

**EFFECT OF CYANOBACTERIA INOCULATION in PRESENCE OR ABSENCE OF DIFFERENT NITROGEN LEVELS ON MAIZE YIELD, YIELD COMPONENTS, SOIL BIOLOGICAL ACTIVITY AND SOIL NATIVE MYCORRHIZAE**

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**ABSTRACT**

A field experiments were carried out at EL- Ismailia Agric. Res. Station (ARC), EL- Ismailia Governorate, Egypt, during the Summer seasons of 2007 to study the influence of cyanobacteria inoculation in different rates (0, 50 and 100% of the recommended dose, i.e., 3.5 kg dried cyanobacteria inoculum fed<sup>-1</sup>) individually or in presence and/ or absence of different nitrogen levels (0, 50 and 100% of the recommended dose, (i.e.), 100 kg N fed<sup>-1</sup>) on maize yield and yield components and the biological activity of the soil remained after maize harvesting as represented by total count bacteria, cyanobacteria count, CO<sub>2</sub> evolution, dehydrogenase (DHA) and nitrogenase (N<sub>2</sub>-ase) activities. As well as, the soil native mycorrhiza were examined at two periods of 45 and 90 days from maize sowing in response to the tested treatments.

Results revealed that all tested treatments increased significantly both maize yield and its components over the control treatment. The highest yield components values were due to 100% N + 100% cyanobacteria and they were comparable to those recorded in presence of 50% N + 100% cyanobacteria treatment. Also the values of maize yield components obtained due to 100% N treatment were not significantly different from those attained due to 100% N + 100% cyanobacteria and 50% N + 100% cyanobacteria treatments. Cyanobacteria inoculation at the rate of 50% combined with 100% N recorded the highest maize protein and carbohydrate percentages. Indefinite trend was noticed of maize oil % in response to cyanobacteria inoculation despite the highest insignificant oil % increments were noticed due to 50% N + 100% cyanobacteria treatment. Also, indefinite response was observed for ash % due to cyanobacteria inoculation. For soil biological activity, cyanobacteria inoculation enhanced significantly any of total count bacteria, cyanobacteria count, CO<sub>2</sub> evolution, dehydrogenase and nitrogenase activities compared to the control treatment received no inoculation. In conclusion, the use of cyanobacteria inoculation technology in cereal crop production such as maize may lead to reduce the amount of mineral nitrogen required for maize production by 50% as well as it ensures good yield quality and safe the environment contaminations resulted from the extensive use of the costly and hazard the so called mineral nitrogen fertilizer.

**INTRODUCTION**

The period since the 1950s has seen exciting advances in understanding biological nitrogen fixation (BNF). Progress in application of BNF technology to agriculture has been slower, but there have been important innovation. While much of the basic BNF research in the last 30 years has been focused on nodulated legumes and rhizobia, there have been relatively rapid advances in knowledge of other N<sub>2</sub>- fixing systems. This

includes the actinomycetes that form nodules on some non-leguminous shrubs and trees, free - living  $N_2$ - fixers associated with cereals and the cyanobacteria. The latter are widely distributed in nature and form prominent autotrophic microbial populations of wetland soils. Reducing the amount of the organic matter, in turn, affect soil aggregates stability. Considering the very low efficiency of applied nitrogenous fertilizer in crop cultivation, may lead to extensive and undue use of chemical fertilizers may lead to serious environmental problems, some of which are accumulation of  $NO_3$  and  $NO_2$  to hazardous levels in the underground water and plant tissues. So, for the production of healthy food, it may be necessary to find out and exploit potential alternative sources of plant nutrients to sustaining soil fertility such as biofertilizers with the minimum addition of chemical fertilizers. Biofertilizers are safe from the environmental point of view, cheaper and at the same time satisfy the nutrient demands of crop plants (Badawy *et al.*, 1996). One of the most promising biofertilizer is cyanobacteria, either as free-living microorganisms or as symbionts with the water *Azolla* fern. Cyanobacteria as biofertilizer utilization in rice fields is common and promising (Venkataraman and Tilak, 1990). Recent researchers have shown that cyanobacteria also help to reduce soil alkalinity and this opened up possibilities for bioreclamation of such inhospital environment. Very recent reports by Thajuddin and Subramanian (2005) showed that cyanobacteria have beneficial effects on a number of other crops rather than rice such as barely, oats, tomato, radish, cotton, sugar cane, maize, chilli and lettuce. They also added that cyanobacteria have received worldwide attention for their possible use in mariculture, food, feed, fuel, fertilizer, and colorant, production of various secondary metabolites including vitamins, toxins, enzymes and pollution abatement. Jagannath *et al.* (2002) found that cyanobacteria inoculation enhanced the overall growth parameters of chickpea. It enhanced all morphological and biochemical characters such as proteins, carbohydrates, total nitrogen uptake, net grain and biomass yield of chickpea. Salem (1999) found that cyanobacteria inoculated to soybean can be successfully overcoming the adverse effect resulted from the saline stress condition. Abd El- Rasoul *et al.* (2004) indicated that inoculation with cyanobacteria to wheat, exhibited an economical view that it can save about 50% of mineral nitrogen amounts required for wheat production. They also showed that this treatment has enhanced the NPK uptake by wheat plants and grains, soil microbial activity in terms of increasing the numbers of soil fungi, *Actinomycetes*, total bacteria, total cyanobacteria count,  $CO_2$  evolution and dehydrogenase activity. El- Gaml (2006) reported that maize inoculation with a mixture of cyanobacteria strains significantly enhanced maize grain yield, NPK uptake by grains and stover, soil organic matter, reduced both soil reaction and soil electrical conductivity, and increased soil particle size aggregates. These benefits achieved due to cyanobacteria inoculation, are in turn increased the nutrients availability to the cultivated plants that ensure high yield and grain quality. Cyanobacteria bring out directly or indirectly a number of changes in the physical, chemical and biological properties of the soil and soil-water interface in inoculated soils. Mandal *et al.* (1999) and Mussa *et al.* (2002) for example revealed that cyanobacteria liberate extra

cellular or organic compounds and photosynthetic O<sub>2</sub> during their growth and contribute biomass. In a cumulative review, Roger and Kulasoorya (1980) reported that besides increasing soil nitrogen fertility, cyanobacteria have been said to benefit rice plants by producing growth-promoting substances. More direct evidence for hormonal effects has come primarily from treatments of rice seedlings with cyanobacterial culture or their extracts. Presoaking of rice seeds in cyanobacteria cultures or extracts has decreased losses from sulphate – reducing processes and this has been attributed to the enhancement of germination and a faster seedlings growth due to cyanobacterial exudates.

This work is designed to study the effect of cyanobacteria inoculation individually to maize variety single hybrid 10 cultivated in sandy soil under different nitrogen levels on maize yield and yield components and the biological activity in soil remained after maize harvesting in terms of total bacteria and total cyanobacteria counts, CO<sub>2</sub> evolution, dehydrogenase and nitrogenase activities. As well as, the soil native mycorrhiza were examined at two periods of 45 and 90 days from maize sowing in response to the tested treatments.

## MATERIALS AND METHODS

A field experiments were carried out at EL- Ismailia Agric. Res. Station (ARC), EL- Ismailia Governorate, Egypt, during the Summer season of 2007 to study the influence of cyanobacteria inoculation in different rates (0, 50 and 100% of the recommended dose, i.e., 3.5 kg dried cyanobacteria inoculum fed<sup>-1</sup>) individually or in combination in presence and/ or absence of different nitrogen levels (0, 50 and 100% of the recommended dose i.e., 100 kg N fed<sup>-1</sup>) on the maize growth, yield and yield components and the biological activity of the soil remained after maize harvesting as represented by total count bacteria, cyanobacteria count, CO<sub>2</sub> evolution, dehydrogenase (DHA) and nitrogenase (N<sub>2</sub>-ase) activities. As well as, the soil native mycorrhiza were examined at two periods of 45 and 90 days from maize sowing in response to the tested treatments. The soil used was sandy in texture, having pH 7.70, available N 21.30 mg kg<sup>-1</sup> (Jackson, 1973), available P 4.20 mg kg<sup>-1</sup> (Olsen *et al.*, 1954) and available K 62.00 mg kg<sup>-1</sup> (Chapman and Pratt, 1961). Prior to maize grains cultivation the uniform recommended practices recommended by the Ministry of Agriculture and Land Reclamation were completed. Phosphate and potassium fertilizers were added at the rates of 30 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> (calcium superphosphate 15.5% P<sub>2</sub>O<sub>5</sub>) and 50 kg K<sub>2</sub>O fed<sup>-1</sup> (potassium sulphate 48% K<sub>2</sub>O<sub>5</sub>).

The experimental area was divided into plots of 3 x 3.5 m and grains of *Zea maize* variety single hybrid 10 were inoculated with cyanobacteria inoculum, which is composed of a mixture of individual strains namely, *Nostoc muscorum*, *Nostoc calcicola*, *Anabaena laxa* and *Clyndrospermum muscicola*. These strains were kindly supplied with Prof. Dr. F. M. Ghazal, Agric. Microbiol. Dept. Soils, Water & Environ. Inst., Agric. Res. Center, Giza, Egypt. Maize grains were then drilled in rows 30 cm apart. Nitrogen fertilizer was applied in 3 levels, i.e., zero, 50 and 100% of the recommended dose

(100 kg N fed<sup>-1</sup>) in the form of urea (46% N). These nitrogen levels were added in two split equal doses, i.e., 20 days after sowing and 50 days later.

The experimental design was in a split plot design with 9 treatments in three replications. Nitrogen fertilization levels of 0, 50, and 100 % N represent the main plot, while the rates of dried cyanobacteria inoculum (0, 50, and 100 %) represent the sub plots. The experiment comprises the following treatments:

- 1- Control without nitrogen and/or cyanobacteria inoculation.
- 2- Zero cyanobacteria 50% N.
- 3- Zero cyanobacteria 100% N.
- 4- Zero N + 50% cyanobacteria.
- 5- Zero N + 100% cyanobacteria
- 6- 50% cyanobacteria + 50% N.
- 7- 50% cyanobacteria + 100% N.
- 8- 100% cyanobacteria + 50%N.
- 9- 100% cyanobacteria + 100%N.

**Analytical procedures:**

At harvest, maize yield and yield components were recorded. The remained soil was sampled and subjected to determine total count bacteria (Allen, 1959), cyanobacteria count (Allen and Stanier, 1968), CO<sub>2</sub> evolution (Pramer and Schmidt, 1964), dehydrogenase activity (DHA) (Casida *et al.*, 1964) and nitrogenase activity (Hardy *et al.*, 1968). Native mycorrhizae were determined by the method described by Phillips and Haynman (1970).

All obtained results for both tested seasons were tabulated and subjected to the combined statistical analysis as described by Gomez and Gomez (1984).

**Examination of soil native mycorrhizae:**

To examine the effect of cyanobacterial inoculation and/or Nitrogen fertilization on the activity of the native VA-mycorrhizal strains in the soil, representative root samples with the surrounding rhizosphere soil were carefully taken, washed and stained in Trypan blue (Phillips and Hayman, 1970). Mycorrhizal colonization ratios were determined after staining the root samples using the gridline intersect method (Giovannetti and Mosse, 1980).

## **RESULTS AND DISCUSSION**

The idea of cyanobacteria inoculation in different rates was monitored in two field experiments conducted at EL- Ismailia Agric. Res. Station (ARC), EL- Ismailia Governorate, Egypt, to investigate its effect of different cyanobacteria inoculation rates in the presence and/or absence of different mineral nitrogen levels on maize yield and yield components, some maize grains technology characters and soil biological activity in terms of total bacteria count, cyanobacteria count CO<sub>2</sub> evolution, dehydrogenase (DHA) and nitrogenase (N-ase) activities. As well as, the soil native mycorrhiza were examined at two periods of 45 and 90 days from maize sowing in response to the tested treatments.

**Maize yield and yield components:**

Data in Table (1) indicate the effect of cyanobacteria inoculation in different rates (0, 50 and 100% of the recommended inoculum rate) in the presence and/or absence of different mineral nitrogen levels (0, 50 and 100% of the recommended –N dose) on maize yield components.

Results revealed that all the tested treatments increased significantly both maize yield and yield components over the control treatment. However, the highest yield components values were 27.30 ardab fed<sup>-1</sup> (grain yield), 2.95 ton fed<sup>-1</sup> (Stover yield), 37.60g (100-grain weight), 24.18cm (ear length) and 228.20 g (grain weight era<sup>-1</sup>) due the treatment received 100% N + 100% cyanobacteria. These high values were not significantly different from those of 26.60 ardab fed<sup>-1</sup> (grain yield), 2.76 ton fed<sup>-1</sup> (stroke yield), 37.30 g (100 – grain weight), 24.06 cm (ear length) and 226.14 g (grain weight ear<sup>-1</sup>) due the treatments received 50% N + 100 % cyanobacteria. Also, it was noticed that the recorded values of maize yield components due to 100% N application were not significantly different from those achieved by both 100% N + 100% cyanobacteria and 50% N + 100% cyanobacteria treatments ( Table1).

Inoculation with cyanobacteria at the rate of 50% combined with any of the different nitrogen levels enhanced all tested maize yield components but without reaching the level of significance between each others. In contrast, inoculation with cyanobacteria at the rate of 100% combined with 50% N increased significantly the maize yield components compared to those recorded due to 50% N + 50% cyanobacteria. These results explain that it is more beneficial to inoculate cyanobacteria at the rate of 100% combined with 50% N in maize production.

**Table (1): Effect of cyanobacteria inoculation and nitrogen fertilization on maize yield components**

Cyanobacteria rate	N. level	Grain yield (ardab fed <sup>-1</sup> )	Stover yield (Ton fed <sup>-1</sup> )	100-grain weight	Ear length (cm)	Grain yield (g ear <sup>-1</sup> )
Zero	Zero	5.30	0.80	31.70	17.20	131.60
	50 %	16.60	1.60	34.00	20.17	189.56
	100 %	26.80	2.70	37.20	22.20	210.95
50 %	Zero	7.20	1.20	32.20	18.60	140.16
	50 %	19.95	2.66	35.60	23.50	200.16
	100 %	21.20	2.80	36.80	23.85	215.85
100 %	Zero	9.30	1.82	32.60	18.92	145.18
	50 %	26.60	2.76	37.30	24.06	226.14
	100 %	27.30	2.95	37.60	24.18	228.20
L. S. D. at 0.05	Nitrogen	2.11	0.52	3.12	2.05	30.60
	Treatment	2.36	0.61	4.65	2.53	40.01
	Interaction	5.01	0.50	NS	NS	NS

Data in Table (2) indicate the soil biological activity for soil remained after maize harvesting in terms of total bacterial count, cyanobacteria count, CO<sub>2</sub> evolution, dehydrogenase (DHA) and nitrogenase (N<sub>2</sub>–ase) activities in response to both cyanobacteria inoculation and nitrogen fertilization, both individually or combined together each in different rates.

In respect to cyanobacteria count, the highest values were recorded due the treatments received 50% N + 50% cyanobacteria and 50 % N + 100% cyanobacteria. The corresponding count values were 14.7 and 16.6 cfu g soil<sup>-1</sup> x 10<sup>3</sup>. However, inoculation with cyanobacteria generally enhanced the cyanobacteria count over those recorded by the control treatment and/or those received different nitrogen levels only.

Same trend was noticed with the soil total bacterial count, since the highest count of 42.10 cfu g<sup>-1</sup> soil x 10<sup>6</sup> was due 50% N + 100% cyanobacteria followed by 30.20 cfu g<sup>-1</sup> soil x 10<sup>6</sup> for 50% N + 50% cyanobacteria. Increasing the nitrogen level up to 100% showed a drastic decrease in the counts of both cyanobacteria and total bacteria count. Also, the treatments received nitrogen only were less in total bacteria count than those received nitrogen combined with cyanobacteria inoculation.

Owing to any of CO<sub>2</sub> evolution, DHA and N<sub>2</sub>-ase, it was detected that inoculation with cyanobacteria led to increase their values over both control (Zero N + zero cyanobacteria) and the treatments received nitrogen only. However, their highest values were 205.00 mg CO<sub>2</sub> 100g soil<sup>-1</sup> (CO<sub>2</sub> evolution), 41.30 mL H<sub>2</sub> g dwt. soil<sup>-1</sup> h<sup>-1</sup> (DHA) and 26.15 μmole C<sub>2</sub>H<sub>4</sub> g dry weight soil<sup>-1</sup> (N<sub>2</sub>-ase). These values were recorded due to 50%N + 100 % cyanobacteria treatment. Generally, inoculation with cyanobacteria enhanced the biological activity of the soil, and this trend was more pronounced in the treatments received 50% N + 50 % cyanobacteria. Also, it is of worth to note that the use of 100% nitrogen caused the soil biological activity to be drastically decreased against the increase noticed with decreasing nitrogen level accompanied with cyanobacteria inoculation.

**Table (2): Effect of cyanobacteria inoculation and nitrogen fertilization on soil biological activity and soil nitrogenase activity**

Cyanobacteria rate	N. level	Cyano count cfu g soil <sup>-1</sup> x10 <sup>3</sup>	Bact. count cfu g soil <sup>-1</sup> x10 <sup>6</sup>	CO <sub>2</sub> evolution mg 100 g soil <sup>-1</sup>	Dehydrogenase activity mL H <sub>2</sub> g soil <sup>-1</sup> h <sup>-1</sup>	Nitrogenase activity μmole C <sub>2</sub> H <sub>4</sub> g dwt soil <sup>-1</sup>
Zero	Zero	5.00	16.00	107	15.20	1.20
	50 %	8.60	24.00	145	18.30	1.60
	100 %	8.10	22.00	121	16.75	0.92
50 %	Zero	9.60	23.80	135	17.75	12.10
	50 %	14.70	30.20	151	22.65	16.20
	100 %	6.20	20.10	195	36.30	14.00
100 %	Zero	12.00	36.00	160	30.20	17.20
	50 %	16.60	42.10	205	41.30	26.15
	100 %	4.30	38.60	140	26.50	17.00

Data in Table (3) indicate the effect of cyanobacteria inoculation in different rates (0, 50 and 100% of the recommended inoculum rate) in the presence and/or absence of different mineral nitrogen levels (0, 50 and 100% of the recommended -N dose) on the soil mycorrhizae colonization.

Results revealed that the sandy soil used in maize cultivation has a remarkable level of effective VA-mycorrhizal fungi (native strains), as indicated from the control treatment being 35.4%, 44.9% and 24.7% for zero,

half dose and full dose of nitrogen fertilization, respectively. The same trend was observed at the second period. Inoculation of soil with cyanobacteria resulted in variable increases in mycorrhizal colonization ratios being more pronounced with those fertilized with half dose of nitrogen at the first period.

On the contrary, at the second period the plants inoculated with cyanobacteria and received zero nitrogen supported highly significant increases in its mycorrhizal colonization ratios as compared to control plants, particularly with those inoculated with full dose cyanobacteria.

**Table (3): Mycorrhizal colonization ratios in broad bean roots as affected by cyanobacterial inoculation and nitrogen fertilization**

Cyanobacteria inoculation rate	Nitrogen Fertilizer levels			Mean
	Zero	½ Dose	Full Dose	
<b>First Period (45 days from sowing)</b>				
Zero	35.4	44.9	24.7	35.0
½ Dose Cyano.	54.9	67.1	52.6	58.2
Full Dose Cyano.	39.3	78.4	60.1	59.3
Mean	43.2	63.4	45.8	
LSD at :	<b>0.05</b>	<b>0.01</b>		
For Cyanobacteria	<b>6.1</b>	<b>8.4</b>		
For Nitrogen	<b>6.1</b>	<b>8.4</b>		
For Cyano. X Nitrogen	<b>10.5</b>	<b>14.5</b>		
<b>Second Period (90 days from sowing)</b>				
				Mean
Control	45.2	55.1	23.6	41.3
½ Dose Cyano.	61.2	77.5	71.7	70.1
Full Dose Cyano.	79.2	91.1	94.3	88.2
Mean	61.9	74.6	63.2	
LSD at :	<b>0.05</b>	<b>0.01</b>		
For Cyanobacteria	<b>5.3</b>	<b>7.3</b>		
For Nitrogen	<b>5.3</b>	<b>7.3</b>		
For Cyano. X Nitrogen	<b>9.2</b>	<b>12.6</b>		

Results of this study emphasized that the inoculation with cyanobacteria to maize at the rate of 100% along with 50 % N dose increased both grain and stover yields over the other tested treatments without significant differences from those obtained by the use of full N dose. This trend stands in well agreement with Abd EL- Rasoul *et al.* (2004) who indicated that all yield wheat parameters increased significantly due to cyanobacteria inoculation combined with 50 % N recommended dose. This may due to that the nitrogen released to soil through nitrogen fixed by cyanobacteria inoculated to soil becomes available to the cultivated plants. Moreover, cyanobacteria are known to excrete extra-cellularly a number of compounds like polysaccharides, peptides, lipids...etc. during their growth in soil, these compounds hold or glue soil particles together in the form of micro-aggregates and hence improve nutrient availability and consequently enhanced the plant growth parameters (Mandal *et al.*, 1999). Dry cyanobacteria surrounded with sheath when inoculated to cereal crops and get moistened due to irrigation and swell up to ten times their dry size and their ability to intercept and store water benefits both the crustal organisms as

well as vascular plants, add to soil organic matter content and increased the soil fertility (Mishra and Pabbi, 2004). Recently, there is a great deal of interest in creating novel association between agronomically important plants, particularly cereals such wheat, maize and rice and N<sub>2</sub>-fixing microorganisms including cyanobacteria (Spiller *et al.*, 1993). The heterocystus cyanobacterium *Nostoc sp.* is usual among characterized cyanobacteria in its ability to form tight association with cereal crops such as wheat and maize roots and penetrate both roots epidermis and cortical intracellular space (Gantar *et al.*, 1991). The N<sub>2</sub>- 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). Inoculation with the nitrogen fixing *Azospirillum* to wheat as biofertilizer combined with ½ recommended N dose increased significantly grain and straw yields and NPK- uptake by grains and straw, improved the grain quality (protein, dry gluten and flour extract percentages) compared to the control without inoculation (AL- Kassas, 2002). Inoculation with cyanobacteria combined with ¼ N dose increased significantly both wheat protein and carbohydrate contents over the control treatment without inoculation and the full nitrogen dose treatments (Gaffar and AL-Kassas, 2005).

Shrivastava and Sinha (1992) showed that the biofertilizers such as cyanobacteria inoculated to cereal crops are likely to assume greater significance as complement and /or supplement to chemical fertilizers in improving the nutrient supplies to cereal crops because of high nutrient turnover in the cereal production system, decreasing cost of fertilizers and greater consciousness on environmental protection. Cyanobacteria have been reported to benefit plants by producing growth promotion substances (the nature of which is said to resemble gibberillin and auxin), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve plant growth and productivity (Zaccaro *et al.*, 2001). Abd El- Rasoul *et al.* (2004) in a field wheat cultivation trial revealed that increasing nitrogen levels from ¼ N to full – N dose increased significantly the carbohydrate wheat grain percentages. They added that cyanobacteria inoculation under different N levels improved wheat grain quality (flour extract percentage, Protein percentage, dry gluten and ash percentage and increased significantly wheat grain carbohydrate percentage over the control treatment without cyanobacteria inoculation. However the highest carbohydrate percentage was due to the treatment inoculated with cyanobacteria + ½ N dose.

Generally inoculation with cyanobacteria increased significantly the soil biological activity in presence or absence of nitrogen over the control treatment with priority of those resulted due to 100% cyanobacteria + 25% N dose treatment. This treatment gave higher values for total cyanobacteria count, total count bacteria, CO<sub>2</sub> evolution, dehydrogenase activity (DHA) and nitrogenase activity compared to the other tested treatments. However, Abd El- Rasoul *et al.* (2004), in wheat, El-Zeky *et al.* (2005) in rice and both Abo EL- Eyoum (2005) and EL-Gmal (2006) in maize found that inoculation with cyanobacteria combined with low level of nitrogen (½ N dose) increased

significantly these biological parameters over the control treatment and their values were comparable to those recorded by the use of the full recommended nitrogen dose. They explained that biofertilization led to increase microorganisms' community in soil through increasing the organic matter, microbial activity and in turn increased dehydrogenase and nitrogenase activities and CO<sub>2</sub> evolution and subsequently improved soil fertility and the plant growth performance. AL- Kassas (2002) reported that Inoculation with the nitrogen fixing *Azospirillum* to wheat increased the soil *Azospirilla* and other microbial population including fungi, actinomycetes and *Azotobacter*, and consequently increased both the dehydrogenase activity and CO<sub>2</sub> evolution, which are considered as index for biological activity and soil fertility (Ghazal, 1980).

Due to the soil native mycorrhizal colonization, the present study indicates that nitrogen fertilization affected more or less the mycorrhizal colonization. In this respect, Gryndler *et al.* (1989) summarized that, the insufficient supply of nitrogen and its high doses caused considerable decreases of mycorrhizal colonization ratios. Also, cyanobacteria inoculation led to increase the soil native mycorrhizal colonization; this can be attributed to the increasing of N<sub>2</sub> fixation achieved by cyanobacteria. It is well observed at the same period that there was no depression effect of nitrogen fertilizers on the mycorrhizal colonization rates particularly with those received cyanobacteria, this may be due to the exhaustion of nitrogen by growing plants to meet their growth demands.

Generally, the positive effect of cyanobacteria on the native mycorrhizal activity was obvious. These results were previously confirmed by the observations of Mollenhauer *et al.* (1996). They found that it is possible to initiate an association between VAM fungi and cyanobacteria. He concluded that the partnership is partly specific; *Nostoc* strains capable of living endocytobiotically are often partners in other symbiosis besides the mycorrhizal fungus *Geosiphon*. The same observation was reported by Schbier (2000) who found that *Glomus Clacoideum* forms an arbuscular mycorrhiza-like symbiosis with the pryophyte *Anthoceros Punctatus*. He found about 1000 mycorrhizal spores were formed in each Petri dish, which was dually inoculated with the two partners.

In conclusion, this work led to take in consideration much attention for establishing the technology of cyanobacteria inoculation to cereal crops with a view of saving partially some of the expensive and none eco-friendly mineral nitrogen fertilizers. Further studies on the other cereal crops rather than maize need to be carried out for more confirmation.

## REFERENCES

- Abd El- Rasoul, Sh. M., Mona M. Hanna, Elham M. Aref and F. M. Ghazal. (2004). Cyanobacteria and effectivemicroorganisms (EM) as possible biofertilizers in wheat production. *J. Agric. Sci. Mansoura Univ.*, 29: 2783 – 2793.

- Abo El- Eyou, A. T. (2005). Studies on the role of cyanobacteria in agriculture. M.Sc. Thesis, Soil Dept. Faculty of Agriculture, Minia University. Minia Governorate, Egypt.
- AL- Kassas, A. R. (2002). Production of wheat and its quality in newly reclaimed lands. Ph. D. Thesis, Fac. of Agric. Al-Azhar University, Cairo, Egypt.
- Allen, M. M. and R. Y. Stanier. (1968). Selective isolation of blue-green algae from Water and Soil. J. Gen. Microbiol., 51: 203 – 209.
- Allen, O. M. (1959). Experiments in soil bacteriology. 1<sup>st</sup> Ed Burgess publishing Co. Minneapolis, Minnesota. USA.
- A.A.O.A.C. (1980). Official Methods of Analysis of the Association of Official Agricultural Chemists, 13<sup>rd</sup> ed. Washington D C., USA.
- Badawy, A. M.; T. M. EL-Katony; M. S. Serag. and M. A. Mousa. (1996). Potentiality of *Azolla filiculoides* Lam. for nitrogen fixation and its use as biofertilizer for rice. Egypt. J. Bot., 36: 109-128.
- Casida, L . E., D. A. Klein and T. Santoro. (1964). Soil dehydrogenase activity. Soil Sci., 98: 371-376.
- Chapman, H. D. and P. E. Pratt (1961). Methods for analysis for soils, plants and waters. Univ. Calif, Div. Agric. Science.
- Dubios, M. A.; J. K. Gilles; P.A. Hamilton and P.A. Smith.(1965). A colorimetric method for determination of sugar and related substances. Anal. Chem., 28: 350.
- El Gaml, Naayem M. M. (2006). Studies on cyanobacteria and their effect on some soil prperties. M.Sc. Thesis, Soil Dept. Faculty of Agriculture, Benha University. Kalubia Governorate, Egypt.
- El- Zeky, M. M., R. M. EL-Shahat, Gh. S. Metwaly and Elham M. Aref. (2005). Using of cyanobacteria or *Azolla* as alternative nitrogen sources for rice production. J. Agric. Sci. Mansoura Univ., 30: 5567 – 5577.
- Gaafar,E. M. and A. R. AL-Kassas. (2005). Do cyanobacteria and Effective microorganisms affect wheat yield and grain quality. J. Agric. Sci. Mansoura Univ., 30: 547 – 559
- Gantar, M., Kerby, N. W. and P. Rowell (1991). Colonization of wheat (*Triticum vulgare* L.) by N<sub>2</sub>-fixing cyanobacteria: I. A survey of soil cyanobacterial isolates forming association with roots. New Phytol., 118: 477-483.
- Gantar, M., Rowell, P. and N. W. Kerby (1995). Role of extracellular polysaccharides in the colonization of wheat (*Triticum vulgare* L.) roots by N<sub>2</sub>-fixing cyanobacteria. Biol. Fertl. Soils. 19: 41-48.
- Ghazal, F. M. (1980). Studies on the enzymatic activity in rice soils inoculated with blue- green algae. M.Sc. Thesis, Fac. Agric., Al-Azhar Univ., Cairo, Egypt.
- Giovannetti, M. and B. Mosse (1980). An evaluation of techniques for measuring Vesicular-arbuscular mycorrhizal infection in roots. New Phytol., 84: 489-500.
- Gomez, K. A. and A. Arturo, Gomez. (1984). Statistical procedures for Agricultural research, (2<sup>nd</sup> ed.), pp. 20-29 & 359-387.

- Gryndler, M.; Lestina, J.; Moravec, V.; Prikryl, Z. and Lipavsky, J. (1989). Colonization of maize roots by VAM fungi under conditions of long-term fertilization of varying intensity. *Agric. Ecosystems and Environ.*, 29: 183-186.
- Hardy, R. W. F., R. D. Holsten and R. C. Burn. (1968). The acetylene-ethylene assay for N<sub>2</sub>-fixation: Laboratory and field evaluation. *Plant. Physiol.*, 43:1185-1207.
- Jackson, M. L. (1973). *Soil Chemical Analysis*. Constable. Co. Lt., London.
- Jagannath, S. B. A., D. Umapati and E. Sedamakar (2002). Algalization studies on chickpea (*Cicer arietinum* L). *Biotechnology of Microbes and Sustainable Utilization*. 145-150.
- Mandal, B. K., P. L. G. Vlek and L. N. Mandal. (1999). Beneficial effects of blue-green algae and *Azolla*, excluding supplying nitrogen, on wetland rice fields: a review. *Biol. Fertl. Soils*. 28: 329-342.
- Mishra, U. and S. Pabbi. (2004). Cyanobacteria: A potential biofertilizer for rice. *Resonance*. 6: 6 -10.
- Mollenhauer, D.; R. Mollenhauer; and M. Kluge (1996). Studies on initiation and development of the partner association in *Geosiphon pyriforme* (K-tz) v. Wettstein, a unique endocytobiotic system of a fungus (*Glomales*) and the Cyanobacterium *Nostoc punctiforme* (K-tz.) Hariot. *Protoplasma*(Historical Archive), 193 (1-4): 3-9.
- Mussa, S. A. I., M. M. Hanna and F. M. Ghazal. (2003). Effect of cyanobacteria wheat association on wheat growth and yield components. *Egypt. J. Biotechnol.*, 14: 164-174.
- Mussa, Sanaa, A.I., S. T. A. Tantawy and F. M. Ghazal. (2002). *Azolla* and cyanobacteria as possible nitrogen biofertilizer source in rice production. *Egyptian J. Phycol.*, 3: 93 -101.
- Olsen, S. R.; C. V. Cok, F.S. Watanabe and L.A. Dean (1954). Estimation of available phosphorus in soil by sodium bicarbonate. *U S. Dept. Agric. Circ.*, USA, 939.
- Phillips, J.M. and D.S. Hayman (1970). Improved procedures for clearing roots and staining parasitic and Vesicular-arbuscular fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 55: 158-161.
- Roger, P. A. and S. A. Kulasooriya. (1980). Blue-green algae and rice. Edt. *International Rice Res. Inst.*, Manila, Loss BÖns, Philippines, pp.51-52.
- Salem, H. and M. Foda. (1999). Cyanobacterial effect on growth and chemical composition of soybean grown under saline conditions. *Arab Univ. J. Agric. Sci.*, Ain –Shams Univ., Kalubia, Egypt. 7: 433 – 446.
- Schbier,, A. (2000). *Glomus claroideum* forms an arbuscular mycorrhiza-like symbiosis with the hornwort *Anthoceros punctatus*
- Shrivastava, U. K. and N. K. Sinha (1992). Response of Zea mays and wheat (*Triticum aestivum*) to *Azotobacter* inoculation and fertilizer application. *Indian J. Agron.*, 37: 356-357.
- Spiller, H., W. Stallings, T. Woods and M. Gunasekaran. (1993). Requirement for direct association of ammonia-excreting *Anabaena variabilis* mutant (SA-1) with roots for maximal growth and yield of wheat. *Appl. Microbiol. Biotechnol.*, 40: 557-566.

- Thajuddin, N. and G. Subramanian. (2005). Cyanobacterial biodiversity and potential applications in biotechnology. Current Science. 89: 47- 57.
- Venkataraman, G. S. and K. B. V. R., Tilak. (1990). Biofertilizer: An important component of itegrated plant nutrient supply in dry lands. In: fifty Years of Dryland Agriculture Research in India(eds. H. P. Singh, Y. S. Ramakrishna, K. L. Sharma and B. Venkateswarlu), Central Research Institute for Dryland Agriculture, Hyderabad, India, Pp. 379-394.
- Walkley, A. and I. A. Black. (1934). An examination of the Degtrarrf method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.
- Zaccaro, M. C., C. Salazar, Zulpa de G. Caire, Stroni de M. Cans and A. M. Stella. (2001). Lead toxicity in cyanobacterial prophyrin metabolism. Environ. Toxocol. and Water Quality. 16: 61 -67.

### أثر التلقيح بالسيانوبكتريا في وجود أو عدم وجود مستويات مختلفة من النيتروجين على محصول الذرة ومكوناته والنشاط البيولوجي لتربة وكذا الميكوريزا المتوطنة بالتربة

السيدة على حسن ، فتحى توفيق ميخائيل و فكرى محمد غزال  
معهد بحوث الأراضو المياه والبيئة- مركز البحوث الزراعية- الجيزة- مصر

- اجريت تجربتين حقلية بمزرعة محطة بحوث الإسماعيلية – محافظة الإسماعيلية (مركز البحوث الزراعية) وذلك لدراسة أثر التلقيح بالسيانوبكتريا بمعدلات مختلفة منفردة أو بمصاحبة مستويات مختلفة من النيتروجين على محصول الذرة ومكوناته وبعض صفاته التكنولوجية وكذا النشاط البيولوجي فى التربة بعد حصاد الذرة. وقد كانت أهم نتائج ما يلي:-
- 1- كل المعاملات تحت الدراسة أدت الى زيادة معنوية فى محصول الذرة ومكوناته.
  - 2- سجلت أعلى قيم لمكونات محصول الذرة استجابة للمعاملة 100% نيتروجين + 100% سيانوبكتريا وكانت هذه القيم غير مختلفة معنويا عن تلك المسجلة استجابة للمعاملة 50% نيتروجين + 100% سيانوبكتريا أو المعاملة 100% نيتروجين فقط..
  - 3- بالنسبة للنشاط البيولوجي للتربة فقد لوحظ أن التلقيح بالسيانوبكتريا أدى الى زيادة أعداد كل من السيانوبكتريا والميكروبات الكلية بالتربة وكذا كمية ثانى أكسيد الكربون المتصاعدة ونشاط كل من انزيمى الديهيدروجينيز والنيتروجينيز.
  - 4- أدى التلقيح ب 100% سيانوبكتريا فى وجود 50% نيتروجين الى زيادة نسبة الميكوريزا المتوطنة بالتربة.
  - 5- من هذه الدراسة يمكن استنتاج أن استخدام تكنولوجيا التلقيح بالسيانوبكتريا فى انتاج محاصيل الحبوب مثل الذرة يمكن أن يوفر حوالى 50% من النيتروجين المعدنى اللازم لانتاج الذرة مع ضمان بيئة نظيفة. وعلى أى حال فإن استخدام تكنولوجيا التلقيح بالسيانوبكتريا يحتاج الى المزيد من التجارب مع محاصيل الحبوب الأخرى لزيادة التأكد من النتائج.