

THE NOVEL OF USING CYANOBACTERIA AND *Azotobacter* AS BIOFERTILIZER FOR WHEAT CROP PRODUCTION

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ABSTRACT

A pot experiment was carried out at the experimental greenhouse of the Faculty of Agriculture, Mnsoura University, Dakahlia, Governorate, to study the effect of *Azotobacter* and/or cyanobacteria inoculation each individually and/or both in combinations under three nitrogen levels (zero N, 1/2 full N recommended dose and full N recommended dose) on wheat (*Triticum aestivum* L.) variety Sakha 93. Some soil biological, physical and chemical properties were also studied. Results indicated that the dual inoculation with both cyanobacteria and *Azotobacter*, generally enhanced wheat plant growth and increased wheat grain and straw yields, NPK uptake by grains and straw, available NPK in soil at three stages of wheat growth (vegetation, panicle initiation and at harvest). As well as the soil biological activity was positively enhanced due to the dual inoculation with both cyanobacteria and *Azotobacter* combined with 1/2 N dose only especially at the second stage (panicle initiation). In this concern, this treatment led to increase the soil dehydrogenase activity, CO₂ evolution, soil microbial community represented by total bacteria count, total cyanobacteria count and *Azotobacter* count. However, the priority was for the second stage (panicle initiation) and the treatment of ½ N + cyano + Azoto compared to the other tested treatments and/or stages.

In conclusion, much attention should be paid to understand the mechanism of dual inoculation with both cyanobacteria and *Azotobacter* that positively affected wheat production and improved biological and chemical characters for the inoculated soil.

INTRODUCTION

Previously, Alexander (1971) reported that *Azotobacter* needs to a simple organic carbon source for its biological activity to fix nitrogen, so it gets into proto-corporation relationship with cyanobacteria formally called blue-green algae, especially *Nostoc* and *Anabaena* to take carbohydrates (resulted from photosynthesis process made by cyanobacteria) that lead to increase the amount of fixed nitrogen by both microorganisms. Recently, and about the same relationship Tantawy (2006) proved that dual inoculation with *Azotobacter* and cyanobacteria combined with 1/4 N dose increased significantly the soil biological activity, which leads to the production of plant growth promoting regulator (PGPR) substances and consequently the amount of fixed nitrogen, available NPK in soil and both maize grain and stover yields over the other tested treatments received single inoculation. However, many authors reported that inoculation with *Azotobacter* and/or cyanobacteria are capable of growing and introducing many active substances, which induce the growth and production many crops. Kumar *et al.* (2001) mentioned that *Azotobacter chroococcum* has the ability to be phosphate solubilizing and phytohormone producing when inoculated to

wheat. Kennedy *et al.* (2004) reported that wheat inoculation with non-symbiotic bacterial diazotrophs (including *Azotobacter*) increased the vegetative growth and grain yield. They added that economic and environmental benefits can include the increase of income due to high yields, the reduction of fertilizers costs and emission of the greenhouse gas (N₂O) with more than 300 times the global warming effect of CO₂. As well as reduced leaching of NO₃⁻ to ground water. Obtaining maximum benefits on farms from diazotrophic, plant growth promoting biofertilizers will require a systematic strategy designed to fully utilize all these beneficial factors, allowing crop yields to be maintained or even increased, in despite of fertilizers application are reduced. Sergeeva *et al.* (2002) established that *Nostoc muscorum* liberated into the culture medium auxin-like substances and demonstrated that a number of cyanobacteria produce, accumulate, and liberate 3-indol acetic acid. Inoculation with cyanobacteria (*Anabaena*, *Nostoc*, *Calothrix*, *Aulosira* and *Cylindrospermum*) genera to rice field soils and urea supplemented plots was investigated by Adhikary (2002) who reported that nitrogenase activity of the soils inoculated with cyanobacteria was higher than the control and N-fertilizer supplemented plots. Most of the inoculated species competed successfully with the indigenous flora and established in the fields contributing higher amount of fixed nitrogen to the soils and an increase of grain yield by over 25 % was obtained in the algalized plots. Also, Mishra and Pabbi (2004) reported that cyanobacteria offer an economically attractive and ecologically sound alternative to chemical fertilizers for realizing the ultimate goal of increased productivity, especially in rice cultivation. In a wetland rice ecosystem, nitrogen fixation by free living cyanobacteria also significantly supplements soil with nitrogen. In very recent reports, Ahmad *et al.* (2008) tested some microbial isolates and found that more than (80 %) of *Azotobacter* isolates produce IAA, whereas (74.47 %) are able to solubilize phosphate and all the tested isolates that produce ammonia.

This work is designed to study the effect of dual inoculation with cyanobacteria and *Azotobacter* either each alone or both in combinations under different nitrogen rates on wheat yield and yield components, NPK uptake for wheat grains and straw. As well as biological activity, physical and chemical characters of clayey soil.

MATERIALS AND METHODS

Wheat experiment:

A pot experiment was carried out at the experimental greenhouse of the Faculty of Agriculture, Mnsoura University, Dakahlia, Governorate, to study the effect of *Azotobacter* and/or *cyanobacteria* inoculation each individually and/or both in combination under three nitrogen levels (zero N, 1/2 full N recommended dose and full N recommended dose) on wheat (*Triticum aestivum* L.) variety Sakha 93. Some soil biological, physical and chemical properties were also considered. Physical, chemical and biological properties of the experimental soil (Black, 1965) are shown in (Table 1 a, b & c).

Table (1 a): Some chemical properties of the studied soil

EC dSm ⁻¹	pH	Soluble ions (meq L ⁻¹)							
		Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁼	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁼
1.62	7.80	1.07	1.82	0.78	0.18	---	1.35	0.96	1.36

Table (1 b): Some physical properties of the studied soil

Coarse sand	Fine sand	Silt	Clay	Texture class
5.60	22.40	23.10	48.90	Clay

Table (1 c): Some biological properties of the studied soil

Total count bacteria cfu g soil ⁻¹ x10 ⁶	Cyanobacteria count cfu g Soil ⁻¹ x 10 ³	<i>Azotobacter</i> cfu g soil ⁻¹ x10 ⁴	<i>Azspirillum</i> cfu g soil ⁻¹ x10 ³	CO ₂ evolution mg CO ₂ 100 g soil ⁻¹	**DHA µg TPF 100 g soil ⁻¹
40	3	6	2	44	420

* cfu = Colony formed unit⁻¹

** DHA = Dehydrogenase activity

Pots with 40cm height and 35cm in diameter were filled with 12 kg clay soil each. Prior to baking the pots with soil, the soil was mixed uniformly with the recommended doses of phosphorus and potassium fertilizers were added uniformly at rates of 15 kg P₂O₅ /fed as super phosphate (15.5% P₂O₅) and 48 kg K₂O/fed as potassium sulphate (48% K₂O) before cultivation. Nitrogen was added in the form of ammonium nitrate (33.5 % N) according to the applied treatments into two equal split doses (after 30 and 60 days from cultivation).

Five Wheat grains were sowed to each pot and upon wheat seedlings were developed approximately after two weeks, one plant was thinned out and four healthy ones were left in each pot. The experiment comprises the following treatment:

- 1- Control
- 2- Cyanobacteria
- 3-*Azotobacter*
- 4-*Azotobacter* +cyanobacteria
- 5-1/2N dose
- 6- 1/2N + cyanobacteria
- 7-1/2N + *Azotobacter*
- 8-1/2N + cyanobacteria + *Azotobacter*
- 9- Full dose N (100%N = 75 kg N fed⁻¹ = 224 kg NH₄ NO₃, 33.5%N).
- 10- Full N + cyanobacteria.
- 11- Full N + *Azetobacter*.
- 12- Full N + cyanobacteria + *Azetobacter*

The treatments were in three replicates each and arranged in a complete randomize design according to Gomez and Gomez (1984).

After 45 (vegetation stage) & 75 days from wheat cultivation (panicle initiation) and at harvest, soil samples were collected to evaluate the available NPK. At harvest wheat plants were cut just above the soil surface

to estimate yield and yield components, i.e., grain and straw yields, 1000-grain weight (g), panicles weight panicles number/pot, and total plant NPK uptake (g pot⁻¹). Straw and grain were sampled and oven dried; ground and digested according to Thomas *et al.* (1967) then subjected to the determination of NPK contents as described by Van Schouwenburg (1968). Available nutrients in the soil after wheat harvesting were extracted as described by Jackson (1976), i.e. nitrogen by 2N potassium chloride, Phosphorus by 0.5 M sodium bicarbonate and potassium by 1N ammonium acetate. As well as some soil biological parameters, i.e., carbon dioxide evolution (mg/100g soil⁻¹), dehydrogenase activity, total count bacteria, total nitrogen fixing cyanobacteria count, and *Azotobacter* were estimated.

All obtained data were subjected to statistical analysis according to Gomez and Gomez (1984), where mean values were compared using L.S.D at 5% level.

Bacterial preparation, inoculation and count methods:

Seedlings (15 days after wheat grains sowing) were inoculated during irrigation by culture broth (24 hours prepared) of *Azotobacter chroococcum* containing 10⁸ cell mL⁻¹, and re-inoculated after two weeks later. *Azotobacter* used was previously isolated from the soil by the Dept. of Agric. Microbiol., Soils, Water, and Environ. Res. Inst. (ARC), Giza, Egypt, using the medium of Hegazy and Neimela (1976), growing and maintenance for *Azotobacter* were done by using the same medium. Total count of *Azotobacter* and was completed by using the most probable number technique (MPN) (Cochran, 1950) using also the same medium., while, cyanobacteria were inoculated to wheat using the soil based inoculum (10¹² cfu g soil⁻¹) prepared as described by Venkataraman (1972). The cyanobacteria inoculum is composed of a mixture of individual strains namely *Nostoc clivicola*, *Nostoc muscorum*, *Anabaena naviculoides*, and *Nostoc maculiforme*. Total count of bacteria enumerated in soil tested after 45 days from sowing (vegetation stage), 75 days (panicle initiation stage) and at harvest stage. For counting total bacteria and nitrogen fixing bacteria the dilution plate method was used on the media of Bridson (1978) and Watanabe and Barraquie (1979), respectively. Cyanobacteria count in soil was carried out by the method described by Allen and Stanier (1968).

RESULTS AND DISCUSSION

Wheat grain yield components:

Results in Table (2) show the effect of the bacterial inoculation on wheat yield and its components. Results showed that the inoculation with a mixture of *Azotobacter* and cyanobacteria combined with ½ N dose attained the superior effect on grain and straw yields compared to that achieved due to single inoculation. The corresponding highest mean values of grain and straw yields were 44.70 and 66.86 g pot⁻¹. These values were not significantly different from those recorded by the use of full N dose either it applied alone or combined with sing and/or dual inoculation. The single inoculation, which

came in the second rank in case of cyanobacterial inoculation plus 1/2 N dose reported a good effect on grain and straw yields, and gave the higher significant values 41.50 g pot⁻¹ against 40.30 for *Azotobacter* plus 1/2 N dose in comparison with the values obtained due to the application of either the single inoculation or 1/2 N dose. Inoculation with a mixture of *Azotobacter* and cyanobacteria inoculation combined with 1/2 N dose, also showed a positive effect on both panicle weight and the number of grains panicle⁻¹. Both the wheat plant height and 1000-grain weight had not significantly affected by inoculation treatments when compared with the treatments received nitrogen only.

These results are in agreement with those obtained by Kenndy *et al.* (2004), who decided that a range of diazotrophic plant growth-promoting rhizobacteria (including *Azotobacter*) participate in interactions with crop plants (e.g. rice wheat, maize, sugarcane and cotton), significantly increased their vegetative growth and grain yield. This result may due to the nitrogen fixation and plant growth promoter bacteria that increased in presence of *Azotobacter* and cyanobacteria together because of the cooperation relation between them mentioned by Alexander (1971) and consequently enhanced the plant growth parameters. In addition, Karthikeyan *et al.* (2007) and Ragab *et al.* (2008) confirmed the novel of association between cyanobacteria and wheat plants and noted that inoculation of wheat with cyanobacteria significantly enhanced the plant growth and crop yield due to their potential in nitrogen fixation and to act as plant growth promoter.

Table (2): Yield and yield components for wheat cultivated in clayey soil as affected by different nitrogen levels and cyanobacteria and *Azotobacter* inoculation

Treatments	1000-grain weight (g)	grain yield (g/pot)	Straw yield (g/pot)	Panicles weight (g/pot)	Plant height (cm)	No. of grains/panicle
Control	42.00	11.20	40.16	31.80	89	40
Cyanobacteria (Cyano)	51.30	11.40	43.20	34.88	93	48
<i>Azotobacter</i> (Azoto)	52.50	11.60	44.05	34.85	94	51
Cyano+ Azoto	55.50	12.20	45.23	35.42	95	56
1/2N	52.00	36.10	55.17	56.07	93	70
1/2N+ cyano	51.00	41.50	56.23	59.83	92	75
1/2N+ Azotor	54.50	40.30	54.09	57.50	95	73
1/2N+cyano+Azoto	57.10	44.70	66.86	67.83	96	85
Full N	55.60	44.40	65.90	63.26	94	81
Full N+cyano	54.50	43.30	63.16	64.40	93	80
Full N+Azoto	54.50	44.30	64.49	65.80	96	82
Full N+cyano+Azoto	56.50	44.42	66.53	66.16	96	83
L. S. D. 0.05	NS	4.21	6.22	8.15	NS	10.12

NPK uptake wheat grain and straw

Data in Table (3) revealed that the superior of dual inoculation (cyanobacteria and *Azotobacter*) was favorable especially when applied in addition to 1/2 N dose. Dual inoculation increased NPK contents in grains and straw over those recorded by the single inoculation treatments. The most affected parameter according dual treatment was nitrogen uptake by grains

and straw, which recorded 136.40 and 81.30 mg pot⁻¹, respectively. The same treatment was also proceeding in available potassium uptake by grains and straw with significant differences when compared with the treatments received single inoculation. Single inoculation with cyanobacterial inoculation plus ½ N dose was proceeding only with nitrogen and potassium uptake by grains and straw and followed by *Azotobacter* plus ½ N dose. However, despite phosphorus uptake by both grains and straw slightly increased over the treatments received nitrogen only, these increases were not significant.

These results are confirmed by those obtained due to Hanna *et al.* (2004) who found that inoculation with cyanobacteria increased significantly the nitrogen, phosphorus and potassium contents of wheat grain and straw. Also, Kumar *et al.* (2001) mentioned that *Azotobacter chroococcum* has the ability to fix nitrogen, Phosphate solubilizing and phytohormone producing when inoculated to wheat.

Table (3): NPK uptake for wheat cultivated in clayey soil as affected by different nitrogen levels and cyanobacteria and *Azotobacter* inoculation

Treatments	Wheat NPK uptake (mg Pot ⁻¹)					
	Straw			Grains		
	N	P	K	N	P	K
Control	37.27	4.62	2.22	12.05	0.79	11.04
*Cyano	46.32	7.35	2.52	51.53	0.91	11.93
<i>Azotobacter</i> (Azoto)	45.49	7.28	2.27	56.60	0.97	12.30
Cyano+ Azoto	42.82	8.65	2.53	53.60	0.76	13.22
1/2N	63.30	7.99	2.57	117.7	1.27	23.76
1/2N+ cyano	65.94	9.51	3.65	129.40	1.28	28.19
1/2N+ Azoto	62.76	10.00	3.80	127.37	1.81	27.48
1/2N+cyano+Azoto	81.30	10.85	5.86	136.4	1.90	35.85
Full N	74.94	10.22	5.60	133.17	1.86	34.33
Full N+cyano	72.10	9.73	4.40	132.00	1.59	35.14
Full N+Azoto	70.50	9.98	5.50	133.33	1.54	35.97
Full N+cyano+Azoto	77.84	9.91	5.40	134.30	1.27	32.17
L. S. D. 0.05	5.02	NS	1.30	12.20	NS	6.12

*Cyano = Cyanobacteria inoculum

Available NPK in soil:

Data in Table (4) show available NPK at three different growth stages of wheat, i.e., vegetation, panicle initiation and harvest stages as affected by cyanobacteria and/or inoculation under different nitrogen levels. Results revealed that in the three tested growth stages, inoculation with either cyanobacteria or *Azotobacter* each alone or both in combination in presence or absence of nitrogen increased significantly the soil available NPK over the control treatments. However, during the vegetation stage up to panicle initiation all the available NPK concentration increased in soil with a priority for the treatment received dual inoculation with cyanobacteria and *Azotobacter* in addition to ½ N dose, which recorded the highest significant values when compared to those of uninoculated treatments and/or those with single inoculation. The values were also not significantly different from those received Full N dose alone or combined with single and/or dual inoculation (Table 4). At harvest, same behavior due inoculation was noticed but the

amounts of the soil available was less than those observed during vegetation and panicle initiation stages. These results could be explained by that both *Azotobacter* and cyanobacteria are known to excrete extra-cellular compounds to soil, these compounds hold or glue soil particles together in the form of micro-aggregates and hence improve nutrients availability in soil (Mandal *et al.* 1999). Also, in long- term studies to evaluate cyanobacteria effect on soil fertility during periods of soil, Pankratova (2006) reported that cyanobacteria contribute to the nitrogen pool of soils that reached 30 kg/ha, and the concept of transforming the organic matter of cyanobacteria in soil and its movement along atrophic chains of the biological cycle has been developed.

Table (4): Available NPK in clay soil as affected by biofertilizers and different nitrogen levels during different wheat growth stages

Treatment	Soil available NPK (mg kg ⁻¹)								
	N			P			K		
	First stage	Second Stage	Third stage	First stage	Second Stage	Third stage	First stage	Second Stage	Third stage
Control	12.06	25.57	9.97	12.30	25.80	8.09	33.50	50.80	29.30
Cyano	15.85	95.82	14.10	28.26	47.90	16.54	48.50	67.80	36.50
<i>Azotobacter</i> (Azoto)	18.24	106.77	17.85	37.30	66.30	28.00	63.80	76.10	43.40
Cyano+ Azoto	22.12	113.93	24.98	56.63	88.46	37.69	44.40	86.20	56.60
1/2N	24.52	118.85	32.13	63.46	46.70	15.26	52.40	76.40	36.20
1/2N+ cyano	34.28	156.11	44.91	74.63	94.50	46.73	64.70	87.10	46.40
1/2N+ Azoto	42.92	154.74	53.31	82.58	96.40	45.50	76.10	95.30	52.20
1/2N+cyano+Azoto	89.03	208.12	90.40	103.10	131.20	67.50	94.07	134.30	61.60
Full N	84.15	200.82	99.86	78.70	55.70	45.30	58.10	84.90	47.00
Full N+cyano	86.97	198.16	86.10	121.40	138.50	73.06	164.10	99.70	54.50
Full N+Azoto	87.20	204.85	98.49	128.57	143.40	80.06	167.60	103.70	63.90
Full N+cyano+Azoto	87.27	203.14	102.30	151.76	151.50	96.66	176.34	145.20	84.30
L. S. D. 0.05	2.97	8.75	4.31	4.26	5.58	4.18	4.90	4.906	4.25

Soil biological activity:

Data in Tables (5 and 6 a, b &c) indicate the soil biological activity in terms of dehydrogenase activity, CO₂ evolution, total bacteria count, total cyanobacteria count and *Azotobacter* count at three different growth stages of wheat, i.e., vegetation, panicle initiation and harvest stages as affected by cyanobacteria and/or inoculation under different nitrogen levels. Results revealed that dehydrogenase activity and CO₂ evolution increased significantly due all the tested treatments over the control treatments at all tested wheat growth stages. However, the values recorded in the second stage (panicle initiation) were significantly higher than those recorded due the other tested stages (vegetative and harvest). Nevertheless, the highest values of 4270.08 mg TPF 100 g soil⁻¹ (DHA) and 356.60 mg CO₂ 100 g soil⁻¹ (CO₂ evolution) were due to the treatment received *Azotobacter* and cyanobacteria inoculation combined with ½ N dose. These two high values

were significantly higher than those attained by the other tested treatments at all stages.

Same trend observed with DHA and CO₂ evolution was true due the soil microbial community represented by total bacteria count, total cyanobacteria count and *Azotobacter* count. However, the priority was for the second stage (panicle initiation) and the treatment of ½ N + cyano + Azoto compared to the other tested treatments and/or stages.

These results are in agreement with those obtained by Abd El- Rassoul *et al.*(2004) in wheat, El-Zeky *et al.*(2005) in rice who found that inoculation with *Azotobacter* combined with low level of nitrogen (1/2 N dose) increased significantly both N₂-ase and dehydrogenase activities over the control as a result of microorganisms count increasing. El-Mohandes (2000) explained that high level of N-fertilizer caused an opposite effect on nitrogen fixation as a result of N₂-ase activity inhibition. Also, dehydrogenase activity increased with bacterial inoculation and this was in agreement with Seagnozzi *et al.* (1995) who reported that there is a positive significant relationship between (DHA) activity and microbial count in soil. In addition, Karthikeyan *et al.* (2007) and Ragab *et al.* (2008) confirmed that dual inoculation with *Azotobacter* and cyanobacteria combined with low nitrogen dose (1/2 recommended N dose) led to increase the soil biological activity in terms of DHA and the count of soil microbial community. Karthikeyan *et al.* (2007) also, demonstrated that cyanobacteria enhanced the plant growth parameters in wheat (plant height, dry weight and grain yields) besides bringing about significant changes in soil microbial community. In this concern, Tantawy (2006) explained that biofertilization of maize with cyanobacteria and *Azotobacter* lead to increase the soil microorganisms' community through increasing the organic matter, microbial activity and in turn increasing dehydrogenase activity, nitrogenase activity and CO₂ evolution.

In the present study, it could be concluded that dual inoculation with *Azotobacter* and cyanobacteria can save approximately 50 % of the nitrogen amount required for wheat crop rather than the improvement released to the biological and chemical properties of the soil. So much attention and further studies should be done to establish this eco-friendly technology towards other cereal crops rather than wheat.

Table (5): Dhydrogenase (DHA) activity and CO₂ evolution in clay soil as affected by cyanobacteria and *Azotobacter* inoculation and different nitrogen levels during different wheat growth stages

Wheat growth stages	DHA (mg TPF 100 g ⁻¹ soil)				CO ₂ evolution (mg CO ₂ 100 g ⁻¹ soil)			
	First stage	Second Stage	Harvest Stage	Mean	First stage	Second Stage	Harvest Stage	Mean
Control	693.15	838.26	237.01	589.47	48.6	85.3	62	65.3
Cyano	843.59	950.01	327.59	707.06	76.3	111.6	104.6	97.5
<i>Azotobacter</i> (Azoto)	888.44	1006.03	533.01	809.16	91	129	114	111.3
Cyano+ Azoto	938.51	1110.95	622.67	890.71	144	186.3	161	136.76
1/2N	906.82	1003.45	551.72	820.66	118	128	121.3	122.43
1/2N+ cyano	1056.08	1217.72	722.90	998.90	122.6	141.3	135	132.96
1/2N+ Azoto	1091.74	1256.64	800.6	1049.66	147	156	144.6	149.2
1/2N+cyano+Azoto	2140.4	4270.08	1157.06	2522.51	233.3	356.6	181	256.9
Full N	1103.49	1449.78	831.83	1128.36	124	195.6	160	159.8
Full N+cyano	1183.52	1503.90	925.37	1204.26	131	211.3	181.6	174.6
Full N+Azoto	1201.54	1637.74	938.98	1259.42	131.3	226	198	185.1
Full N+cyano+Azoto	1570.01	1836.00	1045.56	1483.85	145	253.6	208.6	202.4
Mean	1134.77	1506.71	724.52	_____	126.00	181.71	147.64	_____

stages

L. S. D. 0.05 : Treatments: 3.2154 Stages : 1.6077 Interaction: 4.0721

Table (6 a): Total bacteria count (*cfu x 10⁶) in clayey soil as affected by different nitrogen levels and cyanobacteria and *Azotobacter* inoculation at different wheat growth stages

Levels of nitrogen	Inoculation	First stage**	Second stage***	Third Stage****
Control	Control	0.010	0.028	0.013
	cyano	0.023	0.051	0.035
	Azoto	0.030	0.082	0.043
	Cyano + Azoto	0.023	0.082	0.043
1/2 N	Control	0.015	0.043	0.017
	cyano	0.015	0.035	0.017
	Azo	0.030	0.079	0.51
	Cyano + Azoto	0.010	0.1	0.032
Full N	Control	0.010	0.043	0.032
	cyano	0.015	0.084	0.030
	Azoto	0.015	0.084	0.030
	Cyano + Azoto	0.017	0.061	0.032

* cfu = Colony formed unit⁻¹

** 45 days (vegetation stage)

*** 75 days (panicle initiation stage)

**** Harvest stage

Table (6 b): Total cyanobacteria count (cfu x 10³) in clayey soil as affected by different nitrogen levels and cyanobacteria and *Azotobacter* inoculation at different wheat growth stages

Levels of nitrogen	Inoculation	First stage	Second stage	Third stage
Control	Control	35	45	25
	cyano	45	60	30
	Azoto	45	57	33
	Cyano + Azoto	60	70	41
1/2 N	Control	45	65	27
	cyano	63	75	36
	Azo	61	70	29
	Cyano + Azoto	90	110	70
Full N	Control	65	72	40
	cyano	69	75	33
	Azoto	68	73	26
	Cyano + Azoto	80	95	55

Table (6 c): *Azotobacter* count (cfu x 10⁴) in clayey soil as affected by different nitrogen levels and cyanobacteria and *Azotobacter* inoculation at different wheat growth stages

Levels of nitrogen	Inoculation	First stage	Second stage	Third stage
Control	Control	20	30	10
	cyano	40	60	30
	Azoto	30	40	22
	Cyano + Azoto	60	90	30
1/2 N	Control	20	30	11
	cyano	60	90	54
	Azoto	50	70	34
	Cyano + Azoto	40	98	61
Full N	Control	13	25	14
	cyano	39	53	30
	Azoto	35	49	23
	Cyano + Azoto	33	66	44

REFERENCES

- Abd El-Rasoul, Sh. M., Mona, M. Hanna, Elham, M. Aref and F. M. Ghazal (2004). Cyanobacteria and effective microorganisms (EM) as a possible biofertilization in wheat production .J. Agric. Sci. Mansoura Univ., 29 (5): 2785-2799.
- Adhikary, S. P. (2002). Utilization of region specific cyanobacteria as biofertilizer for rice: A case study. Biotechnology of Microbes and Sustainable Utilization, Editing by, Rajak, R. C. Publisher. India.

- Ahmad Farah, Iqbal Ahmad and M.S. Khan (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research*. 163 (2): 173-181.
- Alexander, M. (1971). *Microbial Biology*, John Wiley & Sons Inc. New York, U.S.A.
- Allen, M. M. and R. Y. Stanier (1968). Selective isolation of blue- green cyanobacteria from water and soil. *J. Gen. Microbiol.*, 51: 203 - 209.
- Black, C. A. (1965). *Methods of Soil Analyses (I and II)*. Amer. Soc. Agron. Inc. Madison, Wiscn., U.S.A.
- Bridson, E. Y. (1978). Natural and synthetic culture media. In: *CRC Handbook series in Nutrition and food*, Section G, vol.111, Miloslay Recheigl, J. Ed. CRC press Inc., Clevel., USA.
- Cochran, W. G. (1950). Estimation of bacterial densities by means of the Most Probable Number. *Biometrics*. 6: 105-116.
- El- Mohandes, M. A.O. (2000). The use of associative diazotrophs with different rates of N fertilization and compost to enhance growth and N₂-fixation of wheat. *International Symposium on Biological Nitrogen Fixation and Crop Production*. Cairo, Egypt 11-13 May, 107-123.
- El-Zeky, M. M.; R. M. El-Shahat; Gh.S. Metwaly and Elham M. Aref (2005). Using of cyanobacteria or *Azolla* as alternative nitrogen source for rice production. *J. Agric. Sci. Mansoura Unvi.*, 30: 5567-5577.
- Gomez, K. A. and A. A. Gomez (1984). *Statistical procedures for Agricultural Research.*, (2nd ed.). 20 - 29.
- Hanna, M. M.; E. M. Aref and F. M. Ghazal (2004). Effect of cyanobacteria-wheat association on wheat production and soil fertility. *J. Agric. Sci. Mansoura Univ.*, 29: 2941-2948.
- Hegazy, N.A. and S. Neimela (1976). A note on the estimation of *Azotobacter* densities by membrane filter technique. *J. Appl. Bacteriol.*, 41:311.
- Jackson, M. L. (1976). *Soil Chemical Analysis*. Prentic-Hall Ins., Engle Wood Cliffs, U.S.A.
- Karthikeyan, N., R. Prasanna, L. Nain and Brahma D. Kaushik (2007). Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *Eur. J. Soil Biol.*, 43: 23 – 30.
- Kemper, W. D. and R. C. Rosenau (1986). Aggregate stability and size distribution. *Methods of soil analysis Part 1. Physical and mineralogical methods*. Agronomy No. 9, 2nd ed. Soil Science Society of America. American Society of Agronomy. Madison, WI., USA. A. Klute and A. L. Page, Eds., 425-442.
- Kennedy Ivan R., A. T. M. A. Choudhury and M L. Kecskés (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited. *Soil Biol. and Biochem.*, 36 (8): 1229- 1244.
- Kumar V., R. K., Behl and N. Neeru Narula (2001). Establishment of phosphate-solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. *Microbiological Research*. 156 (1): 87-93.

- Mandal, B. K.; P. L. G. Vlek and L. N. Mandal (1999). Beneficial effect of blue-green cyanobacteria and *Azolla*, excluding supply nitrogen on wetland rice fields: a review. *Biol. Fertl. Soil.* 28:329-342.
- Mishra, U. and S. pabbi (2004). Cyanobacteria: A potential biofertilizer for rice. *Resonance.* 9 (6): 6-10.
- Pankratova, E. M. (2006). Functioning of cyanobacteria in soil ecosystems. *Eurasian Soil Science.* 39: 118-127.
- Ragab A. A. M; Eman A. Tantawy; A.A. Khalil and A. A. Mahmoud (2008). Effect of combined inoculation with cyanbacteria and *azotobacter* on wheat crop cultivated in sandy soil under different nitrogen levels. *J. Agric. Sci. Mansoura Unvi.*, 33 (6):4417 – 4426.
- Seagnozzi, A. R. Levi-Minzi; R. Riffald, and A. Saviozzi (1995). Changes in organic compounds and dehydrogenase activity in soil amended with crop residues. *Agriculture Mediterranea.*125 (4):427-437.
- Sergeeva, E.; A. Lialmer and B. Bergman (2002). Evidence for production of phytohormone indol-3-acetic acid by cyanobacteria. *Planta.* 215 (2): 229-238.
- Tantawy, Eman A. (2006). Response of maize to *Azotobacter* and cyanobacteria inoculation under sandy soil condition. *Egypt. J. of Appl. Sci.*, 21 (5): 359-374
- Thomas, R.L., R. W. Shearel and Z. R. Mayer (1967). Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analyses of plant material using single digestion. *Agron. J.*, 59: 240.
- Van Schouwenburg, J.Ch. (1968). International Report of Soil and Plant Analysis. Lab. of Soil and Fertilizer Agric., Univ. of Wageningen, The Netherlands.
- Venkataraman, G. S. (1972). Biofertilizer and rice cultivation. *Today and Tomorrow Report*, New Delhy, India. p.81.
- Watanabe, I. and W.S. Barraquie (1979). Low levels of fixed nitrogen required for isolation of free living N₂-fixing organisms from rice roots. *Nature.* 277: 565-566.

فكرة استخدام السيانوبكتريا والأزوتوباكتر كسماد حيوى لانتاج محصول القمح
فتحى اسماعيل على حوقة 1 ، عبدالله العوضى ابراهيم سليم¹ ، جيهان محمد سالم سالم²
و فكرى محمد غزال²

1- قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنصورة
2- قسم بحوث الميكروبيولوجيا الزراعية – معهد بحوث الأراضي والمياة والبيئة – مركز البحوث
الزراعية – الجيزة - مصر

أجريت تجربة لزراعة القمح فى أصص فى كلية الزراعة جامعة المنصورة محافظة
الدقهلية لدراسة اثر التلقيح بالأزوتوباكتر والسيانوبكتريا سوياً او كل على حده فى وجود ثلاث
مستويات مختلفة من النيتروجين المعدنى (صفر_ نصف الجرعة الموصى بها _ جرعة النيتروجين
الكاملة) وذلك لصنف القمح سخا 93 ودراسة بعض الخصائص الحيوية والفيزيائية والكيميائية .
وكانت أهم النتائج كما يلى:

- 1- أن التلقيح باستخدام السيانوبكتريا والأزوتوباكتر شجع نمو نباتات القمح وزاد من محصول
الحبوب والقش كذلك زيادة محتوى الحبوب والقش من النيتروجين والفوسفور والبوتاسيوم
والعناصر المتاحة بالتربة (النيتروجين والفوسفور والبوتاسيوم) وذلك فى ثلاث مراحل لنمو
محصول القمح (مرحلة النمو الخضرى _ مرحلة طرد السنابل _ مرحلة الحصاد).
- 2- كما زاد النشاط الحيوى للتربة بصورة إيجابية نتيجة للتلقيح بالسيانوبكتريا والأزوتوباكتر متحداً
مع نصف كمية النيتروجين خاصة فى المرحلة الثانية (مرحلة طرد السنابل).
- 3- فى هذا الإطار كانت هذه المعاملات تودى لزيادة نشاط انزيم الديهيدروجينيز فى التربة وخروج
ثانى اكسيد الكربون وزيادة المجتمعات الميكروبية فى التربة المتمثلة فى مجموعات السيانوبكتريا
والأزوتوباكتر والأزوسبيريليم .
- 4- على الرغم من ذلك فإن الأولوية كانت فى المرحلة الثانية (مرحلة طرد السنابل) لمعاملة نصف
جرعه النيتروجين المعدنى بالاضافة إلى السيانوبكتريا والأزوتوباكتر بالمقارنة بالمعاملات
الأخرى تحت الدراسة.
- 5- الخلاصة لابد من توجيه كثير من الإهتمام لفهم آلية التلقيح بالسيانوبكتريا أو الأزوتوباكتر الذى
يؤثر إيجابياً على إنتاجية القمح و الأنشطة الحيوية أو الصفات الكيميائية للتربة الملقحة .