concentration of Co increased, where the superior treatment was with using cobalt at concentration of 12.0 mg L^{-1} followed by 8.0 mg L^{-1} and finally the control treatment (0with Co treatment).

Going along with combination treatments, data in Table (4) demonstrated that combination of *Rhizobium* inoculant at rate of 10 g inoculant per 1.0 kg seeds + nitrogen at rate of 100% of NRD as starter (20 kg N fed^{-1}) + cobalt at concentration of 12.0 mg L^{-1} resulted in significant increase in all aforementioned traits of faba bean plants over all other combined treatments , while the lowest values were realized when faba bean plants were fertilized with 50% of NRD as starter (10 kg N fed^{-1}) without inoculation and cobalt treatments. It is worthy to mention that the performance of faba bean plants inoculated prior to sowing with the strain of *Rhizobium* under N fertilization at rate of 75% of NRD as starter (15 kg N fed^{-1}) with cobalt treatment at rate of 8.0 and 12.0 mg cobalt L^{-1} was better than that fertilized with nitrogen at rate of 100% of NRD as starter (20 kg N fed^{-1}) without either *Rhizobium* inoculation or cobalt supplementation.

Table 3. Effect of *Rhizobium* inoculant, nitrogen starter, cobalt element and their interactions on vegetative growth criteria of faba bean plant at 65 days from sowing during seasons of 2019/2020 and 2020/2021.

Characters Treatments			Plant height (cm)		Leaf area index (LAI)		Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})	
			1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Inoculation										
Without inoculation			108.89^{b}	113.02^{b}	4.48^{b}	5.16^{b}	107.97^{b}	110.66^{b}	16.47^{b}	16.81^{b}
With inoculation			112.77^{a}	116.86^{a}	4.64^{a}	5.30^{a}	113.20^{a}	115.65^{a}	17.75^{a}	18.23^{a}
LSD at 5%			0.11	0.10	0.02	0.01	0.42	0.11	0.55	0.19
Nitrogen fertilization										
10 kg N fed^{-1}			103.33^{c}	107.01^{c}	4.27^{c}	4.87^{c}	100.38^{c}	102.48^{c}	14.42^{c}	14.79^{c}
15 kg N fed^{-1}			110.43^{b}	114.66^{b}	4.55^{b}	5.24^{b}	109.93^{b}	112.22^{b}	16.83^{b}	17.35^{b}
20 kg N fed^{-1}			118.29^{a}	122.66^{a}	4.84^{a}	5.56^{a}	120.82^{a}	124.16^{a}	19.90^{a}	20.25^{a}
LSD at 5%			0.22	0.21	0.04	0.05	1.87	0.21	0.33	0.09
Cobalt levels										
0.0 mg Co L^{-1}			104.73^{c}	108.71^{c}	4.31^{c}	4.94^{b}	102.38^{c}	105.04^{c}	14.97^{c}	15.25^{c}
8.0 mg Co L^{-1}			113.33^{b}	117.73^{b}	4.67^{b}	5.36^{b}	113.98^{b}	116.32^{b}	18.08^{b}	18.48^{b}
12.0 mg Co L^{-1}			114.45^{a}	118.39^{a}	4.71^{a}	5.41^{a}	115.40	118.09^{a}	18.30^{a}	18.82^{a}
LSD at 5%			0.34	0.36	0.04	0.05	0.86	0.35	0.29	0.19
Interaction										
Without inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	97.64^{r}	100.78^{p}	3.96^{l}	4.55^{i}	92.99^{n}	95.30^{n}	12.36^{o}	12.60^{n}
		8.0 mg Co L^{-1}	102.59^{o}	107.01^{m}	4.28^{j}	4.88^{gh}	99.35^{l}	101.87^{k}	14.19^{lm}	14.63^{k}
		12.0 mg Co L^{-1}	104.26^{n}	108.02^{l}	4.35^{ij}	4.96^{fg}	101.48^{kl}	104.82^{j}	14.73^{kl}	15.07^{jk}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	101.03^{p}	104.91^{n}	4.18^{k}	4.81^{h}	97.18^{m}	99.02^{l}	13.64^{mn}	13.96^{l}
		8.0 mg Co L^{-1}	111.69^{i}	114.96^{h}	4.60^{fg}	5.33^{cd}	111.71^{gh}	113.93^{h}	17.51^{fg}	17.70^{g}
		12.0 mg Co L^{-1}	113.14^{h}	117.66^{g}	4.65^{f}	5.36^{cd}	113.74^{fg}	115.84^{g}	18.01^{ef}	18.35^{f}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	110.19^{j}	115.17^{h}	4.55^{g}	5.28^{d}	109.76^{hi}	113.37^{h}	16.93^{gh}	17.36^{g}
		8.0 mg Co L^{-1}	118.96^{d}	123.54^{d}	4.86^{cd}	5.65^{ab}	121.66^{c}	124.46^{d}	20.16^{c}	20.47^{d}
		12.0 mg Co L^{-1}	120.55^{c}	125.14^{c}	4.92^{bc}	5.66^{ab}	123.84^{b}	127.31^{c}	20.74^{bc}	21.12^{c}
With inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	99.39^{q}	103.02^{o}	4.09^{k}	4.62^{i}	95.10^{mn}	96.43^{m}	13.01^{no}	13.36^{m}
		8.0 mg Co L^{-1}	107.20^{l}	110.98^{j}	4.44^{hi}	5.11^{e}	105.56^{j}	108.00^{i}	15.82^{ij}	16.27^{i}
		12.0 mg Co L^{-1}	108.90^{k}	112.23^{i}	4.51^{gh}	5.13^{e}	107.79^{i}	108.45^{i}	16.41^{hi}	16.80^{h}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	105.63^{m}	109.89^{k}	4.39^{i}	5.05^{ef}	103.49^{jk}	107.58^{i}	15.30^{jk}	15.49^{j}
		8.0 mg Co L^{-1}	116.10^{f}	120.95^{f}	4.78^{de}	5.43^{c}	117.61^{de}	118.95^{f}	19.06^{d}	19.53^{e}
		12.0 mg Co L^{-1}	117.72^{e}	122.58^{e}	4.82^{cd}	5.60^{b}	119.64^{cd}	121.64^{e}	18.62^{de}	20.05^{d}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	114.49^{g}	118.51^{g}	4.70^{ef}	5.32^{cd}	115.73^{ef}	118.57^{i}	18.56^{de}	18.77^{f}
		8.0 mg Co L^{-1}	121.97^{b}	126.22^{b}	4.99^{ab}	5.73^{a}	125.95^{ab}	128.83^{b}	21.21^{ab}	21.63^{b}
		12.0 mg Co L^{-1}	123.58^{a}	127.39^{a}	5.05^{a}	5.75^{a}	127.95^{a}	132.41^{a}	21.80^{a}	22.16^{a}
LSD at 5%			0.84	0.89	0.10	0.13	2.13	0.88	0.73	0.47

Table 4. Effect of *Rhizobium* inoculant, nitrogen starter, cobalt element and their interactions on chemical constituents in leaves of faba bean plant at 65 days from sowing during seasons of 2019/2020 and 2020/2021.

Characters Treatments			Total chlorophyll (mg g^{-1} F.W)		N (%)		Co (mg kg^{-1})	
			1st season	2nd season	1st season	2nd season	1st season	2nd season
Inoculation								
Without inoculation			1.508^{b}	1.542^{b}	4.23^{b}	4.34^{b}	2.64^{b}	2.70^{b}
With inoculation			1.611^{a}	1.646^{a}	4.40^{a}	4.49^{a}	2.98^{a}	3.05^{a}
LSD at 5%			0.001	0.019	0.16	0.02	0.11	0.02
Nitrogen fertilization								
10 kg N fed^{-1}			1.352^{c}	1.383^{c}	3.99^{c}	4.09^{c}	2.13^{c}	2.18^{c}
15 kg N fed^{-1}			1.550^{b}	1.584^{b}	4.30^{b}	4.38^{b}	2.73^{b}	2.76^{b}
20 kg N fed^{-1}			1.764^{a}	1.805^{a}	4.62^{a}	4.74^{a}	3.49^{a}	3.57^{a}
LSD at 5%			0.016	0.019	0.06	0.03	0.02	0.02
Cobalt levels								
0.0 mg Co L^{-1}			1.393^{c}	1.423^{c}	4.08^{c}	4.16^{c}	1.43^{c}	1.46^{c}
8.0 mg Co L^{-1}			1.628^{b}	1.663^{b}	4.40^{b}	4.52^{b}	3.42^{b}	3.53^{b}
12.0 mg Co L^{-1}			1.657^{a}	1.696^{a}	4.46^{a}	4.56^{a}	3.59^{a}	3.64^{a}
LSD at 5%			0.017	0.015	0.05	0.03	0.05	0.03
Interaction								
Without inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	1.215^{n}	1.244^{l}	3.81^{n}	3.88^{k}	0.85^{q}	0.89^{r}
		8.0 mg Co L^{-1}	1.337^{kl}	1.364^{j}	3.84^{mn}	4.07^{ij}	2.46^{k}	2.52^{l}
		12.0 mg Co L^{-1}	1.372^{jk}	1.401^{j}	4.03^{kl}	4.14^{hi}	2.62^{j}	2.67^{k}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	1.295^{lm}	1.322^{k}	3.93^{lm}	3.98^{jk}	1.33^{o}	1.36^{p}
		8.0 mg Co L^{-1}	1.580^{f}	1.625^{g}	4.37^{gh}	4.43^{f}	3.30^{g}	3.20^{h}
		12.0 mg Co L^{-1}	1.616^{ef}	1.649^{g}	4.43^{fg}	4.51^{ef}	3.22^{g}	3.48^{g}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	1.532^{g}	1.567^{h}	4.28^{hi}	4.45^{f}	1.75^{m}	1.80^{n}
		8.0 mg Co L^{-1}	1.791^{bc}	1.834^{c}	4.62^{cd}	4.77^{bc}	4.02^{d}	4.13^{d}
		12.0 mg Co L^{-1}	1.831^{b}	1.877^{b}	4.71^{bc}	4.80^{bc}	4.20^{c}	4.30^{c}
With inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	1.254^{mn}	1.277^{l}	3.85^{mn}	3.91^{k}	1.10^{p}	1.12^{q}
		8.0 mg Co L^{-1}	1.447^{i}	1.475^{i}	4.18^{ij}	4.25^{gh}	2.77^{i}	2.84^{j}
		12.0 mg Co L^{-1}	1.490^{h}	1.534^{h}	4.23^{i}	4.31^{g}	2.97^{h}	3.04^{i}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	1.406^{ij}	1.439^{i}	4.10^{jk}	4.17^{ih}	1.57^{n}	1.61^{o}
		8.0 mg Co L^{-1}	1.704^{d}	1.746^{e}	4.53^{def}	4.61^{de}	3.62^{f}	3.72^{f}
		12.0 mg Co L^{-1}	1.765^{c}	1.787^{d}	4.58^{de}	4.70^{cd}	3.82^{e}	3.91^{e}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	1.654^{e}	1.690^{f}	4.48^{efg}	4.58^{e}	1.94^{l}	1.98^{m}
		8.0 mg Co L^{-1}	1.874^{a}	1.911^{b}	4.78^{ab}	4.88^{ab}	4.42^{b}	4.50^{b}
		12.0 mg Co L^{-1}	1.903^{a}	1.951^{a}	4.85^{a}	4.96^{a}	4.62^{a}	4.70^{a}
LSD at 5%			0.042	0.037	0.12	0.12	0.12	0.08

Table 5. Effect of *Rhizobium* inoculant, nitrogen starter, cobalt element and their interactions on pods yield measurements of faba bean plant at 90 days from sowing as well as flowering and pods setting during seasons of 2019/2020 and 2020/2021.

Characters / Treatments			Pods yield measurements: Pod weight (g) 1st season	Pod weight (g) 2nd season	No. of pods plant^{-1} 1st season	No. of pods plant^{-1} 2nd season	Green Yield (g plant^{-1}) 1st season	Green Yield (g plant^{-1}) 2nd season	Flowering and pods setting: No of flower plant^{-1} 1st season	No of flower plant^{-1} 2nd season	Pods setting (%) 1st season	Pods setting (%) 2nd season
Inoculation												
Without inoculation			18.94^{b}	19.31^{b}	15.44^{b}	16.85^{b}	304.10^{b}	338.31	21.33^{b}	22.89^{b}	71.02^{b}	72.05^{b}
With inoculation			20.20^{a}	20.59^{a}	17.89^{a}	19.48^{a}	371.84^{a}	413.64	24.04^{a}	25.67^{a}	73.31^{a}	74.97^{a}
LSD at 5%			0.08	0.23	1.26	1.66	21.97	33.54	2.34	2.71	2.49	1.22
Nitrogen fertilization												
10 kg N fed-1			17.21^{c}	17.63^{c}	11.89^{c}	13.00^{c}	207.58^{c}	232.31^{c}	17.72^{c}	19.11^{c}	66.46^{c}	67.76^{c}
15 kg N fed-1			19.42^{b}	19.78^{b}	16.40^{b}	17.73^{b}	325.11^{b}	359.94^{b}	22.47^{b}	23.93^{b}	72.29^{b}	73.06^{b}
20 kg N fed-1			21.92^{a}	22.30^{a}	21.44^{a}	23.61^{a}	474.49^{a}	531.12^{a}	27.56^{a}	29.67^{a}	77.49^{a}	79.27^{a}
LSD at 5%			0.06	0.32	1.15	2.71	22.61	50.99	1.36	4.50	2.54	2.13
Cobalt levels												
0.0 mg Co L-1			17.67^{c}	18.00^{c}	13.11^{b}	13.94^{b}	238.70^{c}	258.08^{b}	18.94^{b}	19.94^{b}	68.01^{b}	69.18^{b}
8.0 mg Co L-1			20.37^{b}	20.81^{b}	18.22^{a}	20.06^{a}	379.80^{b}	428.13^{a}	24.39^{a}	25.94^{a}	73.91^{a}	76.06^{a}
12.0 mg Co L-1			20.67^{a}	21.05^{a}	18.67^{a}	20.50^{a}	395.41^{a}	441.71^{a}	24.72^{a}	26.94^{a}	74.58^{a}	75.28^{a}
LSD at 5%			0.07	0.15	0.80	1.55	15.18	33.35	1.04	1.56	1.72	2.14
Interaction												
Without inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	15.43^{r}	15.72^{m}	9.00^{j}	10.33^{i}	138.91^{m}	162.85^{j}	14.33^{i}	16.33^{g}	62.77^{j}	63.01^{j}
		8.0 mg Co L^{-1}	16.94^{o}	17.42^{j}	11.00^{hi}	12.33^{hi}	186.16^{kl}	214.40^{hij}	16.67^{hi}	18.67^{fg}	66.31^{hij}	66.18^{hij}
		12.0 mg Co L^{-1}	17.42^{n}	17.83^{i}	11.67^{h}	12.67^{hi}	203.10^{k}	225.47^{hij}	17.67^{h}	18.67^{fg}	66.11^{hij}	67.76^{hij}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	16.45^{p}	16.87^{k}	10.67^{hij}	11.33^{hi}	175.55^{klm}	191.75^{ij}	16.33^{hi}	17.33^{fg}	65.32^{ij}	65.53^{ij}
		8.0 mg Co L^{-1}	19.81^{i}	20.00^{g}	17.33^{ef}	17.67^{ef}	343.19^{gh}	352.76^{efg}	23.33^{efg}	24.00^{de}	74.20^{cde}	73.50^{c-g}
		12.0 mg Co L^{-1}	20.32^{h}	20.71^{f}	18.00^{de}	18.67^{de}	365.51^{fg}	387.22^{ef}	24.33^{def}	24.67^{d}	73.81^{c-f}	75.62^{b-f}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	19.35^{j}	19.79^{g}	16.00^{fg}	17.33^{efg}	309.71^{hi}	343.45^{fg}	22.00^{fg}	23.67^{de}	72.69^{d-g}	73.14^{d-g}
		8.0 mg Co L^{-1}	22.14^{d}	22.44^{d}	22.00^{b}	26.33^{a}	487.16^{c}	591.75^{ab}	28.00^{bc}	31.67^{ab}	78.50^{ab}	82.97^{a}
		12.0 mg Co L^{-1}	22.61^{c}	23.03^{c}	23.33^{ab}	25.00^{ab}	527.62^{b}	575.11^{ab}	29.33^{ab}	31.00^{ab}	79.45^{a}	80.71^{ab}
With inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	16.02^{q}	16.42^{l}	9.33^{ij}	10.67^{i}	149.31^{lm}	175.59^{ij}	14.33^{i}	16.00^{g}	63.68^{j}	67.68^{hij}
		8.0 mg Co L^{-1}	18.52^{l}	19.01^{h}	15.33^{g}	14.67^{fgh}	283.86^{ij}	279.48^{gh}	22.00^{fg}	20.67^{ef}	69.67^{fgh}	70.73^{f-i}
		12.0 mg Co L^{-1}	18.93^{k}	19.36^{h}	15.00^{g}	17.33^{efg}	284.13^{ij}	336.04^{fg}	21.33^{g}	24.33^{de}	70.23^{e-h}	71.23^{e-h}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	17.93^{m}	18.15^{i}	14.67^{g}	13.67^{ghi}	262.89^{j}	247.66^{hi}	21.33^{g}	19.67^{fg}	68.64^{ghi}	69.44^{ghi}
		8.0 mg Co L^{-1}	21.25^{f}	21.75^{e}	19.33^{cd}	22.33^{bcd}	410.71^{de}	485.28^{cd}	25.33^{de}	29.00^{bc}	76.40^{a-d}	77.08^{bcd}
		12.0 mg Co L^{-1}	21.66^{e}	22.11^{de}	20.00^{c}	23.67^{abc}	433.20^{d}	522.27^{bc}	26.00^{cd}	29.67^{bc}	76.90^{abc}	79.72^{ab}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	20.83^{g}	21.05^{f}	19.00^{cde}	20.33^{cde}	395.82^{ef}	427.19^{de}	25.33^{de}	26.67^{cd}	74.97^{bcd}	76.29^{b-e}
		8.0 mg Co L^{-1}	23.05^{b}	23.51^{b}	23.67^{ab}	26.00^{ab}	545.41^{ab}	610.63^{a}	30.00^{ab}	31.00^{ab}	78.75^{ab}	83.77^{a}
		12.0 mg Co L^{-1}	23.56^{a}	23.97^{a}	24.67^{a}	26.67^{a}	581.22^{a}	638.59^{a}	30.67^{a}	34.00^{a}	80.59^{a}	78.75^{abc}
			0.17	0.38	1.96	3.81	37.18	81.68	2.53	3.83	4.21	5.26

Table 6. Effect of *Rhizobium* inoculant, nitrogen starter, cobalt element and their interactions on nitrogen and cobalt contents as well as bioconstituents faba bean seeds at 90 days from sowing during seasons of 2019/2020 and 2020/2021.

Characters / Treatments			Chemical constituents: N (%) 1st season	N (%) 2nd season	Co (mg kg^{-1}) 1st season	Co (mg kg^{-1}) 2nd season	bioconstituents: Protein (%) 1st season	Protein (%) 2nd season	T. carbohydrates (%) 1st season	T. carbohydrates (%) 2nd season
Inoculation										
Without inoculation			2.84^{b}	2.90^{b}	1.30^{b}	1.33^{b}	17.74^{b}	18.13^{b}	53.70^{b}	54.39^{a}
With inoculation			3.00^{a}	3.06^{a}	1.42^{a}	1.46^{a}	18.76^{a}	19.14^{a}	54.12^{a}	54.92^{a}
LSD $_{at\ 5\%}$			0.02	0.06	0.01	0.01	0.16	0.37	0.24	n.s
Nitrogen fertilization										
10 kg N fed^{-1}			2.60^{c}	2.66^{c}	1.14^{c}	1.16^{c}	16.25^{c}	16.60^{c}	53.07^{c}	53.48^{b}
15 kg N fed^{-1}			2.91^{b}	2.96^{b}	1.29^{b}	1.33^{b}	18.17^{b}	18.53^{b}	53.84^{b}	54.47^{c}
20 kg N fed^{-1}			3.23^{a}	3.30^{a}	1.60^{a}	1.63^{a}	20.21^{a}	20.64^{a}	54.77^{a}	55.94^{a}
LSD $_{at\ 5\%}$			0.03	0.03	0.02	0.01	0.20	0.19	0.14	0.40
Cobalt levels										
0.0 mg Co L^{-1}			2.66^{c}	2.72^{c}	0.68^{c}	0.69^{c}	16.64^{c}	17.01^{c}	53.23^{c}	53.72^{b}
8.0 mg Co L^{-1}			3.03^{b}	3.10^{b}	1.69^{b}	1.72^{b}	18.94^{b}	19.35^{b}	54.18^{b}	55.00^{a}
12.0 mg Co L^{-1}			3.07^{a}	3.13^{a}	1.72^{a}	1.76^{a}	19.17^{a}	19.53^{a}	54.32^{a}	55.24^{a}
LSD $_{at\ 5\%}$			0.03	0.02	0.01	0.01	0.17	0.14	0.16	0.31
Interaction										
Without inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	2.35^{m}	2.40^{n}	0.53^{q}	0.54^{r}	14.69^{m}	14.98^{n}	52.39^{o}	52.76^{i}
		8.0 mg Co L^{-1}	2.59^{j}	2.65^{k}	1.33^{k}	1.36^{l}	16.17^{j}	16.56^{k}	53.02^{lm}	53.27^{hi}
		12.0 mg Co L^{-1}	2.64^{j}	2.70^{k}	1.38^{j}	1.41^{k}	16.48^{j}	16.85^{k}	53.17^{klm}	53.38^{ghi}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	2.51^{k}	2.56^{l}	0.64^{o}	0.66^{p}	15.67^{k}	16.00^{l}	52.87^{mn}	53.21^{hi}
		8.0 mg Co L^{-1}	2.95^{g}	3.00^{g}	1.59^{g}	1.62^{h}	18.44^{g}	18.77^{g}	53.98^{fgh}	54.82^{def}
		12.0 mg Co L^{-1}	3.02^{f}	3.09^{f}	1.66^{f}	1.69^{g}	18.88^{f}	19.29^{f}	54.14^{efg}	54.93^{de}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	2.90^{gh}	2.97^{g}	0.77^{m}	0.78^{n}	18.10^{gh}	18.56^{g}	53.87^{ghi}	54.60^{ef}
		8.0 mg Co L^{-1}	3.27^{cd}	3.34^{c}	1.89^{c}	1.93^{d}	20.42^{cd}	20.85^{c}	54.84^{bc}	56.18^{bc}
		12.0 mg Co L^{-1}	3.33^{bc}	3.40^{b}	1.95^{b}	1.98^{c}	20.81^{bc}	21.27^{b}	55.05^{ab}	56.31^{b}
With inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	2.42^{l}	2.48^{m}	0.59^{p}	0.61^{q}	15.13^{l}	15.48^{m}	52.63^{no}	53.23^{hi}
		8.0 mg Co L^{-1}	2.77^{i}	2.83^{i}	1.45^{i}	1.48^{j}	17.29^{i}	17.67^{i}	53.50^{ijk}	54.09^{fg}
		12.0 mg Co L^{-1}	2.84^{h}	2.89^{h}	1.53^{h}	1.57^{i}	17.75^{h}	18.06^{h}	53.70^{hij}	54.13^{fg}
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	2.72^{i}	2.76^{j}	0.71^{n}	0.72^{o}	17.00^{i}	17.27^{j}	53.32^{jkl}	53.72^{gh}
		8.0 mg Co L^{-1}	3.16^{e}	3.23^{d}	1.74^{e}	1.78f	19.73^{e}	20.17^{d}	54.42^{de}	55.12^{de}
		12.0 mg Co L^{-1}	3.20^{de}	3.27^{d}	1.80^{d}	1.84e	20.00^{de}	20.42^{d}	54.62^{cd}	55.47^{cd}
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	3.08^{f}	3.16^{e}	0.82^{l}	0.84^{m}	19.23^{f}	19.75^{e}	54.32^{def}	54.83^{def}
		8.0 mg Co L^{-1}	3.38^{ab}	3.45^{ab}	2.08^{a}	2.10^{b}	21.15^{ab}	21.58^{ab}	55.18^{ab}	56.37^{b}
		12.0 mg Co L^{-1}	3.45^{a}	3.49^{a}	2.10^{a}	2.15^{a}	21.54^{a}	21.83^{a}	55.38^{a}	57.32^{a}
LSD $_{at\ 5\%}$			0.07	0.06	0.02	0.01	0.43	0.35	0.38	0.76

3. Available Nutrients in Soil at Period of 90 Days.

Post-harvest soil analysis indicat that available N, P and k (mg kg^{-1}) in soil at period of 90 days pronouncedly differ as a result of treatments studied (Table 7), where the values displayed were the mean of both seasons. Generally, the available nutrients determined in soil increased over that before sowing; as shown in Table 1 (materials section).

Table 7. Average available N, P and k in soil (mg kg^{-1}) as affected by Rhizobium inoculant, nitrogen starter, and cobalt at period of 90 days from sowing the faba bean (combined data over both seasons).

Characters Treatments			Available -N	Available -P	Available -K
			(mg kg^{-1})		
Without inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	43.17	14.40	374.27
		8.0 mg Co L^{-1}	45.63	14.10	365.57
		12.0 mg Co L^{-1}	48.09	13.77	356.67
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	57.84	12.57	321.96
		8.0 mg Co L^{-1}	60.13	12.25	312.91
		12.0 mg Co L^{-1}	65.56	11.91	304.25
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	71.65	10.68	269.54
		8.0 mg Co L^{-1}	73.96	10.39	261.52
		12.0 mg Co L^{-1}	76.16	10.17	253.42
With inoculation	10 kg N fed^{-1}	0.0 mg Co L^{-1}	50.39	13.41	347.62
		8.0 mg Co L^{-1}	52.88	13.13	338.97
		12.0 mg Co L^{-1}	55.34	12.87	330.45
	15 kg N fed^{-1}	0.0 mg Co L^{-1}	67.89	11.64	295.23
		8.0 mg Co L^{-1}	67.14	11.35	286.80
		12.0 mg Co L^{-1}	69.54	11.03	277.76
	20 kg N fed^{-1}	0.0 mg Co L^{-1}	78.41	9.870	244.38
		8.0 mg Co L^{-1}	80.86	9.650	235.28
		12.0 mg Co L^{-1}	83.16	9.450	226.36

Available N gradually increased with increasing cobalt concentration and the rate of nitrogen application rate. On the contrary, available P and K gradually decreased with increasing cobalt concentration and the rate of nitrogen applied. Also, the *Rhizobium* inoculates caused declining available P and K compared to untreated plants with *Rhizobium.*

Discussion

This study intended to reduce the starter synthetic chemical nitrogen and improves the performance of faba bean plants grown under salinity stress by treating with *Rhizobium* and cobalt element.

The pronounced promotional effect of *Rhizobium* inoculant may be attributed to its ability to fix free nitrogen from the air. Faba bean belongs to legumes, which contain nodules on their roots when infected with *Rhizobium* bacteria. So, the inoculation with *Rhizobium* at rate of 10 g inoculant per 1.0 kg seeds led to increasing No. of nodules $plant^{-1}$, thus increasing symbiotic nitrogen fixation in nodules was found on faba bean roots which reflected positively on the performance of faba bean plant grown under salinity stress. Generally, the findings confirmed that treating faba bean with *Rhizobium* inoculant before sowing is beneficial. This result is in accordance with those of Argaw, (2012) and Argaw and Mnalku, (2017). Beside, Youseif *et al*., (2017) reported that faba bean treated with *Rhizobium* inoculant before sowing possessed growth performance better than untreated plants. Also Gaballah and Gomaa (2005) indicated that *Rhizobium* inoculant was beneficial for increasing the productivity of Faba bean, but it did not show protection against oxidative hazard due to salinity. On the contrary, Cordovilla *et al.,* (1996) stated that *Rhizobium leguminosarum* was tolerant of low levels of salt (50 mM) compared to growth in the absence of salinity stress.

All studied traits of faba bean increased as N-fertilizer rate increased with range of zero to 20 kg N fed^{-1} as a starter. This is due to ammonium sulfate is an inorganic salt that contains 21% nitrogen and 24% sulfur. Nitrogen (N) is so vital because it is a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide (*i.e.,* photosynthesis). It is also a major component of amino acids and the building blocks of proteins. Sulfur (s) is of great significance for the structure of proteins and functioning of enzymes and it plays an important role in the defence of plants against stresses and pests. So, healthy and big plant roots were produced to be infected by specified *Rhizobium* and make actual nodules. These findings are in harmony with those of Fageria and Baligar (2005), Heidari *et al.,* (2011) and Abdel-Wahab and Manzlawy (2016).

It is known that cobalt is important for N fixation by the bacteria which associate with legumes such as faba bean. The pronounced promotional effect of cobalt may be attributed to its possible role in the leghaemoglobin synthesis that is essential for active N fixing sites and its role as a cofactor of cobalamine (Vitamin B_6) that functions as a coenzyme involved in nitrogen fixation and nodule growth, thus the seed soaking before sowing in cobalt solution played an important role in N fixation process (Wahab *et al.,* 1996 and Jordan and Reichard, 1998). Cobalt considers as an essential element in the plant-bacteria root nodulation of faba bean due to it possesses a synergistic Impact on nitrogenase enzyme activity. Also, the presence of cobalt in the zone of high salinity roots spread works to reduce the harmful effect of salinity and thus increase the ability of plants to tolerate salinity. The foliar spraying of cobalt gave more vigorous plant growth as a visual score and best performance compared with tap water due to its effective role in increasing and stimulating growth in different physiological stages as was observed in a previous study (Atiia *et al.,* 2016 and Amer *et al.*, 2018).

In respect to available nutrients in soil after 90 days from sowing, it is normal that available N increases as NRD increases. The treating with *Rhizobium* and cobalt caused an increase of available nitrogen in soil due to cobalt element positively affects vitamin B_{12} required for hemoglobin formation in the faba bean root nodules under inoculation by *Rhizobium,* thus raising N-fixation. On the contrary, *Rhizobium* inoculant and raising rate of both cobalt and nitrogen treatments led to decrease the available phosphorus and potassium in the soil after harvest of faba bean plants due to their role in improving plant status, thus the faba bean plants absorbed more P and K, where the plant uptake in presence of *Rhizobium* is more than its absence as well as treating with Co at rate of 12.0 mg L^{-1} led to absorption more than that with 8.0 mg Co $L^{-1}$and 0.0 mg Co L^{-1}, respectively, also, the plant uptake of P and k under 100% of NRD as starter (20 kg N fed^{-1}) is more than that with 75% of NRD (15 kg N fed^{-1}) and 50% of NRD (10 kg N fed^{-1}), respectively may be due to making more plant tissues which need P and K. This explains reducing the residual phosphorus and potassium in soil as a result of improvement of plant performance. On the other hand, increasing available potassium and phosphorus (mg kg^{-1} soil) values in the root zone over that before sowing may be attributed to the impact of roots secretions. These results are in agreement with the obtained results of Sherif *et al.,* (2017) who found that combined treatments of nitrogen as soil addition and cobalt as foliar application significantly increased grain and roots yields of faba bean.

CONCLUSION

The results presented in this investigation confirm that;

1. Combined treatment of *Rhizobium* and cobalt have a positive effect on nodulation traits of faba bean plants.
2. Cobalt is an essential element for legumes such as faba bean.
3. Cobalt helps plants to be more tolerant for soil salinity.
4. Combined treatment of Rhizobium and cobalt can reduce the dose of synthetic nitrogen compounds due to their role in atmospheric N fixation, therefore minimizing environmental hazards resulting from excessive synthetic chemical nitrogen fertilizers, thus protecting the environment.

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Authors' contribution:

1- **Mohamed Atef El-Sherpiny (33.3%)**, conducted the field experiments and wrote the manuscript.

2- **Ahmed Gamal Baddour (33.3%)**, designed and conducted the field experiments.

3- **Hanaa Mohamed Sakara (33.3%)**, analyzed the data.

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REFERENCES

A.O.A.C.(1995). "Method of Analysis". Association of Official Agriculture Chemists. 16th Ed., Washington, D.C.USA.

Abdel-Wahab, T. I and Manzlawy, A. M. (2016). Yield and quality of intercropped wheat with faba bean under different wheat plant densities and slow–release nitrogen fertilizer rates in sandy soil. Journal of Experimental Agriculture International, 1-22.

Amer, A., EL Azab, K., Ismail, A and El-Shazly, M. (2018). Saline soil management to improve its fertility and productivity by some agricultural practices application. Journal of Soil Sciences and Agricultural Engineering, 9(12): 743-751.

Argaw, A and Mnalku, A. (2017). Effectiveness of native Rhizobium on nodulation and yield of faba bean (*Vicia faba* L.) in Eastern Ethiopia. Archives of Agronomy and Soil Science, 63(10):1390-1403.

Argaw, A. (2012). Characterization of symbiotic effectiveness of *Rhizobia* nodulating Faba bean (*Vicia faba* L.) isolated from central Ethiopia. Research Journal of microbiology, 7(6): 280-296.

Atiia,M.A., AbdAlla,M.A and Allam S.M.M (2016). Effect of zinc and cobalt applied with different methods and rates on the yield components of *Vicia faba* L. WWJMRD ; 2(2): 52-58.

Buurman, P., Van Lagen, B and Velthorst, E.J. (1996). ″Manual for Soil and Water Analysis″. Backhuys.

Cordovilla, M. D. P., Ocaña, A., Ligero, F and Lluch, C. (1996). Growth and symbiotic performance of faba bean inoculated with *Rhizobium leguminosarum biovar. viciae* strains tolerant to salt. Soil Science and Plant Nutrition, 42(1):133-140.

CoStat version 6.303 copyright (1998-2004). CoHort Software 798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA.

Dospatliev, L., Georgieva, N. V., Pavlov, A. I and Yaneva, Z. (2010). Extraction-spectrophotometric determination of cobalt in soils by the application of iodine nitrotetrazole chloride (Int). Trakia Journal of Sciences, 8(2): 16-19.

Fageria, N.K. and Baligar, V.C. (2005). Enhancing nitrogen use efficiency in crop plants. Advances in Agronomy, 88: 97-185.

Fixen, P. E and West, F. B. (2002). Nitrogen fertilizers: meeting contemporary challenges. Ambio: a journal of the human environment, 31(2):169-176.

Gaballah, M. S and Gomaa, A. M. (2005). Interactive effect of Rhizobium inoculation, sodium benzoate and salinity on performance and oxidative stress in two faba bean varieties. Int J Agri Biol, 7, 495-498.

Gad, N., Abd El Zaher Fatma, H., Abd El Maksoud, H. K and Abd El-Moez, M. R. (2011). Response of faba bean (*Vicia faba* L.) to cobalt amendements and nitrogen fertilization. The African Journal of Plant Science and Biotechnology. Global Science Books, 41-45.

Gomez; K. A and Gomez, A.A (1984). "Statistical Procedures for Agricultural Research". John Wiley and Sons, Inc., New York.pp:680.

Gotteni, A. L., Verloo, L. G and Camerlynch,G. (1982)."Chemical Analysis of Soil Lap of Analytical and Agro Chemistry", state Univ., Ghent, Belgium.

Hardy, R. W., Holsten, R. D., Jackson, E. K and Burns, R. C. (1968). The acetylene-ethylene assay for N2 fixation: laboratory and field evaluation. Plant physiology, 43(8): 1185-1207.

Heidari, M., Galavi, M and Hassani, M. (2011). Effect of sulfur and iron fertilizers on yield, yield components and nutrient uptake in sesame (*Sesamum indicum* L.) under water stress. African Journal of Biotechnology, 10(44): 8816-8822.

Herridge, D. F., Peoples, M. B and Boddey, R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil, 311(1):1-18.

Jones, J., Wolf, B. J. B and Mills, H. A. (1991). "Plant analysis Handbook: A Practical Sampling, Preparation, Analysis, and Interpretative Guide". Micro-Macro Publishing, Athens, Ga.

Jordan, A. and Reichard P. (1998). Ribonucleotide reductases. Annu Rev Biochem, 67:71–98.

Mady, M. A. (2009). Effect of foliar application with yeast extract and zinc on fruit setting and yield of faba bean (*Vicia faba* L). J. Biol. Chem. Environ. Sci, 4(2): 109-127.

Mudu, P., Terracini, B and Martuzzi, M. (2014). Human health in areas with industrial contamination. WHO Regional Office for Europe.

Official Journal of the European Union. Regulation (Eu) 2019/1009 of the European Parliament and of the Council of 5 June 2019 Laying down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and Repealing Regulation (EC) No 2003/2003. L 170. 25 June 2019, Volume 62. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/ ?uri= OJ:L :2019: 170:FULL& from=EN (accessed on 22 January 2020).

Qados, A. M. A and Moftah, A. E. (2015). Influence of silicon and nano-silicon on germination, growth and yield of faba bean (*Vicia faba* L.) under salt stress conditions. Journal of Experimental Agriculture International, 509-524.

Sadasivam, S and Manickam, A (1996). "Biochemical Methods", 2nd Ed. New age inter. India.

Seadh, A. K. E. G. (2014). Improvement of some wheat varieties productivity under organic and minerals fertilization (Doctoral dissertation, Ph. D. Thesis, Fac. Of Agric. Mansoura Univ., Egypt).

Sherif, A., ElKhalawy, S and Hegab, E. (2017). Impact of nitrogen and cobalt rates on faba bean crop grown on clayey soil. Journal of Soil Sciences and Agricultural Engineering, Mansoura University, 8(9): 459-465.

Soil Survey Staff (2010). Keys to Soil Taxonomy, Eleventh Edition USDA.

Takuwa, D. T., Sawula, G., Wibetoe, G and Lund, W. (1997). Determination of cobalt, nickel and copper in flowers, leaves, stem and roots of plants using ultrasonic slurry sampling electrothermal atomic absorption spectrometry. Journal of Analytical Atomic Spectrometry, 12(8): 849-854.

Wahab, A. A., Abd-Alla, M. H and El-Enany, A. E. (1996). Stimulation of nodulation, nitrogen fixation and plant growth of faba bean by cobalt and copper additions. In Fertilizers and environment (pp. 127-130). Springer, Dordrecht.

Watson, D. J. (1952). The physiological basis of variation in yield. In Advances in agronomy (Vol. 4, pp. 101-145). Academic Press.

Youseif, S. H., El-Megeed, A., Fayrouz, H and Saleh, S. A. (2017). Improvement of faba bean yield using *Rhizobium/Agrobacterium* inoculant in low-fertility sandy soil. Agronomy, 7(1): 2.

تأثير لقاح الريزوبيا وجرعة النيتروجين المنشط والكوبالت على تحفيز العقد البكتيرية وتثبيت النيتروجين وأداء نبات الفول النامي تحت الاجهاد الملحي.

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في أنظمة الزراعة الحديثة، هناك اتجاه عام لتقليل كميات مدخلات النيتروجين المعدني وزيادة كفاءة استخدامها للحد من التلوث البيئي ومخاطره على صحة الإنسان العامة. ومن المعروف أن الكوبالت عنصر نادر ضروري للبقوليات والبكتيريا المرتبطة بها التي تثبت النيتروجين. لهذا السبب، تم إجراء تجربتين بحثيتين حقليتين في تصميم قطعة منشقة مرتين مع ثلاث مكررات خلال موسمي شتاء متتاليين ٢٠٢٠/٢٠١٩ و ٢٠٢١/٢٠٢٠ بهدف دراسة تأثير لقاح الريزوبيا ، معدلات مختلفة من النيتروجين كجرعة تنشيطية عند بداية الزراعة [٥٠ و ٧٥ و ١٠٠٪ وتعادل ٢٠و١٥و١٠ كجم نيتروجين /الفدان علي التوالي] وعنصر الكوبالت (نقع البذور والرش الورقي كطريقة مشتركة) عند تركيزات مختلفة [٠,٠ و ٨,٠ و ١٢,٠ ملجم كوبالت / لتر] على نشاط انزيم النيتروجيناز وأداء نبات الفول النامي بتربة طينية ملحية. أظهرت النتائج أن النباتات التي تم تلقيحها قبل الزراعة بالريزوبيا بمعدل (١٠ جرام لقاح/ ١,٠ كجم بذور) مع جرعة النيتروجين المنشطة بمعدل ١٥كجم/الفدان مع المعاملة بالكوبالت بمعدل ٨,٠ و ١٢,٠ مجم كوبالت / لتر حققت نتائج أفضل من تلك النباتات التي تم تسميدها بكبريتات الأمونيوم كجرعة منشطة بمعدل ٢٠كجم نيتروجين/الفدان في عدم وجود كل من لقاح الريزوبيا والكوبلت. بشكل عام، أدت معاملات الكوبالت إلى تحسين نشاط انزيم النيتروجيناز وحفزت مدلولات تحسن العقد ، مما أدي الي تحسين جميع معايير النمو المدروسة والمحصول الأخضر ومكوناته لنبات الفول النامي تحت الاجهاد الملحي.