The Use of Cyanobacteria as Biofertilizer in Wheat Cultivation under Different Nitrogen Rates

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Abstract: Nowadays, a great attention is paid in establishing concept of the associations between wheat plants and a variety of N_2-fixing microorganisms. This phenomenon has entered the scientific scene arising from the prospects and the possibilities of their potentially application.. In the current study, cyanobacteria inoculation (SBI) was inoculated to wheat in a greenhouse experiment under different rates of nitrogen to find out its impact on wheat yield and its components as well as on some soil properties of the applied sandy soil. Results revealed that inoculation with cyanobacteria generally encouraged the growth of wheat plants. Also, the treatment of 70 % N + SBI gained the highest wheat grain and straw yields, highest total N, P and K contents for both grains and straw. These achieved results were not significantly different from those scored by 100 % N treatment. Due to the soil physical properties, results revealed that inoculation with cyanobacteria increased the proportion of macro-aggregates with a corresponding decrease in the micro-aggregates for the tested sandy soil. Wheat inoculation with cyanobacteria increased both soil organic matter and water holding capacity percentages and in contrast, it decreased the soil bulk density for tested soil. Generally, it is of preliminarily prediction that cyanobacteria inoculation might save almost 30 % of the mineral nitrogen needed for wheat production. Also, it can ameliorate and improve the physical properties of the marginal and poor soils such as the applied sandy soil.
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1. Introduction

The use of the conventional chemical mineral nitrogen fertilizers, which in turn increased crop production, was once considered as a kind of agriculture revolutions which might solve all problems concerning the producing of sufficient food for the ever growing world population. However, this belief was later over-shadowed by the establishment of numerous environmental and social problems associated with the extensive use of agrochemicals in intensive farming systems.

Increasing prices of agrochemicals fertilizers especially nitrogen often leaves farmers with low profit. Uncertain availability of those agrochemicals fertilizers, especially in the developing countries like Egypt, is often a serious constraint for the farmers in their trials to increase crop production. Such problems have attracted the attention of the agriculturists world-wide to look for alternative methods of farming.

In a trial to develop productive, profitable and sustainable agriculture systems, several agriculturalists have been turned to farming methods that are based on biotechnologies. One of the several approaches to achieve this aim is using the nitrogen fixing cyanobacteria (formerly known as blue-green algae) to improve soil fertility and productivity. The use of nitrogen fixing cyanobacteria ensures saving partially the mineral nitrogen required in crop production. Recently, there is a great deal of interest in creating novel association between agronomically important plants, particularly cereal crops such wheat and N_2-fixing microorganisms including cyanobacteria (**Spiller *et al.*, 1993)**. The heterocystus cyanobacteria fellow of the family Nostocacae like *Nostoc* spp. is usual among characterized cyanobacteria in its ability to form tight association with wheat roots and penetrate both roots epidermis and cortical intracellular space **(Gantar *et al.*, 1991)**. The N_2- fixed by *Nostoc* sp. in association with wheat is taken up by the plant and supports its growth, improving grain yields and grain quality **(Gantar *et al.*, 1995)**.

Cyanobacteria comprise a large group of structurally complex and ecologically significant gram-negative prokaryotes that exhibit a wide range of nutritional capabilities ranging from obligate phototrophy to heterotrophy **(Vasudevan *et al.*, 2006 and Prasanna *et al.*, 2009)**, although the majority of forms examined so far exhibit phototrophy. They live wherever there is light in a wide range of terrestrial, freshwater and hypersaline environments, among which soil is the best-studied terrestrial habitat. They are well adapted to a wide range of environmental conditions **a**nd have been widely employed as inoculants for enhancing soil fertility and improving soil structure, besides enhancing crop yields, especially in rice **(Dhar *et al*., 2007)**. The majority of the studies noted in literature do not explain in-depth information regarding the mode of action involved in plant growth stimulation, and only report stimulation of growth yields **(Karthikeyan *et al.*, 2007).**

Therefore, this work aims to investigate the impact of cyanobacterial inoculation with the soil based cyanobacteria inoculum (SBI) on wheat crop grown in sandy soil. The examined parameters were wheat yield, its components, total NPK contents of both wheat straw and grains as well as some physical soil characters.

2. Materials and Methods

A pot experiment was carried out in the greenhouse of Agric. Res. Center, Giza, Egypt to study the impact of cyanobacteria inoculation on and wheat productivity and some soil properties of sandy soil. According to **Jackson (1976)** and **Page (1982)** the experimental soils were sandy in texture with chemical properties of pH 7.5 and EC 0.95, organic matter of 0.30. Pots with 35 cm height and 30 cm in diameter were filled with 8 kg sandy soil each. Before baking the pots, the soil was thoroughly mixed uniformly with phosphate and potassium fertilizers at rates of 100 and 50 kg fed^{-1} in the form of superphosphates (15 % P_2O_5) and potassium sulfate (48 % K_2O), respectively, while nitrogen fertilizer was added to the pots at recommended rates for the treatments. Nitrogen added in two split doses, the first 2/3 N dose was added prior to wheat sowing. The second (1/ 3 N) was added after 30 days from sowing. Six wheat grains were sowed into each pot and when the wheat seedlings developed, two seedlings were thinned out and the other healthy four were left in each pot. Dried flakes from the soil based cyanobacteria inoculum (SBI) that were prepared as described by **Vennkataraman (1972)** and inoculated to pots 10 days after wheat sowing at the rate of 10 kg SBI fed^{-1}. The cyanobacteria inoculation was carried out only for pots received this treatment. SBI composed of different cyanobacteria strains ***viz*** *Nostoc elepsosporum*, *Nostoc linkia* and *Anabaena variabilis*. Pots were irrigated with tap water every two days or when needed.. The experiment includes the following treatment:

1- Control (100 % N).
2- 75 % N + cyanobacteria (SBI).
3- 50 % N + (SBI).
4- Zero N+ (SBI).

The treatments were statistically arranged in a complete randomized design according to **Gomez and Gomez (1984)**.

At harvest, wheat plants in each treatment were collected to determine, wheat yield and its components, N, P and K contents for both grains and straw. As well as, the remained soils in pots after wheat harvesting were sampled to determine some soil properties, i.e.,, soil organic matter **Walkely and Black (1934)**, soil aggregate stability and soil bulk density **(Richards, 1954)** and soil water holding capacity **(Page, 1982).**

All the obtained results were statistically analyzed to compare the means through L. S. D. test at significance probability of 0.05 as according to **Gomez and Gomez (1984).**

3. Results

Wheat yield and its components:

Table (1) show the effect of the soil based cyanobacteria inoculum (SBI) inoculation alone or under the effect of different nitrogen rates on wheat yield and its components cultivated in sandy soil. Results indicated that SBI can compensate partially the nitrogen fertilizer required for wheat production. However, results showed that SBI combined with 75% N rate gained significantly the excellent effect on wheat yield and its components except for both 1000-grain weight and plant height compared to those obtained by the other tested treatments except for those scored by control treatment (100 % N). The corresponding highest significant values were 8 spike $plant^{-1}$, 17.77 g pot^{-1} (dry weight of spikes pot^{-1}), 17.66 g pot^{-1} (grain yield) and 28.43 g pot^{-1} (straw yield). These values were not significantly differed from those given by the use of control (100% N). On the other hand, SBI inoculation had not significantly affected both 1000-grain weight and the plant height of wheat. Although of this trend, both 1000-grain weight and the plant height gave their highest values due to the treatment of 75% N + SBI. Their corresponding highest values were 21.4 g (1000-grain weight) and 69.6 cm (plant height). Nevertheless, these values were not significantly different from those scored by all tested treatments.

Total NPK contents of wheat grains and straw:

Results in Table (2) show that the treatment of 75 % N + SBI inoculation was the superior treatment that increased slightly total NPK contents in grains and straw more than those recorded by 100% N. The results of these two treatments were not significantly different from each other. While, they were significantly higher in total NPK contents for both grains and straw than the other two treatments of 50% N + **SBI** and Zero N% + **SBI**. The highest corresponding total N, P and K contents for 75% N +

SBI treatment were 170.88, 28.74 and 309.89 mg pot^{-1} (straw) and 480.59, 105.96 and 114.79 mg pot^{-1} (grains).

Table (1): Wheat yield components under the effect of SBI inoculation combined with different nitrogen fertilizer rates in sandy soil

Treatments	Spike No. $plant^{-1}$	Spike **DW (g pot^{-1})	Straw DW (g pot^{-1})	Grain DW (g pot^{-1})	1000 - grains (g)	Plant height (cm)
100 % N	7.00	16.73	27.73	16.83	20.60	68.2
75% N+ *SBI	8.00	17.77	28.43	17.66	21.40	69.9
50 % N + SBI	5.00	14.77	22.07	13.23	20.90	68.7
Zero N% + SBI	3.00	11.33	16.27	10.33	20.80	67.90
L.S.D. @ 0.05%	2.65	6.57	4.21	1.29	NS	NS

*SBI = Soil based cyanobacteria inoculum. **DW = Dry weight.

Table (2): Total Nitrogen, phosphorus and potassium contents of wheat plants under the effect of SBI inoculation combined with different nitrogen fertilizer rates in sandy soil

Treatments	Macronutrients uptake (mg pot^{-1})					
	Straw			Grains		
	N	P	K	N	P	K
100 % N	161.25	24.18	302.76	472.65	101.32	106.74
75% N+ *SBI	170.88	28.74	309.89	480.59	105.96	114.79
50 % N + SBI	105.21	17.66	233.94	322.81	52.92	59.90
Zero N% + SBI	51.30	13.01	165.95	216.93	35.83	30.99
L.S.D. @ 0.05%	161.25	24.18	302.76	472.65	101.32	106.74

***SBI = Soil based cyanobacteria inoculum.**

Soil properties:

Soil aggregate stability:

Data in Table (3) show the effect of SBI inoculation either alone or in combination with different nitrogen rates on the sandy soil aggregates stability through using the dry sieving test. Results revealed that inoculation with SBI increased the proportion of macro-aggregates with a corresponding decrease in the micro-aggregates the tested sandy soil. High aggregates proportions were recorded by the aggregates diameter of (0.5 -0.25 mm) for all treatments. The corresponding proportions were 62.33**,** 67.53, 64.5 and 68.61% for the treatments of 100 % N, 75% N + SBI, 50% N + SBI and zero N + SBI, respectively. However, the highest aggregate proportion of 68.61% was for the use of SBI only followed by 67.53 % for 75% N + SBI treatment. On the other respect, the treatment of 100% N + SBI recorded the highest aggregates proportion of 62.33% for the same aggregate diameter of (0.5 -0.25 mm).

Generally, the least soil aggregate proportions were recorded by the soil diameters of 0.125 -0.063 and < 0.063 mm.

Table (3): Dry soil stable aggregates (%) for sandy soil as affected by SBI inoculation combined with different rates of nitrogen fertilizer

Treatments	Fractions of dry sieving aggregate stability						
	Aggregate diameter (mm)						
	> 2	2-1	1-0.5	0.5-0.25	0.25-0.125	0.125-0.063	< 0.063
100% N	0.85	0.68	16.67	62.33	16.08	3.33	0.06
75%N + SBI	0.89	0.63	14.59	67.53	12.45	3.85	0.06
50%N + SBI	0.74	0.66	14.63	64.50	15.77	3.63	0.07
Zero N% + SBI	1.48	0.82	14.75	68.61	11.20	3.09	0.06

***SBI = Soil based cyanobacteria inoculum.**

Soil organic matter, bulk density and water holding capacity percentages:

Table (4) indicates the effect of SBI inoculation on soil organic matter (OM %), bulk density and water holding capacity of the tested sandy soil. Results revealed that SBI inoculation increased OM and WHC % but reduced the bulk density compared to non-inoculated treatment (100% N). Also all the inoculated treatments increased slightly both OM WHC percentages than that recorded by 100% N treatment. The highest percentages of OM and WHC 0.23 and 36.10, respectively, were attained by the treatment of 75 % N + SBI followed by 0.21 and 34.5 % for 50 % N + SBI treatment and the 0.20 and 34.40 % for zero % N + SBI. Also, inoculation with SBI reduced the soil bulk density from 1.72 g cm^{-3} (100 % N) to 1.50 g cm^{-3} (75 % N + SBI). However, both 50 % N + SBI and Zero % N + SBI treatments gave bulk density values of 1.62 and 1.64 g cm^{-3}. These two bulk density values were less than that of 1.72 g cm^{-3} for 100 % N treatment.

Table (4): Soil organic matter, bulk density and water holding capacity percentages as affected by SBI inoculation combined with different rates of nitrogen fertilizer

Treatments	**OM (%)	***BD (g/cm^3)	****WHC (%)
100% N	0.19	1.72	33.90
75%N+ *SBI	0.23	1.50	36.10
50%N + SBI	0.21	1.62	34.50
Zero % N + SBI	0.20	1.64	34.40
LSD @ 0.05%	0.22	0.14	6.33

*SBI = Soil based cyanobacteria inoculum. **OM = Organic matter. *** BD = Bulk density. **** WHC = Water holding capacity.

4. Discussion

Effect of cyanobacteria on wheat growth, yield component and its NPK contents

The impact of cyanobacteria inoculation on the growth, yield productivity and soil physical characteristics was investigated in a greenhouse experiment using sandy soil. Cyanobacteria show a wide ecological and metabolic diversity, and their structural-functional elasticity exhort great versatility, enabling them to adapt and avert a wide range of environments and niches **(Sood *et al*., 2008)**. With the growing realization that chemical based agriculture is unsustainable and is slowly leading to ecological imbalance, the latter part of the last century witnessed the emergence of the concept of ‘‘organic agriculture’’ advocating minimum use of chemical fertilizer and increasing dependence on biological inputs like compost, farmyard manure, green manure and biofertilizers. Amongst the biofertilizers developed for different crops, cyanobacteria constitute the most important inputs in rice cultivation. They form an inexpensive farm grown input, which helps in better crop nutrient management, while working in perfect harmony with nature. Cyanobacteria also raise organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter content of the soil, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants **(Kaushik, 2004).**

However, the limited information is available on their utilization in wheat crop. Most of the work related to cyanobacterial biofertilizers has been in relation to rice crop (**Whitton *et al*., 1988). Gantar *et al*. (1991)** recognized two types of associations with cyanobacteria in wheat were loose associations filaments growing between root hairs, which were typical of the *Anabaena* isolates, and tight associations of micro-colonies in intimate association with the root surface, which were restricted to certain *Nostoc* isolates **Gantar *et al*. (1995)** assessed the role of extracellular polysaccharides in the colonization process by cyanobacteria on wheat roots and characterized the nature of sugars and linkages aiding in the attachment process, but agronomic efficiency was not evaluated. **Karthikeyan *et al.* (2007)** in a pot experiment on wheat using nitrogen fixing cyanobacteria strains under controlled glasshouse conditions found that treatments, which involved cyanobacterial strains (single or in combination) showed visible differences in terms of the appearance of plants. This was accompanied by enhancement in plant height, dry weight and grain yields of wheat crop. Therefore, plant growth stimulation, in terms of plant height, dry weight and grain yields in pot culture experiment can be attributed to IAA-like compounds and photo- eterotrophic/heterotrophic abilities of the cyanobacterial strains. Also they added that in their studies carried out up to harvest stage of wheat crop clearly demonstrated that cyanobacteria enhanced plant growth parameters (plant height, dry weight, grain yields) in addition some significant positive changes in soil microbial biomass carbon. **El-Shinnawy *et al*. (2016) Maqubela and Menkeni (2009)** revealed that application of *Nostoc* caused improvement in the growth and NPK content of maize. They added, increased matter yield by 49% in response to *Nostoc* inoculation, and observed increases in maize tissue NPK following *Nostoc* inoculation mirrored observed improvements in soil N and mineral N content of the soil following inoculation with *Nostoc*. Also, they added that *Nostoc* strain established in the soil improved the soil C, soil N and exo-poymeric substances contents of the soil. The increment of soil N had translated to improved maize growth and N uptake. **EL- Beltagy *et al*. (2016)**

concluded that inoculating wheat with cyanobacteria under 75 % nitrogen increased its dry weight of wheat plants and this results was not significantly different from those recorded by the treatment of 100 % N.

Soil properties:

Good soil structure is important for aeration, root development, and ease cultivation and may minimize or prevent soil erosion losses to wind and water **(Cheshier, 1979)**. Soil conditioners are amendments that either alter soil structure or lower the surface tension of water. Unfortunately, most soil conditioners that alter structure are too expensive for common use in agriculture, and this why the microbiological processes that influence aggregation and soil erosion are accorded attention (**Lynch and Bragg, 1985**). Among these is inoculation with fast growing palmelloid microalgae of irrigated sandy or calcareous soils low in organic matter content that are prone to erosion by wind or water **(Metting and Raburn, 1983)**. In this study cyanobacteria were inoculated into sandy and calcareous soils to improve their expected poor structure. Inoculation led to improve the soil stability aggregates and in turn soil structure. This could be attributed to the heavy growth of cyanobacteria, which exert polysaccharides thereby improving soil aggregation, stimulate some beneficial soil microorganisms, improve soil water holding capacity, soil bulk density and increase soil organic matter **(Rao and Burns, 1990)**. Exopolysaccharide increases the soil organic matter content as a consequence of the sugar derived from the abundant slime mainly secreted by cyanobacteria inoculated to soil in addition to the polymers produced by other microorganisms stemmed in soil in response to cyanobacteria inoculation **(Caire *et al.*, 1997)**. **Omar (1983)** reported that the stability of soil aggregates after potatoes is significantly increased more than that after clover, cotton, tomatoes and corn cultivated without biofertilizer, while inoculation with biofertilizer increased the soil organic matter, soil aggregates stability, water retention and consequently improved the soil structure. He owed these observation noticed with biofertilizer application to the exopolysaccharides secreted by biofertilizers especially those documented in case of algal inoculation applied in tomato cultivation. **Obana *et al.* (2007)** revealed that soil inoculation with *Nostoc* sp. to desert soil has the potential for increasing soil organic matter and reclaiming the degraded soil ecosystems. They added that the developed crust mat of *Nostoc* on the soil surface resulted in reduced evaporation from soil surface and therefore, helped retain the water. **Maqubela *et al.* (2012)** found that the observed improvement in aggregate stability of the soil following inoculation with the three cyanobacteria strains seems to have largely been due to the gluing effect of excreted polysaccharides since the aggregate stability was more strongly associated with exo-polymeric substances than with soil C. Therefore, changes in organic matter content *per se*, due to inoculation seem to have limited effect on aggregation.

Generally, inoculation of cyanobacteria to the marginal poor soil such as sandy or calcareous soils has the ability to ameliorate their aggregates stability especially in sandy soil rather than the calcareous one. This effect is translated to increases improvement in wheat growth, yield and nutrient contents.

In conclusion, it could be predicted that cyanobacteria inoculation can save almost 30 % of the mineral nitrogen required for wheat cultivation. Also it can ameliorate and improve the physical properties of the marginal and poor soils such the sandy soil applied in the present study.

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