Influence Of Different Cyanobacterial Application Methods On Growth And Seed Production Of Common Bean Under Various Levels Of Mineral Nitrogen Fertilization

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ABSTRACT: Nowadays, pollution is considered as the most important problem in Egypt. Moreover using of mineral fertilizers in agricultural production has resulted in serious problems in the soil and contaminates the underground water. It is also accumulated in food chain causing hazard effects. Many solutions were suggested to reduce the previously mentioned problems, out of them using blue green algae (cyanobacteria) such as *Nostoc muscorum*, *Nostoc humifusum, Anabaena oryzae*, *Wollea* sp, *Phormedium* and *Spirulina platensis* can decrease using the mineral form of nitrogen fertilizer. The field experiment was carried out during the two successive summer seasons of 2008 and 2009 at Kaha Research Station, Kalyiobia governorate, Egypt to investigate the response of common bean (*Phaseolus vulgaris* L.) plants to seed inoculation and/or soil drench with cyanobacteria mixture under different nitrogen levels, i.e., 50 or 75 of recommend mineral nitrogen. Most studied traits of bean such as vegetative growth, seed yield and its attributes, NPK and seed sugar (total and reducing) content showed positive significant effects when used the tow different applications. Cyanobacteria also enhance the soil biological activity in terms of increasing the total bacterial, total cyanobacterial counts, CO_2 evolution, dehydrogenase and nitrogenase activities. Results suggested that 1/4 or 1/2 of the recommended dose of nitrogen mineral fertilizer could be saved by using some species of nitrogen fixing cyanobacteria. Obtained results emphasized the prospects and potentials of using cyanobacteria biofertilizers as renewable natural nitrogen resources for bean. They are none polluting, inexpensive, utilize renewable resources in addition to their ability in using free available solar energy, atmospheric nitrogen and water.
[Amal Z. Hegazi, Soha S. M. Mostafa and Hamdino M. I. Ahmed. Influence Of Different Cyanobacterial Application Methods On Growth And Seed Production Of Common Bean Under Various Levels Of Mineral Nitrogen Fertilization. Nature and Science 2010;8(11):183-194] (ISSN: 1545-0740).

Keywords: common bean, nitrogen fertilizer, cyanobacteria, blue green algae, soil properties

INTRODUCTION

Cyanobacteria are a diverse group of prokoryotes possessing oxygen evolving photosynthetic system (Prabina *et al*., 2004). They are known to possess the ability to form associations with vascular/non-vascular plants and produce growth-promoting substances (Nanjappan-Karthikeyan *et al.*, 2007). Many, though not all, non-heterocystous cyanobacteria can fix N_2 and convert it into an available form of ammonia required for the plant growth. Nevertheless, these organisms may make a substantial contribution to the global nitrogen cycle (Bergman *et al.*, 1997).

Using of N_2-fixing cyanobacteria is the ultimate goal of N_2 fixation research which aims to decrease the dependence on chemical N fertilizers for food production. This is due to indiscriminate use of chemical fertilizers for a longer period drastically disturbed the natural ecological balance (Jha *et al.*, 2001).

The role of blue green algal (BGA) as biofertilizers has been limited to its relevance and utilization in rice crops (Prasanna *et al*., 2008). Also Yanni and Abd El-Rahman (1993) stated that rice performance (as assessed by plant height, productive tillers, grain and straw yields and their N-contents and fertilizer N-use efficiency) was enhanced by inoculation with cyanobacteria along with urea fertilizer at 36 or 72kg N ha^{-1} rather than 108 kg N ha^{-1} without inoculation.

Scanty information is available on the use and influence of BGA on other crops or vegetables. However, the present work was devoted to screen the responses of bean (*Phaseolus vulgaris* L.) as an important vegetable crop to different cyanobacterial application methods. Thus because bean is a foodstuff that not only supplies nutrients as protein, vitamins, minerals and fiber (Tatjana Kuto *et al.*, 2003), but also bioactive compounds with antioxidant capacity (Marisela Granito *et al.,* 2008).

Jagannath *et al*. (2002) studied the effect of BGA as potent biofertilizer on chickpea as leguminous vegetable crop solely and also its combined effect with cowdung and chemical fertilizers (NPK). Multistrain BGA, viz., *Nostoc* spp., *Anabaena* spp., *Tolypothrix* spp., and *Scytonema* spp. were isolated and cultured in the laboratory. They found that algalization enhances the overall growth of chickpea. It enhanced all the morphological and biochemical characters such as proteins, carbohydrates, total nitrogen content, net grain yield and biomass of the chickpea.

Also Liu-ShiMing and Liang-ShiZhong (1998) noticed the effectiveness of cyanobactreia extracts. Mung bean (*Phaseolus radiatus* [*Vigna radiata*]) seeds were soaked in extracts from *Nostoc commune* and placed in the dark for 3-4 days before being transferred to the light (1200 Lx). *Nostoc* extracts increased root, epicotyl and hypocotyl growth, number of roots and plant fresh and dry weights.

The effect of cyanobacteria as biofertilizer on seed germination and related processes of wheat, sorghum, maize, lentil and sugarbeet was investigated. Germination of seeds of the tested crop plants either in live inoculum, algal filtrate (exogenous), or boiled algal extract (endogenous) of the nitrogen-fixing cyanobacterium *Nostoc muscorum* was significantly increased, as were growth parameters and content of nitrogenous compounds, compared with controls (Adam, 1999; Aly *et al.*, 2008). Ali and Mostafa (2009) examined the effect of foliar spray or soil application methods of potassium-humate and *Spirulina platensis* alga (individually or combined) as bio-organic fertilizer on sesame yield and its attributes. They found that combined foliar application recorded the highest values of plant height, number of capsule/ plant, number of branches/ plant, seed weight/ plant and 1000 seed weight. While, combined soil application gave the highest values of seed and straw yield.

Hence, cyanobacteria species were recommended to be used as biofertilizers instead of utilizing the expensive industrial chemical fertilizers. This was because of the increased cost of chemical fertilizers that cause soil and water pollution. In comparison, cyanobacteria are a cheap source of N, which does not cause pollution. Thus, current study aimed to investigate the influence of different application methods of cyanobacteria on yield and yield quality of common bean under two levels of mineral nitrogen fertilization.

MATERIALS AND METHODS

This investigation was carried out to evaluate the influence of different application methods of cyanobacteria mixture strains in the presence of different levels of nitrogen mineral fertilizer on performance of bean plants in clay loamy soil (Table,1) under surface irrigation. Common bean seeds (*Phaseolus vulgaris* L.) c.v. Nebraska were obtained from the Vegetable Crops Seed Production And Technology Department, Horticulture Research Institute. The two field experiments were carried out at Kaha (Kalyiobia governorate) Horticulture Research Station.

Table (1): The physiochemical properties of the soil used for bean planting.

SOIL PROPERTIES	2008	2009
Soil texture	Clay loam	Clay loam
pH	8.6	8.3
E.C. (dS/m)	0.82	0.91
Available N (mg/l)	112	133
Available P (mg/l)	5.12	5.26
Available K (mg/l)	321	345
Organic matter (%)	0.59	0.65

Algal strain sources, growth conditions and culture characterizations

N_2-fixing (*Nostoc muscorum*, *Nostoc humifusum*, *Anabaena oryzae* and *Wollea* sp) and non N_2-fixing (*Phormedium* sp. and *Spirulina platensis*) cyanobacteria strains were obtained from the Microbiology Department, Soils, Water and Environment Res. Inst., Agric. Res., Center.

The cyanobacterial strains were grown separately on BG11 medium (Rippka *et al*., 1979) except the *Spirulina platensis*, which was grown on Zarrouk medium (Zarrouk, 1966). The cultures were incubated in growth chamber under continuous illumination (2000 lux) and the temperature of 25˚C± 2˚C for all strains except the mesophilic alga *Spirulina platensis*, which was grown on 35˚C± 2˚C. Equal portions from algal culture suspensions at the stationary phase were mixed together for both dry and fresh inoculation.

Culture growth parameters were shown in Table (2). The pH values and algal dry weight (DW) were estimated according to Vonshak (1986). Culture concentration was determined as optical density (OD) by spectrophotometer at 560 nm (Leduy; Therien, 1977). Chlorophyll-a (Ch-a) was determined spectrophotometrically after extraction by absolute methanol as reported by Vonshak and Richmond (1988).

Table (2): The algal growth parameters for the two seasons (2008 and 2009).

	Nostoc muscorum		***Spirulina platensis***		***Anabaena oryzae***		***Wollea* sp.**		***Nostoc humifusum***		***Phormedium* sp.**	
	2008	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**
pH	**8.16**	**8.06**	**10.05**	**10.27**	**7.5**	**6.77**	**7.52**	**6.11**	**7.4**	**8.7**	**8.01**	**9.33**
OD	**1.18**	**1.198**	**2.55**	**2.99**	**0.81**	**0.933**	**2.31**	**2.48**	**1.53**	**1.8**	**2.01**	**2.16**
Ch-a (mgl^{-1})	**4.76**	**5.76**	**11.02**	**12.23**	**2.19**	**5.87**	**10.12**	**9.51**	**9.88**	**5.23**	**3.14**	**2.86**
DW (mgl^{-1})	**755.2**	**766.72**	**1632**	**1913.6**	**518.4**	**597.12**	**1478.4**	**1587.2**	**979.2**	**1152**	**1286.4**	**1382.4**

Different application methods of algal mixture were used, namely seed coating with air dried algal biomass, soil drench application with algal culture suspension and the combination of seed coating and soil drench in the presence of 50 and 75% of the recommended dose for bean of nitrogen mineral fertilizers. Common bean seeds were coated with the air-dried algal biomass (seed-coating) for one hour before planting. The mixture of algal culture suspension was used for soil drench application at a rate of 20 l fed^{-1} which divided into two equal portions for two doses, the first was with the first irrigation after 21 days from sowing and the second was at the flowering stage.

The experiment comprises the following treatments

1- Control (100 % of nitrogen fertilizer)
2-75 % of the recommended mineral N dose.
3- 50 % the recommended mineral N dose.
4- 50 % N + dry cyanobacteria as seed coat.
5- 75 % N + dry cyanobacteria as seed coat.
6- 50 % N + cyanobacteria as soil drench.
7- 75 % N + cyanobacteria as soil drench.
8- 50 % N + cyanobacteria (seed coat+ soil drench).
9- 75 % N + cyanobacteria (seed coat+ soil drench).

Phosphorus (200 kg/fed.) as super phosphate (15.5 % P_2O_2) and potassium (100 kg/fed.) as potassium sulphate (48 % K_2O) were applied to all treatments at the recommended dose per fadden. While, only the control (100%N) was received the recommended dose (200 kg/fed) of nitrogen as ammonium sulfate (21% N). The experiment was laid out in a randomized complete block design with three replicates.

Sowing was done on 3rd and 4th of March in the two summer season 2008 and 2009, respectively. The seeds were planted 7 cm apart in a single row. Each row was 3 m long and 0.6 m wide. Each plot contains 6 rows. Thus, the area of each plot was 10.8 m^2. Other agriculture practices such as weeding, pest and insect control were carried out as recommended for the convential common bean planting.

Measurements

Vegetative growth: At the end of the flowering stage, ten random plants from each treatment were taken to measure: Plant height (cm), number of leaves/ plant, fresh weight/ plant (g), and dry weight/ plant (g) and leaf area/ plant (cm2) (measured by Li-300 Leaf Area Meter produced by Li-Cor, Pinclivania). Total chlorophyll and carotenoids of the third leaf from top was measured by Chlorophyll Meter (SPAD).

Seed yield: At harvest time, the following measurements are taken as average of ten random plants from each treatment: Number of pods/ plant, pod length (cm), pod weight/ plant (g), seed weight/ plant (g) and number of seeds/ pod. Seed yield (kg)/ fed. and weight of 100 seeds (seed index) were also determined.

Seed quality and components: Germination percentage (%) and rate were carried out according to

ISTA rules (ISTA, 2009). Seedling length (cm), root length of seedling (cm), fresh and dry weigh of seedlings (g) were also determined. Nitrogen, phosphorus and potassium concentration were determined in seeds by the method described by Watanabe and Olsen (1965). Total and reducing sugar were assayed also in seeds and estimated colorimetrically according to the method described by Dubios et al. (1956).

Soil analysis: Soil biological activities and soil chemical properties were measured after the second soil drench addition and before the flowering stage. The CO_2 evolution was determined according to Gaur et al. (1971), total bacterial count was performed on nutrient agar using the spread plate method (APHA, 1992) and total cyanobacterial counts were conducted by plating ten-fold serial soil suspension-dilutions in triplicate onto agarized BG11 medium (Stanier et al., 1971). Soil enzymes, i.e., dehydrogenase activity (DHA), was estimated according to Casida et al. (1964), while nitrogenase activity was measured by acetylene reduction assay as described by Dart et al. (1972).

Available nitrogen in soil was determined according to Black (1982). Available phosphorus was extracted using the method described by Soltanpour (1985) and determined spectrophotometrically as mentioned by Watanabe and Olsen (1965). Available potassium was extracted using the method described by Soltanpour (1985) and determined using flame-photometric method (APHA, 1992). Soil reaction (pH) was measured in 1:2.5 soil water extract using glass electrode pH meter Model (955), and electric conductivity (EC) was measured in 1:5 soil water extract using glass electrode conductivity meter Model Jenway 4310.

Statistical analysis

The obtained data were subjected to statistical analysis of variance according to Gomez and Gomez (1984) and the treatments means were compared using the Duncan Multiple Range test as published by Duncan (1965).

RESULTS AND DISCUSSION

Growth criteria

Cyanobacteria or blue green algae (BGA) play a key role in improving growth of many plants when applied as biofertilizers. This evidence was clearly appeared in growth criteria of bean represented in Table (3). The performance of bean plants, in terms of plant height, number of leaves/plant, leaf area/ plant, fresh and dry weight/ plant, was enhanced by cyanobacteria application in both seasons. Some species of cyanobacteria have the ability of nitrogen fixation, thus the amount of chemical N fertilizer can be decreased. Reducing the chemical N fertilizer up to 50 or 75% of the recommended dose and using BGA either as dry seed inoculation or soil drench gave better results than reducing this amount without any cyanobacteria application (or using the recommended N dose only). The combined application of cyanobacteria, i.e., dry and drench, with using 75% of the recommended chemical N fertilizer was found to be the best treatment for enhancing plant growth followed by same application with 50% N. Moreover, data also demonstrated that dry application method is better than drench method.

In this context, Adam (1999) in Egypt studied the effect of cyanobacteria as biofertilizer on seed germination and related processes of wheat, sorghum, maize and lentil. It was observed that growth parameters were significantly increased compared with controls. These increases could be attributed to the nitrogenase as well as nitrate reductase activities of the alga associated with the surface of plants; or the amino acids and peptides produced in the algal filtrate and/or other compounds that stimulate growth of crop plants. Moreover, Jagannath *et al.* (2002) studied the effect of BGA as potent biofertilizer on chickpea and they found that it enhanced all the morphological characters and biomass of the chickpea. Also, Nanjappan-Karthikeyan *et al.* (2007) stated that cyanobacteria have growth promoting activity as inoculants of wheat.

This stimulation effect of BGA on plant growth may be attributed to their influence on increasing the biological activity (Table 7) and chemical properties of the tested soil (Table 8).

Seed yield characters

Data represented in Table (4) demonstrated that adding BGA as biofertilizers can reduce the amount of using chemical N either by 50 or 25% without affecting seed yield characters. Combined application (dry and drench) of BGA gave the best results with significant increase than chemical N fertilizer in all tested pod and seed characters. Also it is clear from the same table that different application methods of BGA (dry or drench or combined) and using 75% of the recommended N is better than using same applications with the low dose of N amount (i.e. 50%). Dry inoculation of BGA was found to be most effectiveness than drench (at the same N level). But the weight of 100 seeds in both seasons with drench and 75% N treatment was significantly increased than dry application under the same N level. Also length of pod in 2^{nd} season and seed yield in 1^{st} season were increased with drench than dry application under 75% N, but this increase doesn't reach significant level.

Muthukumaravel-Chinnusamy *et al.* (2006) and Dhar *et al.* (2007) concluded that application of biofertilizers (BGA) resulting in significant improvement in rice yield parameters. They added that this investigation clearly emphasizes the need for supplementing chemical fertilizers with the newly developed BGA biofertilizers in rice cultivation for maximizing crop productivity, reducing inputs of chemical fertilizers and sustaining soil fertility.

In this connection, Gholve *et al.* (2004) indicated that the yield (in rice-wheat cropping system) obtained due to application of 75% recommended dose (RD) of N+20 kg BGA/ha was statistically equal to that of 100% RD of N thereby indicating the efficient role of BGA for substitution of nitrogen N up to 25%. This was also confirmed by the assessment of the system in terms of monetary returns. In addition, Nanjappan-Karthikeyan *et al.* (2007) indicated that the cyanobacterial isolates applied along with 1/3 N+P+K gave statistically equivalent results as compared to application of full dose of chemical fertilizers in terms of wheat grain yields.

The favorable effect of BGA application on seed yield characters may be due to their influence on plant growth features (Table3).

Photosynthetic pigments and chemical composition of seeds

Using BGA as biofertilizers, greatly enhance plant growth, yield and concomitant quality of produced seeds. This is emphasis in results represented in Table (5). Data showed significant difference between all forms of BGA application and control (without inoculation) (100, 75 and 50% N) regarding pigments, N, P, K, total and reducing sugar content in the two seasons. Total chlorophyll of plant leaves responded positively to BGA inoculation, but carotenoids were slightly affected. N, P, K content of seeds produced from plants inoculated with different BGA applications in both seasons showed significant increase over the control. Also total and reducing sugar of seeds showed the same trend, but in the second season, dry BGA application showed significant increase than combined application in reducing sugar values (at both N levels).

Prasanna *et al.* (2008) found that BGA and Azotobacter in different combinations with chemical fertilizers gave the highest values of chlorophyll. The increase of plant N concentration following co-cultivation with N-fixing cyanobacteria (*Nostoc* or *Anabaena*) was observed by Zorica Obreht *et al.* (1993). They studied the effect of root-associated N_2-fixing cyanobacteria on the growth and nitrogen content of wheat. They also stated that, this increase in nitrogen appeared to be dependent on the wheat cultivar and the cyanobacterial isolate used. Jagannath *et al.* (2002) also stated that BGA or cyanobacteria as potent biofertilize enhanced all the morphological and biochemical characters such as proteins, carbohydrates, total nitrogen content, net grain yield and biomass of the chickpea.

Generally, major nutrient uptake by plants was enhanced in presence of BGA as biofertilizers. This evidence was observed by Singh *et al.* (2002) who demonstrated that the highest total N, P and K uptake by rice was recorded upon treatment: 30 kg N/ ha+green manure+ FYM+10kg BGA/ ha incorporated in soil 15 days before rice transplantation.

Seed quality

It can be noticed that the quality of seeds produced from plants inoculated with different forms of BGA was enhanced in terms of germination percentage, rate of germination, seedling length and seedling root length (Table 6). Germination percentage, seedling and seedling root length recorded the highest values in both seasons with combined application of BGA under the two N levels (75 and 50% of the recommended N level). The same treatments showed the lowest values for germination rate, i.e., days to complete germination were decreased.

Generally, from data represented in Table (6) it could be stated that, the quality of seeds produced from plants inoculated with BGA is greatly enhanced significantly than control (chemical N without BGA inoculation).

Pathak and Jha (1995) found that when maize, wheat, mustard [*Brassica juncea*] and Lady's Finger [*Abelmoschus esculentus*] seeds were inoculated with *Anabaena* or *Nostoc* (some phytoplanktons) the germination percentage was increased.

Another authors (Liu-ShiMing and Liang-ShiZhong 1998) studied the effect of extract from

Nostoc commune cells on the growth of sprouts and seedlings of mung bean (*Phaseolus radiatus*). Seeds were soaked in extracts from Nostoc commune and placed in the dark for 3-4 days before being transferred to the light (1200 Lx). Nostoc extracts increased root, epicotyl and hypocotyl growth, number of roots and plant fresh and dry weights. El-Nahas and Abd El-Azeem (1999) stated that pretreatment of *Vicia faba* seeds with the extract of *Anabaena variabilis* induced an increase in germination percentage, root growth, seedling dry weight and soluble proteins as compared with untreated seeds.

Thus, the stimulation effect of BGA on plant growth and seed yield might reflect on seed quality leading to an increase in germination percentage and subsequent seedling criteria.

Biological activity and chemical properties of soil

The soil before the flowering stage was analyzed to spot the changes in soil regarding biological and chemical conditions due to the different algal application methods in presence of two nitrogen fertilizer levels (50 and 75% of the recommended dose). These changes in plant rhizosphere could give an approximate vision to the ability of cyanobacteria to compensate the nitrogen limitation and enhance crop production. However, all the treatments affected significantly both of the chemical and biological properties of the soil (Tables 7 and 8). Cyanobacterial inoculation, generally, enhanced the soil biological activity in terms of increasing the CO_2 evolution, dehydrogenase activity, nitrogenase, total cyanobacterial counts and total bacterial counts under both levels of nitrogen (75 and 50%N) compared to the untreated treatments in the two seasons. The maximum microbial activity was achieved by the combined effect of seed coating and soil drench application with 75%N followed by 50%N. These obtained results are in agreement with those of Mahmoud *et al*. (2007) who stated that cyanobacterial inoculation generally enhanced the soil biological activity.

Soil pH and electric conductivity (EC) were slightly decreased by inoculation with cyanobacteria in the first season, while, the second season revealed a significant reduction in these parameters particularly when the combined application of seed coating and soil drench was applied with 75%N (Table 8). These data agree with that of Sutton *et al.* (1991) who observed that the use of nitrogenous fertilizers cause acidification of soils. The application of cyanobacteria to alkali soils resulted in significant improvement in the aggregation status of these soils and also decreased their pH. In addition, Singh (1961) reported that alkaline soils could be reclaimed by using cyanobacteria that neutralize the pH of these soils. It is well documented that cyanobacteria have the capacity to reclaim soil salinity (Hashem *et al*., 1995).

Available N, P and K in soil significantly increased due to the cyanobacterial inoculation compared to the non inoculated treatments. The highest available N, P and K contents were recorded when seed-coating combined with soil drench application was used with 75%N, while the lowest values was observed in the soils that only received the mineral fertilizer during the two seasons (Table 8). Despite the purposed nitrogen reduction in presence of cyanobacterial inoculation, results revealed that the increase in soil available nitrogen was via N_2-cyanobacteria which could compensate about 25-50% of the mineral nitrogen required for bean cultivation, this could be attributed to nitrogen fixation (Çakmakçı *et al*., 2007).

In this study, the used inoculum gathered the beneficial effects of N_2-fixing and non N_2-fixing cyanobacteria. Cyanobacteria can fix about 25 kg N/ ha/ season. Apart from nitrogen fixation, inoculation with cyanobacteria is also reported to reduce considerably the total sulphides and ferrous iron content of the soil (Aiyer, 1965).

The majorities of cyanobacteria are capable of fixing atmospheric nitrogen and are effectively used as biofertilizers (Vaishampayan *et al*., 2001).

The cyanobacterial ability to mobilize insoluble forms of inorganic phosphates is evident from the finding of kleiner and Harper (1977) who reported more extractable phosphates in soils with cyanobacterial cover than in nearby soils without cover. Cyanobacteria is also known to increase soil fertility by enhancing the available N and P levels and exhibited an economical view that it can compensate about 50% of the recommended doses of N, P, K (Singh and Bisoyi, 1989; Mahmoud *et al*., 2007).

The enhancement influence of BGA on the biological activity and chemical properties of the soil positively affected plant characteristics which led to improve in common bean yield and quality of seeds.

Table (3): Growth criteria of common bean (var. Nebraska) cultivated during summer seasons 2008 and 2009 under different levels of N fertilizer with different cyanobacterial application methods.

Treatments	Plant height (cm)		Number of leaves/ plant		Leaves area/ plant $(cm)^2$		Fresh weight/ plant (g)		Dry weight/ plant (g)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
100%N	34.17 c	34.50 d	9.67 cd	10.77 e	472.4 c	479..2 f	29.19 bc	37.57 cd	5.90 c	6.61 cd
75%N	32.50 c	33.17 de	9.00 d	10.50 e	458.0 c	394.0 g	26.47 c	36.90 d	5.57 c	6.60 cd
50%N	31.82 c	32.07 e	8.67 d	10.00 e	347.0 d	379.4 g	25.00 c	35.28 d	5.15 c	6.20 d
50%N + Seed coating	34.73 c	36.83 c	11.33 abc	12.93 cd	608.7 b	625.2 c	35.14 ab	44.79 b	6.05 c	7.13 bc
75%N + Seed coating	34.83 c	38.67 b	12.00 ab	13.50 c	620.1 b	639.7 c	36.81 a	46.03 b	6.49 bc	7.69 b
50%N + Soil drench	34.37 c	36.83 c	11.00 bc	12.23 d	482.5 c	501.6 e	33.12 ab	40.92 bcd	6.04 c	6.06 d
75%N + Soil drench	36.17 c	36.20 c	1.00 bc	12.77 cd	572.2 b	584.4 d	34.59 ab	43.12 bc	6.58 bc	6.19 d
50%N + (Seed coating & soil drench)	41.08 b	39.40 b	12.33 ab	15.47 b	695.4 a	709.3 b	37.90 a	53.05 a	7.82 ab	7.62 b
75%N + (Seed coating & soil drench)	45.33 a	41.20 a	13.00 a	16.50 a	751.1 a	773.9 a	38.07 a	57.75 a	7.99 a	9.12 a

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level.

Table (4): Seed yield characters of common bean (var. Nebraska) cultivated during summer seasons 2008 and 2009 under different levels of N fertilizer with different cyanobacterial application methods (seed coating, soil drench).

Treatments	Number of pods/plant		Length of pod (cm)		Number of seed/pod		Weight of 100 seeds (g)		Seed weight /plant (g)		Seed yield (kg)/fed	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
100%N	16.30 d	15.17 e	10.00 d	11.23 d	3.83 c	3.83 cde	45.36 e	46.07 e	20.27 d	21.96 f	976.5 e	983.5 e
75%N	15.67 d	14.07 e	10.43 cd	11.00 d	3.67 c	3.50 de	44.30 f	45.51 e	17.18 e	20.24 g	876.5 f	889.0 f
50%N	14.83 d	14.00 e	10.77 cd	10.60 e	3.33 c	3.43 e	43.53 f	44.50 f	16.87 e	18.80 h	825.5 g	837.5 g
50%N + Seed coating	18.67 c	22.67 c	12.13 b	12.43 c	4.00 bc	4.23 bc	46.58 d	47.76 d	26.31 b	30.89 c	1101 c	1119 cd
75%N + Seed coating	21.00 b	24.33 b	12.43 b	12.90 b	5.00 ab	4.33 bc	47.49 c	48.99 c	26.94 b	32.49 b	1086 c	1139 c
50%N + Soil drench	19.50 bc	21.83 c	11.23 c	12.80 b	4.00 bc	3.87 cde	48.22 c	49.13 c	22.82 c	24.02 e	1020 d	1093 d
75%N + Soil drench	20.00 bc	20.47 d	12.10 b	12.93 b	4.33 bc	4.13 bcd	49.25 b	50.33 b	23.64 c	25.46 d	1092 c	1110 cd
50%N + (Seed coating & soil drench)	24.67 a	26.00 a	13.27 a	13.77 a	5.67 a	4.67 b	49.94 b	51.44 a	27.40 b	36.85 a	1155 b	1181 b
75%N + (Seed coating & soil drench)	25.73 a	26.27 a	13.80 a	13.83 a	6.00 a	5.33 a	51.41 a	52.20 a	32.46 a	37.12 a	1187 a	1254 a

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level.

Table (5): Photosynthetic pigments (in leaves) and chemical composition of common bean (var. Nebraska) cultivated during summer seasons 2008 and 2009 under different levels of N fertilizer with different cyanobacterial application methods (seed coating, soil drench).

Treatments	Chlorophyll		Carotenoids		N %		P %		K %		Total sugar		Reducing sugar	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
100%N	20.19 d	27.02 d	2.77 a	2.19 bc	2.57 d	2.42 g	0.55g	0.54 g	1.18 g	1.58 de	28.68 f	28.23 d	4.44 e	4.39 d
75%N	17.76 e	27.29 cd	2.72 a	2.13 bcd	2.43 e	2.34 h	0.54 h	0.52 h	1.17 h	1.53 e	25.15 h	28.98 d	4.43 e	4.38 e
50%N	17.50 e	24.42 e	1.83 b	2.17 bc	2.32 f	2.33 i	0.52 i	051 i	1.14 i	1.51 e	25.04 h	28.27 d	4.31 f	4.38 f
50%N + Seed coating	22.87 bc	28.49 bc	2.64 a	2.02 d	3.74 b	2.72 d	0.60 d	0.60 d	1.22 e	1.69 bcd	30.19 d	37.01 b	4.74 d	4.41a
75%N + Seed coating	23.53 b	27.82 cd	2.57 a	2.25 ab	3.79 ab	2.74 c	0.63 c	0.62c	1.26 c	1.73 abc	35.87 c	37.02 b	4.91 c	4.40 b
50%N + Soil drench	21.90 c	29.28 b	2.77 a	2.12 bcd	2.74 c	2.52 f	0.57 f	0.57 f	1.21 f	1.63 cde	27.43 g	35.01 c	4.44 e	4.19 i
75%N + Soil drench	21.81 c	27.60 cd	2.56 a	2.37 a	2.75 c	2.55 e	0.59 e	0.58 e	1.23 d	1.55 e	29.41 e	35.42 c	4.66 d	4.20 h
50%N + (Seed coating & soil drench)	24.18 b	30.87 a	2.31 ab	2.18 bc	3.81 ab	2.90 b	0.65 b	0.64 b	1.30 b	1.78 ab	37.60 b	37.91 ab	5.25 b	4.37 g
75%N + (Seed coating & soil drench)	25.72 a	31.26 a	2.78 a	2.10 cd	3.86 a	3.06 a	0.68 a	0.66 a	1.34 a	1.82 a	37.97 a	38.08 a	5.53 a	4.40 c

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level.

Table (6): Seed quality characters of common bean (var. Nebraska) cultivated during summer seasons 2008 and 2009 under different levels of N fertilizer with different cyanobacterial application methods (seed coating, soil drench).

Treatments	Germination percentage (%)		Germination rate		Seedling length (cm)		Seedling root length (cm)	
	2008	2009	2008	2009	2008	2009	2008	2009
100%N	89.33 c	90.57 c	2.35 bc	2.30 bc	29.00 c	30.33 d	11.00 ef	11.33 de
75%N	86.00 d	86.50 d	2.40 ab	2.35 ab	28.50 c	29.90 d	10.90 ef	11.27 de
50%N	80.67 e	81.83 e	2.48 a	2.44 a	26.17 d	27.13 e	10.23 f	11.07 e
50%N + Seed coating	90.67 bc	92.43 bc	2.27 c	2.23 cd	32.83 ab	33.77 bc	11.33 cde	11.93 cd
75%N + Seed coating	91.33 abc	93.40 ab	2.27 c	2.21 cd	34.33 a	35.33 ab	12.17 c	12.43 c
50%N + Soil drench	90.67 bc	91.33 bc	2.31 bc	2.28 bc	31.67 b	32.50 c	11.17 def	11.77 cde
75%N + Soil drench	92.00 abc	92.17 bc	2.37 bc	2.27 bc	33.00 ab	33.63 bc	12.00 cd	12.07 cd
50%N + (Seed coating & soil drench)	94.00 ab	94.83 a	2.14 d	2.13 de	34.50 a	36.00 a	14.10 b	14.17 b
75%N + (Seed coating & soil drench)	94.67 a	95.50 a	2.04 e	2.07 e	34.50 a	35.63 a	16.77 a	16.43 a

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level.

Table (7): Soil biological activity one week after the second soil drench addition and before the flowering stage of common bean (var. Nebraska) cultivated during summer seasons 2008 and 2009 under different levels of N fertilizer with different cyanobacterial application methods (seed coating, soil drench).

Treatments	CO_2 evolution (mg100g soil^{-1} day^{-1})		Dehydrogenase activity (µg TPFg^{-1} dry soil Day^{-1})		Nitrogenase activity (µ mole C_2H_4 g soil^{-1}hr.$^{-1}$)		Cyano. counts (10^2 cfu g soil^{-1})		Bact. counts (10^5cfu g soil^{-1})	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
100%N	17.43 de	18.30 e	29.09 f	39.27 f	15.56 d	19.83 efg	80.00 e	75.00 e	152.68 e	162.86 e
75%N	14.61 f	14.40 f	22.66 g	30.36 h	10.31 e	16.68 g	40.00 g	44.50 f	71.25 g	81.43 g
50%N	15.18 f	15.33 f	27.38 f	33.54 g	14.89 de	17.89 fg	65.00 f	45.00 f	91.61 f	132.32 f
50%N + Seed coating	15.75 ef	19.18 e	32.99 e	41.81 e	13.19 g	20.88 def	50.00 h	77.50 e	101.79 f	157.78 e
75%N + Seed coating	18.12 d	21.57 d	37.43 d	44.18 d	15.42 d	22.78 de	92.00 d	82.50 e	167.95 d	187.29 d
50%N + Soil drench	19.029 d	23.28 c	43.93 c	45.09 d	20.92 d	24.50 d	100.00cd	105.00 d	213.75 c	203.57 d
75%N + Soil drench	21.00 c	26.79 b	45.16 c	49.27 c	31.62 b	36.28 c	108.00 c	125.00 c	254.43 c	264.46 c
50%N + (Seed coating & soil drench)	25.65 b	27.18 b	47.20 b	55.45 b	33.04 b	40.66 b	130.00 b	182.50 b	219.86 b	371.52 b
75%N + (Seed coating & soil drench)	28.97 a	32.64 a	55.06 a	62.36 a	60.99 a	90.35 a	210.00 a	240.00 a	427.50 a	488.57 a

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level.

Table (8): Some chemical properties of soil one week after the second soil drench addition and before the flowering stage of common bean (var. Nebraska) cultivated during summer seasons 2008 and 2009 under different levels of N fertilizer with different cyanobacterial application methods (seed coating, soil drench).

Treatments	pH (1:2.5)		EC (dSm-1) (1:5)		Available-N		Available-P		Available-K	
					g.Kg^{-1} dry soil					
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
100%N	7.99 a	7.65 c	3.66 a	3.41 a	0.14 c	0.15 bc	1.48 bc	1.51 ef	2.23 bcd	2.28 d
75%N	7.23 b	7.80 b	3.10 b	2.90 b	0.09 d	0.11 c	1.41 c	1.44 f	1.87 cd	1.91 fg
50%N	7.22 b	8.01 a	2.99 b	2.32 cd	0.09 d	0.09 c	1.24 c	1.27 g	1.75 d	1.79 g
50%N + Seed coating	7.65 ab	7.40 de	2.90 b	2.91 b	0.09 d	0.18 ab	1.58 bc	1.62 de	2.01 cd	2.05 ef
75%N + Seed coating	7.29 b	7.40 de	2.83 b	2.81 b	0.11 cd	0.19 ab	1.75 ab	1.76 cd	2.13 cd	2.18 de
50%N + Soil drench	7.63 ab	7.42 d	2.51 c	2.32 cd	0.18 b	0.20 ab	1.77 ab	1.81 bc	2.42 bcd	2.47 c
75%N + Soil drench	7.26 b	7.35 e	2.48 c	2.45 c	0.20 ab	0.20 ab	1.79 ab	1.83 bc	2.54 bc	2.59 c
50%N + (Seed coating & soil drench)	7.26 b	7.35 e	2.25 cd	2.11 de	0.20 ab	0.21 ab	1.91 ab	1.95 b	2.89 b	2.93 b
75%N + (Seed coating & soil drench)	7.23 b	7.25 f	2.00 d	1.95 e	0.22 a	0.24 a	2.22 a	2.26 a	3.56 a	3.63 a

Values within the same column followed by the same letters are not significantly different, using Duncan's Multiple Range Test at 5% level.

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تأثير الطرق المختلفة لإضافة الطحالب على نمو وإنتاجية الفاصوليا تحت مستويات مختلفة من التسميد النتروجينى المعدنى

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فى الآونة الأخيرة يعتبر التلوث من أهم المشكلات فى مصر ، فإن الإسراف في استخدام الأسمدة المعدنية في الإنتاج الزراعي ينتج عنه مشكلات خطيرة بالتربة وتلوث الماء الأرضى. و تراكم الأسمدة المعدنية فى الغذاء يسبب مخاطر صحية.
وعليه فإن هناك عديد من المقترحات لتقليل حدة هذه المشكلة منها إستخدام بعض أنواع السيانوبكتريا *Nostoc muscorum* - *Nostoc humifusum* - *Anabaena oryzae* - *Wollea* sp- *Phormedium* - *Spirulina platensis* كمخصب حيوي لتقليل استخدام الأسمدة النتروجينية المعدنية.
تم إجراء هذا البحث خلال الموسمين الصيفيين 2008 ، 2009 بمزرعة قها محافظة القليوبية- مصر ، لدراسة إستجابة نبات الفاصوليا للطرق المختلفة لإضافة هذه الطحالب ومنها تلقيح البذور و/أو الإضافة الأرضية تحت مستويات مختلفة (50 ، 75%) من الجرعات النتروجينية المعدنية الموصى بها.
أوضحت النتائج أن معظم صفات الفاصوليا المدروسة مثل : النمو الخضرى, إنتاجية البذور ومكوناتها من NPK و السكريات (الكلية والمختزلة) قد زادت زياده معنوية بإستخدام كلا الطريقتين.
كما شجع إستخدام الطحالب النشاط البيولوجى للتربة مثل زيادة عدد البكتريا ,عدد الطحالب, تصاعد ثانى أكسيد الكربون, نشاط إنزيمي الديهيدروجينيز و النيتروجينيز.
وأوضحت النتائج أيضا أنه أمكن توفير ربع أو نصف الجرعات الموصى بها من الأسمدة النتروجينية المعدنية بإستخدام السيانوبكتريا المثبتة للنتروجين.
وتؤكد النتائج أهمية استخدام الطحالب كأسمدة حيوية موصى بها كبدائل للأسمدة النتروجينية المعدنية عند زراعة الفاصوليا لأنها غير ملوثة للبيئة(50%,75%) ورخيصة الثمن ، فقط تحتاج إلى الطاقة الشمسية والماء لتثبيت النتروجين الجوى.

2010/11/10