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IMPACTS OF CYANOBACTERIA AND *BRADYRHIZOBIUM* INOCULATION ON LUPINE PLANTS UNDER DIFFERENT NITROGEN RATES IN SANDY SOIL

[134]

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ABSTRACT

A field experiment was conducted in sandy soil at Ismailia Agricultural Research Station, (Ismailia Governorate, Egypt, in two successive winter seasons of 2015/2016 and 2016/2017. The experiment aimed to study the response of lupine plants (*Lupinus albus* L.) variety Giza 2 to cyanobacteria applied with different methods under different nitrogen fertilizer rates and inoculated with rhizobia. Results revealed that application of cyanobacteria generally increased the mean values of nodules number, dry weight of nodules and shoot dry weight of lupine plants, nitrogen uptake, biological activity of the soil rhizosphere lupine plants, yield, yield components and seed protein content as compared to those recorded by the control treatment without cyanobacteria. Increasing nitrogen rates decreased the mean values of both nodules number and dry weight of nodules. On the contrary, increasing nitrogen rate up to 100% of the recommended one increased all the other tested parameters. The highest values of these parameters were recorded when cyanobacteria were applied as dry + soaking combined with 75 % N rate expect for those of number and nodules the dry weight of nodules that gave their highest values when the lupine plants received the treatment of dry + soaking combined with 50 % N rate. In conclusion, the use of cyanobacteria along with rhizobia as renewable nitrogen source for lupine production can save 25% N from that required for lupine.

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INTRODUCTION

The use of conventional chemical farming methods, which is substantially increased crop production, was once regarded as a kind of agriculture revolutions, which would solve all problems of producing sufficient food for the ever-growing world population. However, this belief is accompanied with numerous environmental and social problems due to the heavy use of agrochemicals in intensive farming systems. Moreover, increasing prices of agrochemicals especially nitrogen, often leaves the marginal farmers with low profits. Uncertain availability of those agrochemicals such as nitrogen (the most essential nutrient required for plant growth), especially in the developing countries such as Egypt, is often a serious constraint for the farmers in their attempt to increase crop production. Such problems have pushed the agriculturalists world-wide to look for alternative methods for farming. Amongst of these trials to develop productive, profitable and sustainable agriculture systems, the agriculturalists have turned to the farming methods, which are based on biotechnologies. One of such bio-technologies to achieve this goal is the use of the biological nitrogen fixation through cyanobacteria to improve soil fertility and crop productivity. The use of nitrogen fixing cyanobacteria ensure entirely or partially the mineral nitrogen and/or enhances the availability of soil nutrients **(Abbas, 2015).**

Cyanobacteria formerly called blue-green algae are group of ubiquitous photosynthetic prokaryotes, since they capture energy from sunlight and possessing the ability to fix the atmospheric nitrogen. They contribute in agriculture where they used as biofertilizer and soil stabilizers **(Abdel-Raouf et al 2012)**. Cyanobacteria are known by their ability to excrete growth-promoting substances such as hormones (Auxin, Gibberellins), vitamins and amino acids. They also increase the water- holding capacity through their jelly structure, increase in soil biomass after their death and decomposition, preventing weeds growth **(Alam et al 2014)**. Cyanobacteria excrete a great number of substances that influence plant growth and development. These microorganisms have been reported to benefit plants by producing growth promoting regulators (the nature of which is said to resemble gibberellins and auxins), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity **(Zaccaro, 2000)**. Cyanobacteria are known to commonly be used in rice paddy field rather than other crops. However, few works reported beneficial effects of cyanobacteria for some other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chili and lettuce (**Thajuddin and Subramanian, 2005)**.

Lupine (*Lupinus albus* L.) is considered one of the most important leguminous crops. It is cultivated around the Mediterranean and along the Nile valley, where it is used for human consumption and for medical and industrial purposes. It is also full of the amino acid arginine, which also helps lower blood pressure, lowers cholesterol & trigs, and lowers blood sugar levels **(Maknickiene, 2001)**. Lupini beans are a legume that grow all over Europe and is a common snack food in Portugal, Spain, France, Italy, Greece, and Egypt. Lupini beans are full of protein, fiber, and low in oil and starch and because of that, weight loss is commonly related with eating lupini beans **(Radwan et al 2012)**. They also added that lupine can be considered as a friendly crop to the environment related to its efficient nitrogen fixation system, in addition to its improvement to the traditional cereal rotation and protein supply in low input farming system.

The use of cyanobacteria to legumes along with rhizobia is very rare, **Bidyarani et al (2016)** reported that the inoculant of the cyanobacterium *Anabaena laxa* along with the biofilmed-*Rhizobium* proved to be the top-ranking treatments and achieved the highest grain yield compared to the use of either cyanobacteria or rhizobium each alone.

The aim of the current work is to study the effect of cyanobacteria inoculation along with rhizobia under different nitrogen fertilizer rate on nodulation status, rhizosphere biological activity, nitrogen uptake, yield and yield components of lupine plants.

MATERIALS AND METHODS

A field experiment was conducted in sandy soil at Ismailia Agricultural Research Station, (ARC), Ismailia Governorate, Egypt, in two successive winter seasons of 2015/2016 and 2016/2017, to study the response of lupine plants (*Lupinus albus* L.) variety Giza 2 to cyanobacteria applied with different methods under different nitrogen fertilizer rates and inoculated with rhizobia. The physical and chemical properties of the experimental soil are shown in **Table (1)** according to **Page et al (1982).**

Table 1. Some chemical and physical analyses of the investigated soil

pH (1:2.5) Soil extract	EC (dSm^{-1}) (1:2.5) Soil extract	Soluble cations ($meqL^{-1}$)				Soluble anions ($meqL^{-1}$)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
8.10	0.30	0.30	0.50	1.90	0.30	0.00	0.80	1.10	1.10

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	Texture class
85.18	10.17	2.25	2.40	1.30	Sandy

Cyanobacteria strains were obtained from Agric. Microbiol. Dept., Soils, Water & Environ. Res. Inst., ARC, Giza, Egypt. Cyanobacteria cultures (Cyano) for different cyanobacteria strains, i.e., *Nostoc muscorum*, *Anabaena laxa, Calothrix fertilissima and Anabaena oryzae* was applied as culture filtrate. To obtain the cyanobacteria culture filtrate, each cyanobacterium strain was grown and propagated for 5 weeks on the free nitrogen BG 11_0 medium (**Allen and Stanier, 1968)**. The developed cyanobacteria cultures were centrifuged (3000 rpm min^{-1}) and the supernatant were used as cyanobacteria filtrate by mixing the supernatant for each strain together to have the cyanobacteria culture filtrate. The filtrate was used in soaking treatment for lupine seeds preplanting. As well as, these cyanobacteria strains were prepared as dry cyanobacteria soil based inoculum as described by **Venkataraman, (1972)** to be used for lupine as seeds side dressing (dry inoculum) when drilled along the rows. Cyanobacteria are introduced in three forms, i.e., 1) Soaking lupine seeds in Cyano filtrate for 24 h, 2) Side dressing along the row (dry inoculum).

Bradyrhizobium strain ARC 408 were obtained from Nitrogen Fixation Research Unit, Agric. Microbiol. Dept., Soils, Water & Environ. Res. Inst., ARC, Giza, Egypt. *Bradyrhizobium* (ARC 408) inoculant was prepared by growing *Bradyrhizobium* on yeast extract mannitol broth medium **(Vincent, 1970)**, incubated at 28°C for three days until early log phase (5 x 10^9 cfu/ ml culture). Vermiculite supplemented with 10% Irsh peat was packed in polyethylene bags (300 g carrier per bag), then sealed and sterilized by gamma irradiation (5.0 x 10^6 rads.). Bradyrhizobia culture was injected into the carrier to satisfy 60% of water holding capacity.

The *Bradyrhizobium* inoculum was applied as seed coating at the rate of 300 g inoculants /40 kg seeds using Arabic gum as adhesive agent. Nitrogen was introduced in three rates of 25 (50 % N), 37.5 (75 % N), and 50 kg N ha^{-1} (100% full N) in the form of ammonium sulphate (20.5 % N), while phosphorus as superphosphate (15 % P_2O_5) was added basically during soil preparation at the rate of 500 kg ha^{-1} and potassium as potassium sulphate (48 % K_2O) was applied after 45 days from sowing at a rate of 125 Kg ha^{-1}. Lupine seeds due to all treatments were inoculated with rhizobia bacteria before sowing as recommended by the Egyptian Ministry of Agriculture and Land Reclamation.

Lupine seeds (*Lupinus albus* L.) variety Giza 2 (Field Crops Res. Inst., ARC. Giza, Egypt) were sowed in the rate of 100 kg ha^{-1} Irrigation was conducted by using sprinkler system. The experimental Plot was of 5 rows, 60 cm apart and 3x 3.5 m (10.5 m^2 area). The experimental design was split-plot and comprises 12 treatments with three replications, where nitrogen rates represent the main plots (three treatments) while cyanobacteria treatments (four treatments) were assigned to the sub plots.

Five plants were randomly gently uprooted from each plot at 70 days from sowing to determine number and dry weight nodules (mg $plant^{-1}$), shoot dry weights (g $plant^{-1}$) and total nitrogen content of shoot (mg N $plant^{-1}$) (Nitrogen uptake). As well as at the same stage lupine plant rhizosphere soil samples were collected to determine total bacterial count (**Allen, 1959**), dehydrogenase activity (**Casida et al 1964**) and CO_2 evolution (**Pramer and Schmidt, 1964**).

At harvest, ten guarded plants were randomly collected from each plot to determine yield and yield components, i.e., seed and straw yields (ton ha^{-1}), 100-seed weight (g) and seed crude protein percentages calculated by multiplying N% by 6.25 **(Tripathi et al 1971)**

Statistical analysis of the data was performed according to **Gomez and Gomez (1984).**

RESULTS

Effect of cyanobacteria treatments and nitrogen rates on nodulation status

Data in **Table (2)** showed the effect of different cyanobacteria treatments and different nitrogen rates and their interaction on nodulation status of the lupine plants after 70 days from sowing. Results revealed that inoculation with cyanobacteria increased the mean values of nodules number, dry weight of nodules and shoot dry weight of lupine plants compared to that recorded by the control treatment without cyanobacteria. The highest mean value of nodules number (18.89) was recorded with the cyanobacteria treatment (dry + soaking) has significantly surpassed those of the other tested treatments. In respect to the effect of N rates, results indicated that increasing the nitrogen rates decreased significantly the mean values of nodules number, the highest one of 22.33 was recorded with 50% N followed by 18.58 (75 % N) and 3.00.for 100 % N treatment. Results also, showed the significant interaction effect, which means that the response of nodulation status was not the same under the studied nitrogen rates.

Variations among cyanobacteria treatments reached the significant level with 50 and 75 % N rates, but this was not the case with the highest rate (100 %) of N fertilizer.

Similar trend that achieved with the number of nodules was also noticed with the nodules dry weight, since cyanobacteria treatments increased significantly the mean values of the nodules dry weight of the lupine plants compared to that obtained by the control treatment.

Table 2. Effect of cyanobacteria application under different nitrogen rates on nodulation status of lupine plants (Data are a mean of two seasons)

Cyanobacteria treatment (cyano.)	**Nitrogen rates (%)**			
	50	**75**	**100**	**Mean**
	Nodules number			
*Control	17.33	14.00	3.33	11.56
Dry	20.00	15.67	3.00	12.89
Soaking	23.00	20.00	2.67	15.22
Dry + soaking	29.00	24.67	3.00	18.89
Mean	22.33	18.58	3.00	
L. S. D. @ 0.05				
N Rate	**1.36**			
Cyano.	**1.57**			
Cyano. x N	**2.71**			
Nodules dry weight (mg plant^{-1})				
Control	716.00	630.00	19.00	455.00
Dry	744.30	658.70	22.00	475.00
Soaking	800.00	743.00	23.70	522.00
Dry + soaking	935.00	821.30	26.00	594.10
Mean	798.80	713.30	22.67	
L. S. D. @ 0.05				
N Rate	**12.40**			
Cyano.	**14.40**			
Cyano. x N	**24.90**			
Shoot dry weight (g plant^{-1})				
Control	11.17	11.99	12.62	11.93
Dry	11.99	12.90	13.74	12.88
Soaking	13.78	14.27	14.90	14.32
Dry + soaking	15.80	16.65	17.11	16.52
Mean	13.18	13.95	14.59	
L. S. D. @ 0.05				
N Rate	**0.18**			
Cyano.	**0.21**			
Cyano. x N	**0.36**			

***Control = without cyanobacteria.**

Without cyanobacteria treatment consequently, the highest mean nodules dry weight of 594.10 mg $plant^{-1}$(dry + soaking) was significantly higher than those of 522.00 and 475.00 mg $plant^{-1}$ for both soaking and dry cyanobacteria treatments, respectively. In contrast, increasing the nitrogen rate from 50, 75 up to 100% decreased significantly the mean values of the nodules dry weight. The highest mean nodules dry weight of 798.80 mg $plant^{-1}$ was obtained with 50% N treatment followed by 713.30 and 22.76 mg $plant^{-1}$ in respective to 75 and 100% N rates.

For the interaction effect of both cyanobacteria treatments and nitrogen rates on the nodules dry weight of lupine plants, the results confirmed significant interaction with the cyanobacteria treatments when accompanied with either 50% or 75 % N rates. While the behavior was true when the cyanobacteria treatments accompanied with 100% N rate that was not significant.

Moreover, **Table (2)** illustrated the effect of different cyanobacteria treatments and different nitrogen rates and their interaction on the shoot dry weight of lupine plants. Results revealed that cyanobacteria treatments increased the mean values of the shoot dry weight of lupine plants compared to the control treatment without cyanobacteria. However, the highest mean value of shoots dry weight (16.52 g $plant^{-1}$) was obtained with the cyanobacteria treatment of dry + soaking that was significantly higher than the other mean values achieved by the other tested cyanobacteria treatments and the (control without cyanobacteria). The corresponding mean values were 14.32 g $plant^{-1}$(soaking), 12.88 g $plant^{-1}$ (dry) and 11.93 g $plant^{-1}$ (control).

On the other respect, increasing nitrogen rate increased the mean values of shoot dry weight of lupine plants. The highest nitrogen rate of 100 % gave the highest mean value of shoot dry weight of 14.59 g $plant^{-1}$, which was significantly higher than those of 75 and 50 % N rates.

In respect to the interaction effect between cyanobacteria treatments and nitrogen rates on shoot dry weight of lupine plants, results postulated that the dry + soaking cyanobacteria treatment along with 100 % N rate gave the highest value of 17.11 g $plant^{-1}$ that surpassed significantly those of dry +soaking cyanobacteria treatment along with 75 and 50 % N rates.

Generally, either of dry or soaking cyanobacteria treatment alone and\or combined with any of the applied nitrogen rates gave less values for all the tested parameters including the nodulation status compared to those of dry + soaking cyanobacteria treatment alone or combined with any of the N rates.

Effect of cyanobacteria treatments and nitrogen rates on the biological activity in the soil rhizosphere of the lupine plants

Data in **Table (3)** showed the effect of different cyanobacteria treatments and different nitrogen rates and their interaction on the biological activity of the soil rhizosphere activity of the lupine plants after 70 days from sowing. This biological activity is expressed as total count bacteria, carbon dioxide evolution and dehydrogenase activity (DHA). Results revealed that the use of different cyanobacteria treatments increased significantly the mean values of the soil rhizosphere activity of the lupine plants in terms of total count bacteria, carbon dioxide evolution and DHA. The highest mean values of 72.48 x 10^5 cfu g dry rhizosphere $soil^{-1}$ (total count bacteria), 80.49 mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1} (CO_2 evolution) and 164.17 mg TPF dry rhizosphere $soil^{-1}$ day^{-1} (DHA) were recorded by dry + soaking cyanobacteria treatment. These values were significantly higher than those recorded by the other tested cyanobacteria treatments and the control treatment without cyanobacteria.

For nitrogen rates treatments, the 75 % N rate recorded the highest mean values of total bacterial count, CO_2 evolution and DHA compared to those recorded by both 50 and 100% N rates. In respect to the interaction effect between the two factors under investigation was significant. This means that the effect of cyanobacteria treatments on the above studied traits was not the same under the different N rates. However, it could be noticed that 50 % N rate recorded slightly higher mean values of soil rhizosphere biological activity of the lupine plants than those recorded by 100 % N rate.

Table 3. Effect of cyanobacteria application under different nitrogen rates on total count of bacteria, CO_2 evolution and dehydrogenase activity of the soil rhizosphere of lupine plants (Data are a mean of two seasons)

Cyanobacteria treatment (cyano.)	Nitrogen rates (%)			
	50	75	100	Mean
	Total count bacteria (**cfu X 10^5) g soil^{-1}			
*Control	50.97	55.70	54.23	53.63
Dry	60.30	68.13	58.62	62.35
Soaking	64.07	78.13	60.93	67.71
Dry + soaking	68.70	87.20	61.53	72.48
Mean	61.01	72.29	58.83	
L. S. D. @ 0.05				
N Rate	**2.58**			
Cyano.	**2.98**			
Cyano. x N	**5.16**			
CO_2 Evolution (mg CO_2 100 g dry rhizosphere soil-1 day^{-1})				
Control	63.67	67.77	54.37	61.94
Dry	69.53	71.50	60.10	67.04
Soaking	72.33	80.47	68.87	73.89
Dry + soaking	76.53	88.63	76.32	80.49
Mean	70.52	76.98	64.92	
L. S. D. @ 0.05				
N Rate	**9.42**			
Cyano.	**10.88**			
Cyano. x N	**18.85**			
Dehydrogenase activity (mg ***TPF g dry rhizosphere soil^{-1} day^{-1})				
Control	91.43	104.17	98.23	97.94
Dry	110.40	129.70	122.28	120.79
Soaking	126.20	148.60	125.80	133.53
Dry + soaking	157.30	198.80	136.40	164.17
Mean	121.34	145.32	120.68	
L. S. D. @ 0.05				
N Rate	**8.07**			
Cyano.	**9.32**			
Cyano. x N	**16.14**			

*Control = without cyanobacteria. **cfu = Colony forming unit. ***TPF= Tri phenyl formazan.

Effect of cyanobacteria treatments and nitrogen rates on total nitrogen content of lupine plant (nitrogen uptake)

Data in **Table (4)** showed the effect of inoculation with cyanobacteria alone and\or combined with different nitrogen rates, i.e., 50, 75 and 100% from the recommended N rate compared to the traditional N fertilization rate on total nitrogen content of lupine plants (nitrogen uptake). Results revealed that inoculation of lupine plants with cyanobacteria using different methods increased the mean values of N- uptake of lupine plants compared to that recorded by the control treatment without cyanobacteria. The highest mean value of the of dry + soaking treatment was significantly higher than those given by the other two cyanobacterial treatments including the control treatment.

Concerning the nitrogen rate treatments, the 100 % N rate recorded the highest mean value of N- uptake (477.08 mg N plant^{-1}) that was not significantly higher than 470.63 mg N plant^{-1} for 50 % N rate. However, the N-uptake mean values recorded by 75 and 100 % N rates were significantly higher than that of 50 % N rate. The effect of the interaction between inoculation treatments and N rates was significantly on total N contents. The inoculation treatment (dry + soaking) showed significant superiority over the control and other treatments under the under the N rates of 75 and 100%, but this was not the case under the lowest rate of N (50 %).

Table 4. Effect of cyanobacteria treatments and nitrogen rates on total nitrogen content of lupine plant (nitrogen uptake) (Data are a mean of two seasons)

Cyanobacteria treatment (cyano.)	**Nitrogen rates (%)**			
	50	**75**	**100**	**Mean**
mg N plant^{-1}				
*Control	377.80	420.90	435.70	411.47
Dry	394.10	432.40	437.20	421.23
Soaking	405.30	460.10	470.10	445.17
Dry + Soaking	416.20	569.10	565.30	516.87
Mean	398.35	470.63	477.08	
L. S. D. @ 0.05				
N Rate	**53.30**			
Cyano.	**61.60**			
N x Cyano.	**92.60**			

***Control = without cyanobacteria.**

Effect of cyanobacteria treatments and nitrogen rates on lupine yield, yield components and crude protein:

Data in **Table (5)** indicated the values of lupine yield component, i.e., seed yield, straw yield and 100-seed weight as well as the crude protein content of lupine seeds as influenced by the different cyanobacteria treatments and different nitrogen rates and their interaction. Results revealed that all methods for cyanobacteria inoculation increased the mean values of the lupine seed and straw yields. The treatments of dry + soaking recorded the highest mean values of 1.90 and 3.32 t h-1 for seed and straw yields, respectively. These high mean values were significantly higher the other cyanobacteria treatments.

As for nitrogen rates on lupine seed and straw yields, results noted that increasing the nitrogen rate more than 50 % N increased significantly both the mean values of lupine and straw yields. The highest mean values of lupine seeds and straw yields were recorded by 100% N rate. The relative mean values were 1.95 t ha^{-1} for seed yield against 3.23 t ha^{-1} straw yield. These high mean values were significantly higher those recorded by 75% N rate.

For the interaction effect of both cyanobacteria treatments and nitrogen rates on lupine seed yield, straw yields, results showed that the interaction between 75% N rate and dry + soaking treatment gave significantly the highest seed and straw yields amount as compared to the other interaction treatments. The corresponding high interaction values were 1.98 ton ha^{-1} (seed yield) and 3.49 t ha^{-1} (straw yield).

Same trend noticed for the effect of cyanobacteria treatments, nitrogen rates and their interaction on seed and straw yields was achieved for 100-seed weight, since the highest significant mean value of 49.73 g was recorded by the cyanobacteria treatment dry + soaking compared to those recorded by the other cyanobacteria treatments and the highest significant one of 49.72 g that recorded by 100%N rate compared to those recorded by the tested nitrogen rates. While for the interaction between cyanobacteria treatments and N rates, the interaction treatment of cyanobacteria dry + soaking along with 75% N scored the highest 100-seed weight of 50.52 g compared to the other tested interaction treatments.

Table 5. Effect of cyanobacteria application under different nitrogen rates on lupine yield, yield components and protein content (Data are a mean of two seasons)

Cyanobacteria treatment (cyano.)	**Nitrogen rates (%)**			
	50	**75**	**100**	**Mean**
	Seed yield (t ha^{-1})			
*Control	1.57	1.72	1.96	1.75
Dry	1.64	1.69	1.96	1.77
Soaking	1.68	1.76	1.92	1.79
Dry + soaking	1.79	1.98	1.94	1.90
Mean	1.67	1.79	1.95	
L. S. D. @ 0.05				
N Rate	**0.02**			
Cyano.	**0.03**			
Cyano. x N	**0.05**			
Straw yield (t ha^{-1})				
Control	1.97	2.82	3.15	2.65
Dry	2.81	2.85	3.19	2.95
Soaking	2.93	3.02	3.20	3.05
Dry + soaking	3.12	3.49	3.35	3.32
Mean	2.71	3.05	3.23	
L. S. D. @ 0.05				
N Rate	**0.02**			
Cyano.	**0.03**			
Cyano. x N	**0.05**			
100-Seeds weight (g)				
Control	41.65	45.85	49.28	46.59
Dry	43.78	46.55	50.31	46.87
Soaking	46.15	47.74	49.49	47.79
Dry + soaking	48.88	50.52	49.80	49.73
Mean	45.12	47.67	49.72	
L. S. D. @ 0.05				
N Rate	**0.30**			
Cyano	**0,35**			
Cyano x N	**0,60**			
Crude protein content of lupine seeds (%)				
Control	26.71	32.71	30.40	29.94
Dry	24.03	24.56	24.44	24.35
Soaking	23.94	28.42	26.59	26.31
Dry + soaking	28.23	31.70	26.44	28.79
Mean	25.73	29.35	26.97	
L. S. D. @ 0.05				
N Rate	**1.99**			
Cyano	**2.29**			
Cyano x N	**3.97**			

***Control = without cyanobacteria.**

The crude protein content expressed as mean percent of lupine seeds recorded the highest one of 28.79 in response to the cyanobacteria treatment of dry + soaking compared to the other tested cyanobacteria treatments. While, for nitrogen rate, the 75% N rate gave the highest mean percent of 29.35 compared to those recorded by the other tested N rates. For the interaction effect initiated from the action of cyanobacteria treatments and N rates resulted in the highest significant crude protein content of 31.70 % for the interaction effect of dry + soaking cyanobacteria treatment along with 75% N rate compared to the other tested interaction treatments.

Combined effect of season's variation on the tested parameters along both studied seasons

The analysis of variance for both tested seasons due to the treatments and their interactions of all studied parameters are given in **Table (6)**. Results revealed that there were significant differences in growth, nodules number and dry weight of nodules, shoot nitrogen contents, total count of bacteria, CO_2 evolution, dehydrogenase activity, weight of 100 seeds as well as seed and straw yields and seed crude protein. The second season of 2016/2017 recorded the highest significant values of these parameters than those recorded by the first season of 2015/2016. These results indicated that the season effect is important to be in consideration. The differences between seasons reflecting the differences in weather and the environmental conditions along both studied seasons, suggesting the possibility to rise yield level by choosing the proper time of agriculture and other agronomic practices.

Table 6. Combined statistical for the mean values of tested parameters in both seasons (2015/2016 and 2016/2017)

Parameters	Seasons		L. S. D. @ 0.05
	First	Second	
Shoot dry weight (g plant^{-1})	12.9	14.90	0.08
Nodules numbers plant^{-1}	11.17	17.94	1.32
Nodules dry weight (mg plant^{-1})	480.5	542.7	12.27
Shoot N-content (mg plant^{-1})	335.4	562.0	8.74
Total Count (*cfu X 105 g soil^{-1})	67.1	61.0	1.45
CO_2 Evolution (mg CO_2 100 g dry rhizosphere soil-1 day^{-1})	66.3	75.4	2.54
**DHA (mg ***TPF g dry rhizosphere soil^{-1} day^{-1})	112.3	145.9	11.07
100- Seeds weight (g)	47.20	48.00	0.18
Seeds yield (t ha^{-1})	1.69	1.91	0.05
Straw yield (t ha^{-1})	2.8	3.13	0.04
Crude protein yield in seed (%)	24.54	30.17	1.97

***cfu = Colony forming unit. **DHA= Dehydrogenase activity ***TPF= Tri phenyl formazan.**

DISCUSSION

Effect of nitrogen rates

Results in the current work revealed that application of nitrogen was observed to have a significant influence on number of nodules/plant. Increasing nitrogen rate led to decrease significantly both number and dry weight of nodules of lupine plants. The nitrogen rate of 50 % recorded the higher significant mean values than those recorded by 75 and 100 % N rates. In this respect, **Ntambo et al (2017)** pointed out that increasing nitrogen fertilizer in inoculated plants decreased nodule number and nodule dry weight but enhanced plant height and stem dry weight that led to increase the shoot dry weight of soybean plants. Moreover, **Fagam et al (2007)** also reported decrease in number of nodules with increased level of nitrogen at 30, 60 and 90 kg N ha^{-1} of soybean varieties. However, it is generally accepted that the presence of sufficient levels of nitrogen in the soil, nodulation is inhibited **(Laws and Graves, 2005).** Increasing the nitrogen rate from 50 up to 200 kg urea ha^{-1} increased significantly the crude protein content of sorghum plants **(Almodares et al 2009)**. This trend agrees with those of the current study. At high nitrogen levels, the sorghum roots showed an increase in biomass accompanied by an increase in protein content **(Mrid et al 2016)**. Due to the seed yield of lupine crop, the results of the present study are consistent with **Ntambo et al (2017)** who reported that the application of 200 kg N ha^{-1} significantly increased the soybean yield than 0, 50 and 100 kg N ha^{-1}.

Effect of cyanobacteria inoculation

Inoculation with cyanobacteria increased significantly number of nodules, nodules dry weight, shoot dry weight, biological activity of the rhizosphere lupine plants, yield and yield components. The cyanobacteria treatment of dry + soaking was better than those of either dry or soaking treatment each alone and all compared to the control treatments without inoculation. These results are in accordance with **Haroun and Hussein (2003)** postulated that soaking the seeds of *Lupinus termis*, in cyanobacteria filtrate increased chlorophyll a & b contents in leaves compared to the other treatments without cyanobacteria application. Also. **Ghazal and Teilep (2014)** who reported that canola seeds treated with cyanobacteria as dry + soaking increased significantly all studied parameters including the vegetative parameters and the canola yield as well as the rhizosphere biological activity of canola plants in terms of total bacteria, dehydrogenase activity, and CO_2 evolution. **Zulpa et al (2008)** found that the biomass and extracellular products of the cyanobacteria *Tolypothrix tenuis* and *Nostoc muscorum* increased significantly the soil microbial activity and its nutrients availability. *N. muscorum* and *T. tenuis* biomasses increased the soil oxidizable C (15%; 14%), total N (10%; 12%) and available P (22%; 32%), respectively. In addition, *T. tenuis* extracellular products increased oxidizable carbon by 28% and *N. muscorum* extracellular products increased the available phosphorus by 15%. These increases caused the soil biological activity to be increased also because they are a continuously renewable carbon source. Production of bioactive substances, which accelerate the decomposition process in the soil due to the increase of microbial activity and because they are a continuously renewable organic matter source **(Caire et al 2000)**. They also added that cyanobacteria can increase the soil enzymatic activity. Besides, exopolysaccharide secreted by cyanobacteria are a source of organic carbon for the soil microflora increasing microbial activity **(Storni de Cano et al 2002** and **Ghazal et al 2013)**). Cyanobacteria inoculation enhanced significantly any of total count bacteria, cyanobacteria count, CO_2 evolution, dehydrogenase and nitrogenase activities compared to the control without inoculation. They explained that biofertilization with cyanobacteria increased microorganisms' community and in turn soil biological activity through increasing the organic matter and microbial activity. Cyanobacteria can excrete extracellular polymers of different chemical composition particularly exopolysaccharides that enhance microbial growth that consequently improve soil structure and exoenzyme activity **(Sharma et al 2012). Burjus et al (2014)** inoculation of chickpea with cyanobacteria along with rhizobia resulted in a significant improvement in grain yield and plant biomass as compared to control without inoculation. They also added that the highest increase of total protein content of the chickpea due the same inoculation treatments.

Generally, cyanobacteria as biofertilizer can reconfigure the tiny soil particles into larger spaces between the particles. The larger spaces between the particles improve the flow of water, oxygen, and nutrients to roots, on the other words, increasing the nutrients availability, and in turn increased the soil biological activity **(Boraste et al 2009)**. Consequently, cyanobacteria are recommended to be applied as renewable nitrogen sources for different crop in agriculture. They are nonhazardous, cost effective, utilize renewable resources in addition to solar energy, water and atmospheric nitrogen (**Dobhal and Singhal, 2017)**.

Effect of nitrogen rates and cyanobacteria inoculation interaction

Results in the current study revealed that the interaction between the nitrogen rate 75% and cyanobacteria (dry + soaking) treatment gave the values of all tested parameters, i.e., soil rhizosphere biological activity, total nitrogen content of lupine plants, yield, yield components and lupine seeds content except for nodules number and the dry weight of nodules that recorded their highest values in response to the interaction between 59 % and cyanobacteria (dry + soaking) treatment. **Ghazal et al (2015)** confirmed that the use of cyanobacteria with different methods along with 75 % N improved the pepper yield and its components, its plants N, P and K concentration and content. The pepper yield obtained by the treatment of dry + soaking + billets + 75% N was not significantly differed from those recorded by the control treatment (100 % N). As well as, the use of Dry + Soaking + Billets + 75% N treatment enhanced the soil rhizosphere pepper plants biological activity in terms of total bacterial count, dehydrogenase activity and CO_2 evolution amount. They explained that the use of full nitrogen recommended rate inhibits the biological nitrogen fixation by the diazotrophic bacteria such as cyanobacteria and *Rhizobia*. Even so, the cyanobacteria and rhizobia can do well in the presence in lower nitrogen rate such as that in the current study 75 % N and cyanobacteria (dry + soaking) treatment.

CONCLSION

This study concluded that the use 75 % N and cyanobacteria (dry + soaking) in Lupine production can be comparable to those of using 100% N recommended rate. So, the use of cyanobacteria along with rhizobia as renewable nitrogen source for lupine production can save 25% N from that required for Lupine. The use of biofertilizers are eco-friendly and cost effective. However, more studies are needed to establish this phenomenon.

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مجلة اتحاد الجامعات العربية
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تأثير التلقيح بالسيانوبكتريا والبرادى ريزوبيا على نباتات الترمس فى وجود مستويات مختلفة من النيتروجين فى تربة رملية

[134]

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الكلمات الدالة: نبات الترمس، السيانوبكتريا، برادى ريزوبيم، التلقيح، معدلات النيتروجين، التربة الرملية

الموجـــــــز

تم اجراء تجربة حقلية بمحطة بحوث الاسماعيلية بمحافظة الاسماعيلية خلال موسمين شتويين متتاليين (2016/2015 و2017/2016) لدراسة استجابة نباتات الترمس (صنف جيزة 2) للتلقيح بالسيانوبكتريا بطرق مختلفة فى وجود التلقيح بالريزوبيا. أوضحت النتائج بصفة عامة أن استخدام السيانوبكتريا أدى الى زيادة عدد العقد البكتيرية والوزن الجاف لها والوزن الجاف للمجموع الخضرى ومحتوى النبات من النيتروجين وكذا النشاط الميكروبى فى منطقة ريزوسفير جذور النباتات وكمية الحاصل وبعض مكوناته ومحتوى البذور من البروتين بالمقارنة مع معاملة المقارنة (النباتات غير المعاملة بالسيانوبكتريا). كما أوضحت النتائج أن زيادة معدل التسميد النيتروجينى أدى إلى تقليل عدد العقد الجذرية والوزن الجاف لها. وعلى العكس من ذلك فقد أدى زيادة معدل التسميد النيتروجينى إلى 100% (المعدل الموصى به) الى زيادة القياسات الأخرى تحت الدراسة. كما أوضحت النتائج أن المعاملة (سيانوبكتريا + النقع) فى وجود معدل التسميد النيتروجينى 75% حققت أعلى القيم فى كل القياسات ما عدا عدد العقد والوزن الجاف لها والتى حققت أعلى القيم فى النباتات التى أضيف لها المعاملة (سيانوبكتريا الجافة + النقع) فى وجود معدل التسميد النيتروجينى 50%. وعموما فإنه يمكن الاستنتاج أن التلقيح بالسيانوبكتريا فى وجود التلقيح بالريزوبيا يمكن أن يوفر 25% من كمية السماد النيتروجتنى التى يحتاجها نبات الترمس.

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