J. Agric. Sci. Mansoura Univ., 29(5): 2941 - 2948, 2004

EFFECT OF CYANOBACTERIA-WHEAT ASSOCIATION ON WHEAT PRODUCTION AND SOIL FERTILITY

Mona M. Hanna; Elham M. Aref and F. M. Ghazal
Agric. Microbiol. Dept., Soils, Water and Environ. Res. Institute, Agric. Res. Center, Giza, Egypt

ABSTRACT

At present, a great interest in establishing novel associations between higher plants and a variety of N_2-fixing microorganisms has entered the scientific scene arising from the prospects and the possibilities of their potentially application. Data presented is obtained during the co-cultivation of local cyanobacteria strains previously isolated from the Egyptian rice soils and wheat. Results revealed that cyanobacteria inoculation (SBI) exhibited an economical view that it can save about 30 % of the mineral nitrogen amounts required for wheat crop production. The trend was noticed when SBI inoculation was applied at the rate of 3 kg ha^{-1} along with 80 kg N ha^{-1} which recorded a grain yield not significantly different from that obtained by 120 kg N ha^{-1} the recommended nitrogen dose in wheat cultivation. Cyanobacteria inoculated to wheat crop have also improved the soil microbial community, and soil fertility.

Keywords: Cyanobacteria, wheat association, soil fertility.

INTRODUCTION

The modern day intensive crop cultivation requires the use of nitrogen fertilizers. However, fertilizers are short supply and expensive in developing countries such Egypt. Therefore, it is important to explore the possibility of supplementing nitrogen fertilizers with biofertilizers of microbial origin. Microbial processes are fast and consume relatively less energy than industrial processes.

Microbial inoculants are carrier-based preparations containing beneficial microorganisms in a viable state intended for seeds or soil application and designed to improve soil fertility as well as plant growth.

The application of N_2-fixing cyanobacteria as biofertilizers in the cultivation of wet-land rice has beneficial effect on growth and yield (De 1939; Venkataraman, 1979; Swaminathan, 1982; Grant *et al.*, 1986 and Mule *et al.*, 1999). Reports on the effect of cyanobacteria on growth of other crops other than rice are, however, scarce (Henricksson 1971; Pachpande 1990, Nanda *et al.*, 1991 and Gantar *et al.*, 1995).

On the other respect, many microorganisms bear extra-cellular sheaths of considerable thickness external to their outer membrane, providing a protective and favorable microenvironment. These sheaths are usually composed mainly of polysaccharides. In plant-microbe associations, polysaccharide, regardless of whether it is plant or microbial origin, may enable a close contact to take place between the two partners that required for their symbiotic or parasitic relationships. N_2-fixing cyanobacteria are capable of forming symbiotic associations with various plants and fungi (Bergman *et al.*, 1992). Recently, there is a great deal of interest in creating novel associations between agronomically important plants, particularly cereals, and N_2-fixing microorganisms including cyanobacteria (Spiller *et al.*, 1993). The heterocystous Cyanobacterium *Nostoc sp.* is usual among characterized cyanobacteria in its ability to form tight associations with wheat roots and to penetrate both root epidermis and cortical intracellular space (Gantar *et al.*, 1991 a&b). The N_2 fixed with *Nostoc* sp. in association with wheat is taken up by the plant and supports its growth (Gantar *et al.*, 1995).

The aim of this work is to study the possibility to substitute a part of the mineral nitrogen applied in wheat production by the nitrogen fixed by cyanobacteria as biofertilizer as well as to explore the effect of cyanobacteria on wheat yield components and soil fertility.

MATERIALS AND METHODS

A field experiment was carried out in a private farm at Kashish, Shibin EL-Kanter, Qualubiya Governorate during the winter season of 2001 / 2002. The soil of the experimental plots was clayey with pH 7.55, organic matter 2.19 % (Walkley and Black, 1934), total nitrogen 0.20 % (Jackson, 1976) and available P 0.02% (Olsen *et al.*, 1954).

The field was prepared by ploughing and puddling. It was then divided into 21 plots (3.5 m × 3.5 m each) representing 7 treatments with three replicates in randomized block design. The treatments consisted of control (no nitrogen), full recommended dose (RD) of the dried flakes of cyanobacteria soil based inoculum SBI (10 kg ha^{-1}), urea nitrogen at 120 kg Nha^{-1}, 5 kg SBI ha^{-1} + 60 kg Nha^{-1}, 3 kg SBI ha^{-1} + 80 kg Nha^{-1}, 6 kg SBI ha^{-1} + 40 kg Nha^{-1} and 10 kg ha^{-1} SBI + 120 kg Nha^{-1}.

Wheat seeds variety Giza 168 were sown on December 20, 2001 and harvested on May 15, 2002. Uniform application of super-phosphate (15 % P_2O_5) at a rate of 100 kg ha^{-1} was done as basal to each plot. Cyanobacteria inoculation was executed 7 days after sowing of wheat seeds. Urea nitrogen treatments were applied in two equal doses 10 days after sowing and 35 days later. Irrigation was carried out from sowing time and then at every 20 days intervals.

At harvest wheat yield components such as straw and grain yield (tons ha^{-1}), 1000-grain weight (g), weight of grains m^{-2}, plant height (cm), number of grains $spike^{-1}$, number of spikes m^{-2}, total nitrogen uptake (kg Nha^{-1}) and harvest index in percent (Yanni,1991).

$$\frac{\text{Kg grain}}{\text{kg grain yield} + \text{kg straw yeild}} \times 100$$

Carbon di-oxide evolution (Pramer and Schmidt, 1964) and dehydrogenase activity (DHA) (Casida, *et al.*, 1964) in soil after were also estimated. All obtained data were then subjected to the statistical analysis according to Gomez and Gomez, (1984).

The cyanobacteria soil based inoculum (SBI) was prepared in the field by the method of Vennkataraman, (1981) as a mixture of nitrogen fixing cyanobacteria strains viz. *Anabaena* sp. and *Nostoc* sp. which were locally isolated, purified and identified as described by (Ghazal, 1992).

RESULTS AND DISCUSSION

1- Wheat yield components:

Table (1) indicates the effect of cyanobacteria inoculation and / or urea-N fertilization either individually or combined together with different levels on the growth and the yield components of wheat crop variety Giza 168.

Results revealed that all the tested treatment increased significantly straw and grain yields over the control treatment except for the grain yield due to the 10 kg SBI ha^{-1} treatment. The highest grain and straw yields were attained by the use of 120 Kg N ha^{-1} treatment. The corresponding yield amounts were 9.02 and 9.85 tha^{-1}, respectively. However, the highest grain yield was not significantly different from those of 8.34 and 8.95 tha^{-1} for both 3 kg SBI + 80 kg Nha^{-1} and 10 kg SBI + 120 kg Nha^{-1}, respectively. These two treatments were also not significantly different from each other. The inoculation with 10 kg SBI (cyanobacteria inoculum) alone slightly raised the grain yield insignificantly over the control treatment.

Owing the straw yield, the highest yield of 9.85 tons ha^{-1} was not significantly different from those of 9.00 and 9.75 tons ha^{-1} due to the treatment of 3 kg SBI + 80 kg Nha^{-1} and 10 kg SBI + 120 kg Nha^{-1}, respectively. However, these two straw yield amounts were not significantly different from each others.

1000-grain weight recorded for 10 kg SBI ha^{-1} (48.58 g) was significantly higher than those recorded by the other treatment. However no definite trend could be observed in response to the tested treatment.

The plant height of wheat plants exhibited significant increases over the control treatment (80.8 cm) except for 10 kg SBI treatment (86.50 cm). The highest plant height measurement (95.50 cm) was recorded for 10 kg SBI + 120 kg Nha^{-1} treatment. This high plant height value was significantly different from both control, 10 kg SBI and 6 kg SBI ha^{-1} + 40 kg N ha^{-1}.

The number of grains $spike^{-1}$ recorded the highest value of 60 grains $spike^{-1}$ by 10 kg SBI + 120 kg Nha^{-1} treatment. This high number of grain $spike^{-1}$ was not significantly different from those of 58,55 and 53 grains $spike^{-1}$ due to 120 kg N, 5 kg SBI + 60 kg N and 3 kg SBI + 80 kg Nha^{-1}, respectively.

In view of straw and grain yields m^{-2}, it was observed that they followed identically the trend noticed with both straw and grain yields ha^{-1}.

Harvest index percentage fluctuated within a relatively narrow range indicating no definite trend for the treatments effects. However, the highest harvest index percentage (48.10) was due to 3 kg SBI ha^{-1} + 80 kg Nha^{-1} treatment. Also, no significant differences were noted amongst all nitrogen and SBI treatments either each alone or combined together.

In respect to the nitrogen uptake by wheat crop, all treatments exceeded significantly the N-uptake amount over the control treatment (50.32 kg Nha^{-1}). The highest total N-uptake by wheat crop of 165.88 kg Nha^{-1} was significantly higher than those recorded by 3 kg SBI ha^{-1} + 80 kg-N ha^{-1} and 10 kg SBI ha^{-1} + 120 kg N ha^{-1} treatments. This attitude led to conclude that the use of 3 kg SBI ha^{-1} + 80 kg Nha^{-1} (148.62 kg Nha^{-1}) could economically satisfy the recommended level of nitrogen (120 kg Nha^{-1}).

Table (1): Effect of cyanobacteria (SBI) inoculation and nitrogen fertilization on wheat yields components.

Treatments	Yields ($t\ ha^{-1}$)		1000-grain weight (g)	No. of grains spikes $^{-1}$	Plant Height (cm)	Nitrogen %		Total N Uptake kg N ha^{-1}	Harvest index %
	Straw	Grains				Straw	grains		
Control	6.30	5.00	46.33	43	80.80	0.14	0.83	50.32	44.31
10 kg SBI ha^{-1}	7.00	6.00	48.58	47	86.50	0.15	1.10	79.50	46.15
120 kg-N ha^{-1}	9.85	9.02	43.59	58	90.50	0.28	1.50	165.88	47.80
5kg SBI ha^{-1} + 60 kg-N ha^{-1}	8.20	7.40	42.67	55	93.20	0.25	1.38	122.62	47.43
3kg SBI ha^{-1} + 80 kg-N ha^{-1}	9.00	8.34	41.59	53	95.20	0.27	1.48	147.73	48.10
6kg SBI ha^{-1} + 40 kg-N ha^{-1}	8.00	6.60	40.41	45	87.15	0.22	1.35	106.70	45.21
10kg SBI ha^{-1} + 120 kg-N ha^{-1}	9.75	8.95	43.12	60	95.50	0.23	1.42	149.52	47.86
L . S . D . < 0.05	2.01	1.30	2.24	10.59	6.14	0.11	0.53	27.38	4.34

2- Soil microorganisms:

In respect to microorganism community in soil (Table 2), all the tested treatments significantly increased the numbers of microorganism count over the control treatment. However, 3 kg SBI ha^{-1} +80 kg N ha^{-1} treatment gave the highest bacteria count compared to other treatments. The corresponding values were 38.5 x 10^{-6} (total bacteria). But 5 kg SBI ha^{-1} + 60 kg-N ha^{-1} gave the highest count of total fungi, actinomycetes and *Azotobacter*. While, 6 kg SBI ha^{-1} + 40 kg-N ha^{-1} was gave maximum count of *Azospirillium*.

Table (2): Effect of cyanobacteria (SBI) inoculation and nitrogen fertilization on soil microbial community.

Treatments	Total bacteria x 10^{-6}	Total fungi $x10^{-3}$	Total Actinomycetes $x10^{-4}$	Nitrogen fixing bacteria	
				Azospirillum $x10^{-5}$	Azotobacter $x10^{-5}$
Control	7.70	12.60	25.80	2.30	1.60
10 kg SBI ha^{-1}	9.80	4.20	11.90	6.70	4.80
120 kg-N ha^{-1}	12.40	8.90	7.20	4.20	2.40
5kg SBI ha^{-1} + 60 kg-N ha^{-1}	28.20	29.10	36.30	7.90	8.80
3kg SBI ha^{-1} + 80 kg-N ha^{-1}	38.50	2.40	15.90	5.20	6.30
6kg SBI ha^{-1} + 40 kg-N ha^{-1}	20.20	5.80	9.70	9.00	7.70
10kg SBI ha^{-1} + 120 kg-N ha^{-1}	16.70	18.50	19.40	3.20	3.40

3- Soil Biological activity:

The soil biological activity as represented by dehydrogenase activity and CO_2 evolution are indicated in (Table 3). A similar behavior to that observed in soil microorganism numbers in response to the tested treatments. Herein, 3 kg N ha^{-1} +80 kg N ha^{-1} gave the highest dehydrogenase and CO_2 evolution values compared to other treatments. The corresponding DHA and CO_2 evolution values were 64.33 mg TPF 100 g $soil^{-1}$ and 829.67 mg CO_2 100 g $soil^{-1}$, respectively.

Such results are in agreement with those described by (Abd-Alla *et al.*, 1994) who attributed the increase in wheat growth parameters to the substantial increases of N_2 fixation in soil due to nitrogenase activity of the cyanobacteria inoculation. Consequently, this could explain that the reduction of the recommended nitrogen dose required for wheat production, cyanobacteria could compensate this reduction either it was ½ (60 kg Nha^{-1}) or 2/3 (80 kg Nha^{-1}) the nitrogen recommended dose. They also added that inoculation of wheat with cyanobacteria either alive or killed led to a significant increase in wheat dry-matter accumulation over control treatments. El-Mancy *et al.*, (1997) revealed that cyanobacteria inoculation (SBI) to rice significantly increased both rice grain and straw yields to the extent of 2.07 and 17.06 % over the control, respectively. Combination of SBI inoculation with N and P chemical fertilizers can lead to saving chemical N fertilizer (about 50 %) improving NPK uptake and N and P recovery, reducing the environmentally bad effects of the high doses from chemical fertilizers and consequently increasing the possibility for producing high and good rice yield.

Inoculation of rice fields with cyanobacteria (SBI) might help to regenerate quickly and improve the soil structure. SBI are known to excrete extracellularly a number of compounds like polysaccharides, peptides, lipids etc. during their growth in soil particles and hold /glue them together in the form of micro-aggregates (Mandal *et al.*, 1999). This soil improvement due to cyanobacteria inoculation being reflected on soil fertility and consequently on cultivated crop. Gantar (2000) emphasized the cyanobacteria-wheat association and stated that when wheat seedling are co-cultivated with *Nostoc* sp. In hydro-ponics, the cyanobacteria colonizes the endorhizosphere at low frequency. He suggested that mild sonication of the roots dramatically increased the number of cyanobacteria within the root tissues. The cyanobacteria penetrated the roots in the form of motile filaments (hormogonia), at once inside, they divided and transformed into a seriate packages, which showed nitrogenase activity. Thus, co-cultivation of wheat with cyanobacteria could partially meet the wheat nitrogen needs.

Table (3): Effect of cyanobacteria (SBI) inoculation and nitrogen fertilization on soil dehydrogenase activity (DHA) and CO_2 evaluation.

Treatments	DHA mg *TPF 100 g $soil^{-1}$	mg CO_2 evolution 100 g $soil^{-1}$
Control	9.71	408.00
10 kg SBI ha^{-1}	24.36	500.33
120 kg-N ha^{-1}	17.53	507.00
5kg SBI ha^{-1} + 60 kg-N ha^{-1}	64.33	704.00
3kg SBI ha^{-1} + 80 kg-N ha^{-1}	47.88	829.67
6kg SBI ha^{-1} + 40 kg-N ha^{-1}	31.81	629.33
10kg SBI ha^{-1} + 120 kg-N ha^{-1}	38.95	582.00
L . S . D . < 0.05	6.38	46.90

*TPF (tri- phenyl formazan)

EL-Kasas, (2002) reported that *Azospirillum* inoculation to wheat increased Azospirilla and other microbial population including fungi, actinomycetes and Azotobacter, and consequently increased the dehydrogenase activity and CO_2 evolution as indices for biological activity and soil fertility (Abd EL-Aal *et al.*, 1982).

Reducing nitrogen level up to 80 kg N ha^{-1} obviously encouraged the soil microorganisms to proliferate and increase in number. This trend could be explained by that increasing nitrogen level over 80 kg N ha^{-1} lead to inhibit the growth of microorganisms especially the nitrogen fixing ones (EL-Kasas, 2002).

Generally, cyanobacteria as biofertilizers are a promising alternative to avoid soil pollution caused by agrochemicals and recover the nutrient content and Soil structure lost after as they bring to soil combined nitrogen (some of them are N- fixers and secrete exopolysaccharides) that improve soil structure and bio-active substances that enhance the plant growth.

REFERENCES

Abd-Alla, M. H., A. L. A. Mahmoud and A. A. Issa (1994). Cyanobacterial biofertilizer improved growth of wheat. Phyton, 34, 1: 11-18.

Abd El Aal, R. S., A. M. Shatta, M. S. Khadr and F. M. Ghazal, (1982). Comparative studies on N-fixing blue-green algae and N-fertilization on rice growth, N-balance and dehydrogenase activity in two types of soil. Ain Shams Universty, Agric. Faculty Res. Bull. 2014: 1-15.

Bergman, B., Rai, A. N., C. Johansson and E. Soderbak (1992). Cyanobacterial-plant symbioses. Symbioses. 14: 61-81.

Casida, L . E., D. A. Klein, and T. Santoro (1964). Soil dehydrogenase activity. Soil Sci. 98: 371-376.

De, P. K. (1939). The role of Blue-green algae in nitrogen fixation in rice fields, Proc. R. Soc., 127 B, 121-139.

EL- Kasas, A. R. (2002). Production of wheat and its quality in newly reclaimed lands. Ph.D Thesis, Fac. Of Agric. Al-Azhar University., Cairo, Egypt.

El-Mancy, M. H., M. Th, A. Kotb, Kh, H. El-Hamdi and S. A. Hammad (1997). N, P and K contents of rice crop in relation to algalization combined with N, P fertilization. J. Agric. Sci., Mansoura Univ., 22 (9): 3053-3065.

Gantar, M. (2000). Mechanical damage of roots provides enhanced colonization of wheat endosphere by the dinitrogen-fixing Cyanobacterium *Nostoc* strain 2S9B. Biol. Fertil. Soils. 32: 250-255.

Gantar, M., N. W. Kerby and P. Rowell (1991a). Colonization of wheat (*Triticum egetable* L.) by N_2-fixing cyanobacteria: I. A survey of soil cyanobacterial isolates forming association with roots. New Phytol. 118: 477-483.

Gantar, M., N. W. Kerby and P. Rowell (1991b). Colonization of wheat (*Triticum _egetable* L.) by N_2-fixing cyanobacteria: II. An ultrastructural study. New Phytol. 118: 485-492.

Gantar, M., P. Rowell and N. W. Kerby (1995). Role of extra- cellular polysaccharides in the colonization of wheat (*Triticum _egetable* L.) roots by N_2-fixing cyanobacteria. Biol. Fertil. Soils. 19: 41-48.

Ghazal, F. M. A. (1992). Algae culture collection. In: Biofertilizer germplasm collection at IRRI. (International Rice Research Institute, Manila Phlippines. 53-59.

Gomez, K. A. and A. Arturo, Gomez (1984). Statistical procedures for Agricultural research, (2nd ed), pp. 20-29 & 359-387.

Grant, I. F., P. A. Roger and I. Watanabe (1986). Ecosystem manipulation for increasing biological N2 fixation by Blue-green algae (cyanobacteria) on lowland rice fields. Biol. Agr. Horticul. 3: 299-315.

Henricksson, E. (1971). Algal nitrogen fixation in temperate regions. In: Lie T. A. and Mulder E. D. (eds.) Biological nitrogen fixation in natural and agricultural habitats. Plant and Soil, Special volume pp. 415-419.

Jackson, M. L. (1976). Soil chemical analysis. Prentice-Hall, Englewood Califfs, New Jersey.

Mandal, B. K., P. L. G. Velk and L. N. Mandal (1999). Beneficial effects of blue-green algae and *Azolla*, excluding supplying nitrogen, on wetland rice fields: a review. Biol. Fert. Soils, 28: 329-342.

Mule, M. C. Z., G. Z. Caire, M. S. Cano, M. R. Palma and Karina Colombo. (1999). Effect of cyanobacterial inoculation and fertilizers on rice seedlings and postharvest soil structure. Commun. Soil, Sci. Plant Anal., 30: 97-107.

Nanda, B., S. K. Tripathy and S. Padhi (1991). Effect of algalization on seed germination of vegetable crops. W. J. Microbiol. Biotech. 7: 622-623.

Olsen, S. R.; C. V. Colc, F. S . Watanabe and L. A . Dean (1954). Estimation of available phosphorus in soil by sodium bicarbonate. U. S. Dep. Agric. Circ., 939.

.Pachpande, R. R. (1990). Role of algal biofertilizer for increasing yield of irrigated plantation crops. In: National Symposium on Cyanobacterial Nitrogen Fixation (abstracts), Ind. Res. Inst. New Delhi, P. 29.

Pramer, D. and E. L. Schmidt (1964). Experimental soil microbiology. Burgess Publisher Company. Minnesota, U. S. A.

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.

Swaminathan, M. S. (1982). Biotechnology research in third world agriculture. Science 218: 967-972.

Venkataraman, G. S. (1979). Algal inoculation of rice fields. In: Nitrogen and Rice. International Rice Research Institute, Los Banos, Philippines, 311-321.

Venkataraman, G. S. (1981). Blue- green algae and rice production. FAO / Soils Bull. No. 46, P. 31.

Walkley, A. and I. A. Black (1934). An examination of the Degtrarff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.

Yanni, Y. G. (1991). Potential of indigenous cyanobacteria to contribute to rice performance under different schedules of nitrogen application. World J. Microbiol. Biotech. 7: 48-52.

تأثير مصاحبة السيانوبكتريا علي إنتاج القمح و خصوبة التربة

منى ميخائيل حنا - الهام محمد عارف - فكرى محمد غزال
معهد بحوث الأرا ضى والمياة والبيئة - مركز البحوث الزراعية - الجيزة - مصر

يهدف هذا البحث إلي دراسة أثر التلقيح بالسيانوبكتريا علي إنتاج القمح و كذلك علي خصوبة التربة. لذلك أجريت تجربة حقلية في موسم ٢٠٠١/٢٠٠٢ أستخدم فيها السيانوبكتريا كلقاح للقمح بمعدلات مختلفة إما منفردا أو تحت تأثير معدلات مختلفة من النيتروجين.و كانت أهم النتائج المتحصل عليها ما يلي :

١- أدت جميع المعاملات سواء النيتروجين بمعدلاته أو السيانوبكتريا بمعدلاتها كلاهما منفردا أو مع بعضهما إلي زيادة مكونات محصول القمح بالمقارنة مع معاملة المقارنة.

٢-أعطت المعاملة ٨٠ كجم نيتروجين / هكتار + ٣ كجم سيانوبكتريا / هكتار أعلي محصول و كان هذا المحصول لا يختلف معنويا عن المحصول المتحصل علية من التسميد بالمعدل النيتروجيني الموصى به (١٢٠ كجم / هكتار).

٣-أدى التلقيح بالسيانوبكتريا إلي زيادة عدد الميكروبات بالتربة و كذا نشاط إنزيم الديهيدروجينيز و كمية ثاني أكسيد الكربون المنطلقة و كان ذلك أيضا مع المعاملة ٨٠ كجم نيتروجين / هكتار + ٣ كجم سيانوبكتريا / هكتار.

٤-أدى استخدام السيانوبكتريا إلي توفير حوالي ٣٠% من كمية النيتروجين المعدني المستخدم في إنتاج القمح.

٥-يحتاج هذا البحث إلي مزيد من الدراسة و البحث لعدة مواسم متتالية وفى عدة مواقع أخرى و هو ما يجري تنفيذه الآن.