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CYANOBACTERIA AND *Rhizobium radiobacter* AS POSSIBLE BIOFERTILIZERS IN WHEAT PRODUCTION

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ABSTRACT

Recently, a great attention is paid in establishing concept of the associations between wheat plants and a variety of N_2-fixing and/or growth promoting producer microorganisms. This phenomenon has entered the scientific scene arising from the prospects and the possibilities of their potentially application. In this work, cyanobacteria (cyano) and/or *Rhizobium radiobacter* (R) inoculation each alone or both in combination were applied to wheat in a field experiment under the effect of different of nitrogen levels. Results revealed that cyanobacteria inoculation (cyano) combined with R exhibited an economical view that it can save about 25 % of the mineral nitrogen amounts required for wheat crop production especially. The trend was noticed when Cyano was combined with R that recorded a wheat grain yield not significantly different from that obtained by the full recommended nitrogen dose in wheat cultivation. Cyanobacteria inoculation to wheat crop along with R has also enhanced the NPK- uptake by wheat plants, soil microbial community, dehyderogenase activity and CO_2 evolution and fluorescein diacetate activity (FDA) as index for soil fertility.

INTRODUCTION

The use of the conventional chemical farming methods, which substantially increased crop production, was once regarded as a kind of agriculture revolutions which would solve all problems relating to producing sufficient food for the ever growing world population. However this belief was later over-shadowed by the emergence of numerous environmental and social problems associated with the heavy use of agrochemicals in intensive farming systems. Conventional farming methods are generally associated with degradation of the environment. Among other things, soil degradation is one of the most serious problems which affect crop production. Increasing prices of agrochemicals especially nitrogen often leaves farmers with low profit. Uncertain availability of those agrochemicals, especially in the developing countries such Egypt, is often a serious constraint for the farmers in their attempt to increase crop production. Such problems have directed the attention of the agriculturists world-wide to seek alternative methods of farming.

In attempting to develop productive, profitable and sustainable agriculture systems, several agriculturists turn to farming methods, which are based on biotechnologies. One of the several approaches to achieve this goal is using the nitrogen fixing cyanobacteria (cyano) and/or *Rhizobium radiobacter* (R) inoculation each alone or both in combination in order to improve soil fertility and crop productivity. The use of nitrogen fixing cyanobacteria ensures entirely or partially the mineral nitrogen, while, *R. radiobacter* is recommended to produce plant growth promoting substances and its ability to create active association with the no leguminous crops such as wheat (Green *et al*., 2006).

Recently, there is a great deal of interest in creating novel association between agronomically important plants, partially cereals such wheat 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 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 takes up by the plant and supports its growth, improving grain yields and grain quality (Gantar *et al.,* 1995).

The aim of this study is to:

1. Improve wheat yield production and wheat through cyanobacteria and *Rhizobium radiobacter* (R) inoculation, either each alone applied alone or both in combination.
2. Reduce the hazard environmental mineral nitrogen amounts used in wheat cultivation.
3. Exploring of the effect of both cyanobacteria and *Rhizobium radiobacter* inoculation on soil fertility status through the measuring of the soil microbial counts, dehyderogenase activity, CO_2 evolution and fluorescein diacetate hydrolysis activity (FDA) after wheat harvesting as soil fertility index.

MATERIALS AND METHODS

A field trial was conducted at El Ismailia Research Station, Agric. Res. Center (2008 / 2009) to study the effect of both cyanobacteria and *Rhizobium radiobacter* inoculation each applied alone or both in combination on wheat (*Triticum aestivum* cv. Sakha 69) growth under different mineral nitrogen fertilizer levels as urea of full recommended dose (FRD) (80 kg N), 75% (FRD) (60 kg N) and 50 % (FRD) (40 kg N). The physico-chemical analyses (Black, 1965) of the experimental soil is as shown in Table (1).

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

pH (1:2.5)	**EC dS/m**	**Soluble cations (meq/L)**				**Soluble anions (meq/L)**			
		Ca^{++}	**Mg^{++}**	**Na^+**	**K^+**	**CO_3^-**	**HCO_3^-**	**Cl^-**	**$SO_4^=$**
8.14	1.4	4.56	2.60	3.07	0.36	--	6.60	2.83	1.16

Coarse sand %	**Fine sand %**	**Silt %**	**Clay %**	**$CaCO_3$ %**	**Textural class**
76.18	15.17	2.35	6.30	1.5	Sandy

Available N (ppm)	**Available P (ppm)**	**Available K (ppm)**
20	4	49

The experimental field was prepared by ploughing and puddling, and then divided into 36 plots (3m x 3m each) to represent 12 treatments in three replicates arranged in statistical split plot design. Herein, nitrogen fertilizer represents the main plot in three treatments, while cyanobacteria inoculation (cyano) and *Rhizobium radiobacter* (R), their combination and the control treatment without cyano and/ or R inoculation represent the sub plots. Uniform application of phosphorus @ 30 P_2O_5 kg fed^{-1} as superphosphate (15 % P_2O_5) and potassium @ 48 K_2O kg fed^{-1} as potassium sulphate (48% K_2O) were done as basal to each plot. Nitrogen as urea (46.5 % N) was applied in five split doses. Cyanobacteria inoculation (cyano) at the rate of 15 kg dried mixture *Nostoc* sp., *Anabaena* sp., *A. laxa* and *Nostoc muscorum*. Cyanobacteria inoculation was executed 7 days after sowing of wheat seeds, While *Rhizobium radiobacter* (R) was inoculated through wheat seed coating by using the rhizobium liquid culture (10^9 cfu ml^{-1}) two hours before sowing. In addition, rhizobium inoculation was applied also twice as soil drench at a rate of 10 L fed^{-1} (10^9 cfu ml^{-1}) after 30 and 55 days of sowing. Irrigation was carried out every three days using the sprinkler system.

Both cyanobacteria strains and *Rhizobium radiobacter* L. M. were obtained from Agric. microbiol. Dept., Soils, Water & Environ., Inst., Agric. Res. Center, Giza, Egypt. *Rhizobium radiobacter* L M. was grown and propagated in the Lab on yeast extract mannitol broth medium (YMB) for 48hr. to ensure the required cell viable count (10^9 cfu ml^{-1}) as described by Vincent (1970). While cyanobacteria strains were also propagated in the Lab. by using liquid BG 11 medium (Rippika *et al*., 1979) to ensure the log. Phase, and the harvested through centrifugation (5000 rpm for 10 min), air dried, gently crushed, mixed and finally used as cyanobacteria inoculum as mentioned above.

At harvest, wheat plants were cut gust above the soil surface to determine the wheat yield components, NPK by wheat grains and straw (Chapman and Pratt, 1961). The remained soil was sampled and subjected to evaluate the available NPK (Jackson, 1962) as well as to compute the total microbial count (Allen, 1959), *Actinomycetes* (Williams and Davis, 1965), total fungi (Martin, 1950), *Azotobacter* and *Azospirillum* (Cochran, 1950), cyanobacteria (Allen and Stanier, 1968) CO_2 evolution (Pramer and Schmidt, 1964) and both dehydrogenase (Casida *et al*., 1964) activity and fluorescein diacetate hydrolysis activity (FDA) (Schnurer and Rosswall, 1982) as index for the soil fertility.

All obtained results are exposed for statistical analysis as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The nitrogen fixing cyanobacteria inoculation to wheat is recently established to alternate partially or entirely the mineral nitrogen utilization (Mussa *et al*., 2003), while, *R. radiobacter* is more recently recommended to produce plant growth promoting substances and its ability to create active association with the no leguminous crops such as wheat (Green *et al*., 2006).

Herein, a field experiment is laid out to detect the influence of association both cyanobacteria and/or *R. radiobacter* wheat associations either each inoculated alone or both in combination under different levels of mineral nitrogen on wheat yield components, soil characteristics, soil microbial community, CO_2 evolution, dehyderogenase activity and fluorescein diacetate hydrolysis activity (FDA) as soil fertility index.

Wheat yield components:

Data in Table (2) indicate the effect of cyanobacteria association, *R. radiobacter* and N fertilization levels on wheat yield components. All the treatments increased significantly the wheat grain yield over the control treatments. The highest grain yield (1295.34 kg fed^{-1}) attained by cyanobacteria plus *R. radiobacter* treatments combined with full dose of mineral N (80 kg N fed $^{-1}$) followed by 1274.77 kg fed^{-1} for cyanobacteria + *R. radiobacter* combined with 3/4 full mineral nitrogen (60 kg N fed^{-1}) dose treatment . However, there was no significant difference between these two treatments.

Table (2): Effect of cyanobacteria and *Rhizobium radiobacter* inoculation *and* N-fertilization on wheat yield components

N-fertilization	Treatments				
	Control	**Cyanobacteria**	***R. radiobacter***	**Cyanobacteria +*R. radiobacter***	**Means**
	Grain yield kg fed^{-1}				
50% N	574.28	780.96	845.49	962.25	790.75
75% N	736.09	869.57	866.46	1274.77	936.72
full dose N	872.10	993.48	1014.70	1295.34	1043.91
Means	**727.49**	**881.34**	**908.88**	**1177.45**	
L.S.D.at 5%					
N:	**96.765**				
Treatment:	**72.257**				
Interaction:	**144.000**				
	Straw yield ton fed^{-1}				
50% N	2.61	2.33	2.59	2.28	2.45
75% N	2.41	3.03	2.42	2.97	2.71
full dose N	2.69	2.49	2.21	3.31	2.68
Means	**2.57**	**2.62**	**2.41**	**2.85**	
L.S.D.at 5%					
N:	**0.313**				
Treatment:	**0.286**				
Interaction:	**0.530**				
	1000-grains weight (g)				
50% N	45.87	53.30	48.77	49.00	49.24
75% N	46.97	53.83	54.50	48.30	50.90
full dose N	42.63	49.97	40.10	50.90	45.90
Means	**45.16**	**52.37**	**47.79**	**49.40**	
L.S.D.at 5%					
N:	**2.264**				
Treatment:	**3.922**				
Interaction:	**4.440**				

The application of full nitrogen dose gave significantly the highest wheat grain yield (1043.91 kg fed^{-1}) compared with the other two levels of nitrogen (50 and 100% N dose).

Same behavior exhibited by grain yield was observed for straw yield indicating the highest straw yield (3.31 tons fed^{-1}) for the treatment cyanobacteria plus *R. radiobacter* under full nitrogen dose and followed by

2.97 tons fed $^{-1}$ for cyanobacteria plus *R. radiobacter* under 75% N level treatment without significant different between each others.

Towards nitrogen application also full dose recorded the highest significant straw yield (2.68 tons fed^{-1}) due to full N dose application compared with the other two nitrogen levels.

1000-grain weight showed an indefinite trend in response to the tested treatments. However, this notice depends on the number of panicles plant^{-1}, which is correlated drastically with the grain yield.

NPK uptake by wheat grains:

Table (3) reveal that inoculation with cyanobacteria plus *R. radiobacter* under full nitrogen dose gave the highest N uptake amount (22.03 kg N fed $^{-1}$) for wheat grains with no significant difference with that recorded by cyanobacteria plus *R. radiobacter* under 75% N dose (21.67 kg N fed $^{-1}$). Due to nitrogen application alone, there was no significant difference in N uptake values obtained by either 75% or full N dose treatments,their respective mean N uptake values were 15.71 and 16.87 kg N fed $^{-1}$.

Table (3): Effect of cyanobacteria and *Rhizobium radiobacter* and N-fertilization on NPK uptake by wheat grains

N-fertilization	Treatments				
	Control	**Cyanobacteria**	***R. radiobacter***	**Cyanobacteria +*R. radiobacter***	**Means**
	N-uptake kg fed^{-1}				
50% N	8.04	10.15	15.20	16.36	12.44
75% N	12.51	14.78	13.86	21.67	15.71
full dose N	18.31	11.92	15.22	22.03	16.87
Means	**12.95**	**12.28**	**14.76**	**20.02**	
L.S.D.at 5%					
N:	**1.451**				
Treatment:	**1.197**				
Interaction:	**2.290**				
	P-uptake kg fed^{-1}				
50% N	0.75	1.17	1.18	1.35	1.11
75% N	1.18	1.74	1.39	2.17	1.62
full dose N	1.35	1.49	1.93	2.07	1.71
Means	**1.09**	**1.47**	**1.50**	**1.86**	
L.S.D.at 5%					
N:	**0.160**				
Treatment:	**0.125**				
Interaction:	**0.244**				
	K-uptake kg fed^{-1}				
50% N	2.99	3.75	4.22	4.62	3.90
75% N	3.98	4.87	3.99	6.63	4.87
full dose N	4.45	5.17	5.38	6.35	5.34
Means	**3.81**	**4.60**	**4.53**	**5.87**	
L.S.D.at 5%					
N:	**0.516**				
Treatment:	**0.379**				
Interaction:	**0.762**				

Phosphorus uptake and K uptake indicated the same trend in response to the tested treatments as shown in N uptake. In case of P uptake the highest value of 2.17 kg P fed $^{-1}$ was not significantly different from 2.07 kg P fed^{-1} for cyanobacteria plus *R. radiobacter* plus 75% N dose and cyanobacteria plus *R. radiobacter* plus full N dose, treatments, respectively. While with K uptake, the highest value of 6.63 kg K fed $^{-1}$ was not significantly different from that of 6.35 kg K fed $^{-1}$ for cyanobacteria plus *R. radiobacter* + 75% N dose and cyanobacteria + *R. radiobacter* + Full dose treatments, respectively.

For nitrogen application means, the priority in NPK uptake by wheat grains was for the use of full N dose. Their respective mean values, were 16.87 kg N fed $^{-1}$ (N uptake), 1.71 kg P fed $^{-1}$ (p-uptake) and 5.34 kg K fed $^{-1}$ (K-uptake).

NPK uptake by wheat straw

Table (4) indicate that N uptake value recorded by the cyanobacteria + *R. radiobacter* + 75% N dose (14.83 kg N fed $^{-1}$) was not significantly different from that recorded by Cyanobacteria + *R. radiobacter* + full N dose treatment (16.55 kg N fed $^{-1}$). In contrast, P uptake value of 4.15 kg P fed $^{-1}$ (cyanobacteria + *R. radiobacter* + 75% N dose) was significantly higher than that recorded by cyanobacteria + *R. radiobacter* + full N dose treatment (2.98 kg P fed $^{-1}$).

Table (4): Effect of cyanobacteria and *Rhizobium radiobacter* and N-fertilization on NPK uptake by wheat straw

N-fertilization	Treatments				
	Control	**Cyanobacteria**	***R. radiobacter***	**Cyanobacteria +*R. radiobacter***	**Means**
	N-uptake kg fed^{-1}				
50% N	13.05	27.94	10.37	11.95	15.83
75% N	24.18	30.27	14.54	14.83	20.96
full dose N	16.16	14.94	13.38	16.55	15.26
Means	**17.80**	**24.38**	**12.76**	**14.44**	
L.S.D.at 5%					
N:	**2.649**				
Treatment:	**2.147**				
Interaction:	**4.135**				
	P-uptake kg fed^{-1}				
50% N	1.55	3.51	2.34	1.36	2.19
75% N	4.10	4.52	2.91	4.15	3.92
full dose N	2.24	1.24	4.01	2.98	2.62
Means	**2.63**	**3.09**	**3.09**	**2.83**	
L.S.D.at 5%					
N:	**0.594**				
Treatment:	**0.429**				
Interaction:	**0.869**				
	K-uptake kg fed^{-1}				
50% N	16.18	12.82	14.65	17.55	15.30
75% N	25.34	19.37	12.60	16.91	18.56
full dose N	17.77	19.67	15.84	22.18	18.87
Means	**19.76**	**17.29**	**14.36**	**18.88**	
L.S.D.at 5%					
N:	**3.147**				
Treatment:	**2.32**				
Interaction:	**4.663**				

Same observations were noticed by K –uptake, that the K- uptake value recorded by the treatment of cyanobacteria + *R. radiobacter* + full N dose (22.18 kg K fed $^{-1}$) was significantly higher than that recorded by cyanobacteria + *R. radiobacter* + 75% N dose treatment (16.91 kg k fed $^{-1}$) .

In respect to available NPK amounts remained in soil after wheat harvesting, results in Table (5) indicate that available-N significantly increased in response to increasing nitrogen fertilizer doses over 50% N dose with priority to 50% N-dose treatment, which recorded 126.25 mg kg^{-1} available N.

Apart from nitrogen doses, cyanobacteria inoculation combined with *R. radiobacter* had achieved the highest available-N amounts (116.6 mg kg^{-1}). This high available-N amount was significantly different from that recorded by either cyanobacteria (105.00 mg kg^{-1} -N) or *R. radiobacter* (103.33 mg kg^{-1} -N) treatments each applied alone.

Both cyanobacteria, *R. radiobacter* each alone or in combination when being affected with the different fertilizer-N dose showed the highest available-N amount (140.00 mg kg^{-1}) for the treatment received cyanobacteria + *R. radiobacter* + 75%N dose. However, this high amount was significantly exceeded all other interacted treatments.

Available phosphors amounts decreased significantly in response to nitrogen fertilizer doses, since they recorded less amounts of 6.61 and 6.99 mg kg^{-1} than that of 8.43 mg kg^{-1} for 75%, full and 50% N-dose treatments, respectively.

Due to cyanobacteria and *R. radiobacter* inoculation, each alone or both in combination, results revealed that the highest significant available phosphorus amount of 8.63 mg kg^{-1} (*R. radiobacter* treatment alone) was higher than those of 6.82 and 7.93 mg kg^{-1} for cyanobacteria + *R. radiobacter* and *R. radiobacter* treatments, respectively. However, the nitrogen, cyanobacteria and *R. radiobacter* relation gave the highest significant available-P amount (11.4 mg kg^{-1}) for *R. radiobacter* combined with 50% N-dose compared to the other interaction treatments except for cyanobacteria combined with 50%N-dose (9.00 mg kg^{-1} -P) treatment.

Available potassium amounts had fluctuated between decrease in response to 75% and full nitrogen dose compared to 50% N-dose application. Nevertheless, the highest available-K amount of 91.75 mg kg^{-1} (full-N dose) was significantly higher than that recorded by 75% N dose (76. 50 mg K kg^{-1}) but did not higher than that of 89.25 mg kg^{-1}K for 50% N-dose treatment. On the other hand, *R. radiobacter* applied alone had achieved the available-K amount (95.00 mg kg^{-1}) being significantly higher than those of cyanobacteria (89.33ppm-K) and cyanobacteria + *R. radiobacter* (68.00 mg K kg^{-1}) treatments. Nitrogen, cyanobacteria and *R. radiobacter* interactions resulted in the highest significant available-K amount of 118.00 mg kg^{-1} more than those recorded by the other interaction relations.

Table (5): Effect of cyanobacteria and *Rhizobium radiobacter* and N-fertilization on soil available nitrogen, phosphorus and potassium after wheat harvesting

N-fertilization	**Treatments**				
	Control	**Cyanobacteria**	***R. radiobacter***	**Cyanobacteria +*R. radiobacter***	**Means**
	Available nitrogen (mg kg^{-1} soil)				
50% N	90	100	80	100	92.50
75% N	120	125	120	140	126.25
full dose N	80	90	100	110	95.00
Means	**96.66**	**105**	**103.33**	**116.6**	
L.S.D.at 5%					
N:	**2.649**				
Treatment:	**2.14**				
Interaction:	**4.135**				
	Available phosphorus (mg kg^{-1} soil)				
50% N	7.4	9.0	11.4	5.90	8.43
75% N	4.16	8.3	6.9	7.07	6.61
full dose N	6.34	6.5	7.6	7.50	6.99
Means	**5.97**	**7.93**	**8.63**	**6.82**	
L.S.D.at 5%					
N:	**1.40**				
Treatment:	**1.16**				
Interaction:	**2.50**				
	Available potassium(mg kg^{-1} soil)				
50% N	103	110	86	58	89.25
75% N	70	79	81	76	76.50
full dose N	100	79	118	70	91.75
Means	**91**	**89.33**	**95**	**68**	
L.S.Dat 5%					
N:	**3.540**				
Treatment:	**3.091**				
Interaction:	**6.930**				

Soil micro-organisms counts:

Total fungal count in soil after wheat harvesting (Tables 6&7) exhibited no significancy in response to nitrogen fertilizer doses. However, the highest total fungal count (32.90 x 10^{2} cfu g^{-1} soil) obtained by 3/4 N-dose treatment.

Apart from nitrogen doses, cyanobacteria + *R. radiobacter* treatment gave the highest insignificant total fungi count of 36.33 x 10^{2} cfu g^{-1} soil compared to those recorded by the other treatments.

Interaction effect due to the treatments under the influence of N-doses resulted in higher significant total fungi count of 57.3 x 10^{2} cfu g^{-1}soil due to cyanobacteria + *R. radiobacter* + 75% N-dose compared to the other interaction influences except for those recorded by cyanobacteria + *R. radiobacter* + 50% N-dose (38.90 x 10^{2} cfu g^{-1} soil). and *R. radiobacter* + 50% N dose (53.70 x 10 ^{2} cfu g^{-1} soil).

Actinomycetes count indicates significant response due to N-dose application, when the highest count (42.03 x 10^{3}) cfu g^{-1} soil) recorded by the use of 45% N- dose compared with the other applied N-doses.

Table: (6): Effect of cyanobacteria and *Rhizobium radiobacter* and N-fertilization on N_2 - fixers (*Azotobacter*, *Azospirillum* and total cyanobacteria) counts in soil after wheat harvesting

N-fertilization	**Treatments**				
	Control	**Cyanobacteria**	***R. radiobacter***	**Cyanobacteria+ *R. radiobacter***	**Means**
	***Azotobacter* x10^5**				
50% N	5.60	4.40	9.10	2.50	5.40
75% N	2.00	5.40	2.90	6.30	4.15
full dose N	5.40	2.30	3.10	7.00	4.45
Means	4.33	4.03	5.03	5.27	
L.S.D.at 5%					
N:	**0.2145**				
Treatment:	**0.2270**				
Interaction:	**0.4000**				
	***Azospirillum* x 10^5**				
50% N	5.20	1.20	6.30	1.70	3.60
75% N	1.10	4.80	2.20	6.00	3.53
full dose N	4.60	1.60	2.20	7.90	4.08
Means	3.63	2.53	3.57	5.2	
L.S.D.at 5%					
N:	**0.227**				
Treatment:	**0.156**				
Interaction:	**0.354**				
	N_2-fixing cyanobacteria x 10^3				**Means**
50% N	1.70	11.70	2.00	27.70	10.775
75% N	6.00	4.00	19.00	43.30	18.075
full dose N	0.63	3.30	1.00	1.00	5.93
Means	2.77	6.33	7.33	24.00	
L.S.D.at 5%					
N:	**7.191**				
Treatment:	**6.49**				
Interaction:	**9.34**				

Cyanobacteria + *R. radiobacter* had recorded the highest significant actinomycetes count (71.00 x 10^3 cfu g^{-1}soil) compared with those recorded by either cyanobacteria or *R. radiobacter* each alone.

On the other hand, when cyanobacteria combined with *R. radiobacter* under the effect of 75% N-dose gave the highest actinomycetes count of (84.10 x 10^3 cfu g^{-1}soil) compared to the other treatments under the influence of N-doses application.

Due to the total bacterial count, the highest significant values were recorded by the 50% N-dose, *R. radiobacter* and cyanobacteria combined with *R. radiobacter* + 75% N-dose treatments. The corresponding total bacterial cont were 106.42, 117.66 and 131.40 x 10^6 cfu g^{-1} soil.

Azotabacter gave its highest count (6.30 x 10^5 cfu g^{-1} soil) in response to cyanobacteria *R. radiobacter* + 75% N dose treatment. This high count was significantly higher than those given by all the other treatments except for that recorded by cyanobacteria + *R. radiobacter* + full- N dose treatment (7.00 x 10^5 cfu g^{-1} soil).

Azospirillum had recorded its favorite count number of 7.9 x10^5 cfu g^{-1} soil with the use of cyanobacteria + *R. radiobacter* + full- N dose treatment, which was significantly different from that attained by cyanobacteria + *R. radiobacter* + 75% N dose treatment (7.00 x 10^5 cfu g^{-1} soil).

Due to the number of the nitrogen fixing cyanobacteria, it was obvious that increasing the nitrogen levels to full- N dose drastically suppressed the presence of cyanobacteria in soil. However, the treatment of cyanobacteria + *R. radiobacter* + 75% N dose had achieved the highest significant cyanobacteria count (43.30 x10^3 cfu g^{-1} soil) in soil compared to the other tested treatments.

Table (7): Effect of cyanobacteria and *Rhizobium radiobacter* and N-fertilization on total fungi, actinomycetes and total bacterial counts after wheat harvesting

N-fertilization	Treatments				
	Control	**Cyanobacteria**	***R. radiobacter***	**Cyanobacteria +*R. radiobacter***	**Means**
	Total fungi x10^2				
50% N	7.20	29.10	53.70	38.90	32.23
75% N	22.60	36.00	15.70	57.30	32.90
full dose N	15.60	25.50	33.20	12.80	21.77
Means	15.13	30.20	34.20	36.33	
L.S.D.at 5%					
N:	**n.s**				
Treatment:	**6.49**				
Interaction:	**14.193**				
	Actinomycetes x10^3				
50% N	1.00	38.20	58.10	31.80	32.275
75% N	18.70	46.70	18.60	84.10	42.025
full dose N	8.30	31.60	38.80	7.10	21.450
Means	9.30	38.83	38.50	71.00	
L.S.D.at 5%					
N:	**5.22**				
Treatment:	**9.01**				
Interaction:	**14.14**				
	Total bacteria x10^6				
50% N	26.10	38.70	273.2	45.10	106.42
75% N	38.20	77.60	32.0	131.40	69.80
full dose N	28.50	34.70	47.8	17.70	32.18
Means	30.93	50.30	117.66	64.73	
L.S.D.at 5%					
N:	**25.255**				
Treatment:	**15.715**				
Interaction:	**24.196**				

CO_2 evolution:

Carbon dioxide evolution by soil (Table 8) calculated after wheat harvesting showed its highest significant amounts of 391.33, 394.30 and 470.00 mg CO_2 100 g $^{-1}$ soil due to the application of *R. radiobacter* treatment alone, 75% N-dose alone and cyanobacteria combined with *R. radiobacter* under the influence of 50% N-dose, respectively, in comparison to their related treatment without nitrogen.

Dehydrogenase activity (DHA):

Dehydrogenase activity in soil after wheat harvesting (Table 8) expressed its highest significant values of 47.30, 26.00 and 22.90 µg TPF 100 g^{-1} soil by *R. radiobacter* combined with 50% N-dose, *R. radiobacter* treatment alone and full-N dose alone, respectively. These DAH values were significantly higher than those recorded by the other related treatment.

Fluorescein diacetate hydrolysis activity (FDA):

Concerning nitrogen levels effect on FDA, increasing nitrogen level increased FAD activity and gave its highest significant value of 25.75 µg fluorescein g^{-l} soil due to full nitrogen dose. Also, due to inoculation effect, the inoculation with cyanobacteria + *R. radiobacter* recorded the highest significant FDA value of 25.66 µg fluorescein g^{-l} soil compared to the treatments inoculated with either cyanobacteria or *R. radiobacter* each alone. For the interaction effect, the combination between cyanobacteria + *R. radiobacter* + 75% full N dose gave its highest significant value of 34.00 µg fluorescein g^{-l} soil without significant difference with 31 µg fluorescein g^{-l} soil that recorded due to cyanobacteria + *R. radiobacter* + full N dose treatment.

These results are in agreement with those described by (Abd-Alla *et al.,* 1994 and Mussa, *et al.,* 2003) who attributed the increase in wheat yield components in the cyanobacteria inoculated treatments to the substantial increases in N_2 fixation in soil due to nitrogenase activity of the cyanobacteria succeeded to create tight association with the roots of wheat plants. They also added that cyanobacteria inoculation led to soil structure improvement, which being reflected on soil fertility and consequently on cultivated crop.

These observations are in parallel with the results in this study. On other respect, Increasing the nutrient uptake by wheat grain and straw in response to the use cyanobacteria as biofertilizer separately was confirmed by those of Abd El Rasoul *et al.* (2003) and Mussa *et al.* (2003) who indicated that the use of nitrogen fixing biofertilizers had significantly increased N, P and K uptake by wheat grains and straw over the control treatments. The use of cyanobacteria along with some nitrogen fixing microorganisms plus full-N dose had achieved grains and straw yields, NPK uptake by grains and straw very close to and not significantly different from those achieved by same treatments under the influence of ½ N dose. They added that these nitrogen fixing microorganisms release plant growth promoting substances. This trend are in parallel to what revealed by El Mancy *et al.* (1997) who found that combination between biofertilizers with reduced amount of the mineral nitrogen can lead to saving chemical-N fertilizer (about 50 %) and improving NPK uptake by rice grains and straw. Inoculation with the nitrogen fixing *Azospirillum* to wheat as biofertilizer combined with ½ recommended N dose increased significantly grains and straw yields and NPK- uptake by wheat grains and straw, improved the grain quality (protein, dry gluten and flour extract percentages) compared to the control without inoculation (EL- Kasas, 2002).

The use of cyanobacteria enhanced the chemical properties of the wheat post harvest remained soil. Mandal *et al.* (1999) emphasized that inoculation with cyanobacteria (SBI) might help to regenerate quickly and improve soil structure. Albeit, 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 being a reason to improve the nutrient availability in soil. 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, 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. EL- Kasas (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_2 evolution, which are considered as index for biological activity and soil fertility (Ghazal, 1980). 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, especially in sandy soil (Mishra and Pabbi, 2004). NPK uptake again increased significantly with the use of $^1/_4$ N dose + *Azotobacter* and cyanobacteria. This result was in parallel with those of Hanna *et al.* (2004) who found that inoculation with cyanobacteria increased significantly the nitrogen, phosphorus and potassium contents of wheat grains and straw, and El- Gaml (2006) in maize. *Rhizobia* can associate with roots of non-legumes also, without forming true nodules, and can promote their growth by using one or more of the direct or indirect mechanisms of actions. Phytohormone production, secretion of other chemicals like lipo-chito-oligosaccharides (LCOs) and lumichrome, solubilization of precipitated phosphorus and mineralization of organic P, improvement in uptake of plant nutrients by altering root morphology, production of siderophores to meet the iron requirements of the plant under iron-stressed conditions and lowering of ethylene level through ACC deaminase enzyme, are some examples of the rhizobial mechanisms with direct positive effects on non-leguminous plant growth. Indirectly, rhizobia improve the growth of non-legumes through biocontrol of pathogens via antibiosis, parasitism or competition with pathogens for nutrients and space, by inducing systemic resistance in the host plant and through increasing root adhering soil by releasing exopolysaccarides, which regulate the water movement and facilitate the root growth. However, no influence or even inhibitory effects of rhizobial inoculation on non-legumes has also been demonstrated in some cases. Plant growth promoting mechanisms of rhizobia and its practical application in non-legumes are the major focus of this review, recorded by Mehboob *et al.* (2009).

Table (8): Effect of cyanobacteria and *Rhizobium radiobacter* and N-fertilization on CO_2- evolution, Dehydrogenase activity and Fluorescein diacetate hydrolysis activity (FDA) in soil after wheat harvesting

N-fertilization	Treatments				
	Control	**Cyanobacteria**	***R. radiobacter***	**Cyanobacteria + *R. radiobacter***	**Means**
	CO_2- evolution (mg CO_2 / 100g soil)				
50% N	199	368	470	372	352.2
75% N	370	431	315	461	394.3
full dose N	257	358	389	249	313.3
Means	**280.33**	**358.66**	**391.33**	**360.66**	
L.S.D.at 5%					
N:	**2.75**				
Treatment:	**2.94**				
Interaction:	**5.17**				
	Dehydrogenase activity (µg TPF / 100g soil /day)				
50% N	9.2	15.0	47.3	18.9	2.6
75% N	13.8	9.30	18.3	16.2	14.4
full dose N	26.0	30.8	14.8	19.9	22.9
Means	**16.36**	**18.36**	**26.8**	**18.33**	
L.S.D.at 5%					
N:	**2.69**				
Treatment:	**2.60**				
Interaction:	**4.70**				
	Fluorescein diacetate hydrolysis activity (FDA) (µg fluorescein g $soil^{-1}$)				
50% N	12.00	15.00	17.00	20.00	16.00
75% N	15.00	19.00	22.00	34.00	20.25
full dose N	19.00	23.00	29.00	31.00	25.75
Means	**15.33**	**19.00**	**23.66**	**25.66**	
L.S.D.at 5%					
N:	**3.12**				
Treatment:	**2.98**				
Interaction:	**4.70**				

El-Zeky *et al.* (2005) in rice and Abo El- Eyoun (2005) in maize found that inoculation with *Azotobacter* and cyanobacteria combined with low level of nitrogen ($^1/_2$ N dose) increased significantly these 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 increasing dehyderogenase and nitrogenase activities and CO_2 evolution and subsequently improved soil fertility and the plant growth performance.

Generally, It could be concluded that the use of cyanobacteria inoculation technology combined with *R. radiobacter* along with 75% of the recommended nitrogen dose in wheat production (especially in the areas with new reclaimed soil such as in this study) can enhance grains and straw yields, grains quality, NPK-uptake by grains and straw, and improve the soil nutrient status of the soil remained after wheat harvesting. However, this study needs to be repeated in different locations in Egypt, with special concern with the combination between *Rhizobia* and cyanobacteria as biofertilizer for cereal crops.

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إستخدام السيانوبكتريا وريزوبيا الراديوباكتر كسماد حيوى فى إنتاج القمح

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لقد أجريت تجربة حقلية بمحطة البحوث الزراعية بالاسماعيلية والتابعة لمركز البحوث الزراعية وذلك فى موسم 2009/2008 لتقييم امكانية استخدام كل من السيانوبكتريا وريزوبيا الراديوباكتر كسماد حيوى يوفر جزء من السماد النيتروجينى اللازم لعملية انتاج القمح وتأثير ذللك على محصول القمح من الحبوب والقش، المحتوى النيتروجينى والفوسفورى والبوتاسيومى لكل من الحبوب والقش ،الصفات التكنولوجية للحبوب، النيتروجين المتاح بالتربة بعد حصاد القمح ، المحتوى الميكروبى بالتربة بعد حصاد القمح.

فى هذة التجربة تم تلقيح القمح بالسيانوبكتريا جافة بمعدل 15 كجم / للفدان وكذلك التلقيح بريزوبيا الراديوباكتر عن طريق تغليف بذور القمح بمزرعة الريزوبيا قبل الزراعة ثم بعد ذلك السرسبة لمحلول مزرعة الريزوبيا على التربة بجوار النباتات بمعدل 10 لتر للفدان وذلك بعد 30 و 55 يوم من الزراعة. هذا وقد تم استخدام أى منهما منفردا أو مجتمعين تحت تأثير مستويات مختلفة من النيتروجين هى المستوى الموصى به و50% المستوى الموصى به و 75% المستوى الموصى به. وكانت أ هم النتائج المتحصل عليها كما يلى:

أولا: مكونات محصول القمح (الحبوب والقش):

1- لقد تحقق أعلى محصول للحبوب (1295,34 كجم/ فدان) عند استخدام السيانوبكتريا + ريزوبيا الراديوباكتر + المستوى النيتروجينى الكامل الا أن هذا المحصول كان غير مختلف معنويا مع ذلك المتحصل علية عند استخدام نفس المعاملة + 75%المستوى النيتروجينى الكامل (77, 1274 كجم/ فدان).
2- أعطى استخدام المستوى النيتروجينى الكامل منفردا أعلى محصول للحبوب بالمقارنة مع استخدام مستويات النيتروجين الأخرى منفردة.
3- لقد أظهر محصول القش نفس الاتجاه المتحقق مع محصول الحبوب.
4- لم يكن هناك اتجاه محدد لتأثير المعاملات تحت الدراسة على وزن ال 1000 حبة.

ثانيا :محتوى العنا صر (نيتروجين – فوسفور- بوتاسيوم) لكل من الحبوب والقش:

1- لقد تحقق أعلى محتوى نيتروجينى للحبوب (03, 22كجم نيتروجين/ فدان) عند استخدام السيانوبكتريا + ريزوبيا الراديوباكتر + المستوى النيتروجينى الكامل الا أن هذا المحتوى النيتروجينى كان غير مختلف معنويا مع ذلك المتحصل علية عند استخدام نفس المعاملة + 75% المستوى النيتروجينى الكامل (67, 21كجم نيتروجين / فدان).
2- لقد أظهر محتوى الحبوب من الفوسفور والبوتاسيوم نفس الاتجاه المتحقق مع المحتوى النيتروجينى.
3- لقد أظهر محتوى القش من النيتروجين نفس الاتجاه المتحقق مع المحتوى النيتروجينى للحبوب.

4- لقد كان محتوى القش من الفوسفور عند استخدام نفس المعاملة + 75%المستوى النيتروجينى الكامل (4,15 فوسفوركجم/ فدان) أعلى معنويا من ذلك المتحصل علية عند استخدام نفس المعاملة + المستوى النيتروجينى الكامل (2,98 كجم فوسفور/ فدان) هذا وعلى العكس من ذلك كان محتوى القش من البوتاسيوم أعلى معنويا لمعاملة السيانوبكتريا + ريزوبيا الراديوباكتر + المستوى النيتروجينى الكامل من معاملة السيانوبكتريا + ريزوبيا الراديوباكتر + 75% المستوى النيتروجينى الكامل.

ثالثا: أعداد الميكروبات بالتربة بعد حصاد القمح:

1- لقد كان عدد الميكروبات بالتربة بعد حصاد القمح نتيجة المعاملات المختلفة متأثرا بأنواعها حيث أدى التلقيح بالسيانوبكتريا أو ريزوبيا الراديوباكتر الى زيادة غير معنوية فى أعدادها كما فى أعداد الفطريا ت أو زيادة معنوية فى أعدادها كما فى أعداد الأكتينوميسيتات.

رابعا : تركيز غاز ثانى أكسيد الكربون ونشاط انزيم الديهيدروجينيز وفاعلية تحلل الفلوروسين بالتربة بعد حصاد القمح:

1- لقد حققت المعاملة السيانوبكتريا + ريزوبيا الراديوباكتر + 50% المستوى النيتروجينى الكامل أعلى تركيز لغاز ثانى أكسيد الكربون بالتربة بالمقارنة مع المعاملات الأخرى.

2- لقد حققت المعاملة ريزوبيا الراديوباكتر + 50% المستوى النيتروجينى الكامل أعلى نشاط لانزيم الديهيدروجينيز بالتربة بالمقارنة مع المعاملات الأخرى.

3- لقد حققت المعاملة المستوى النيتروجينى الكامل من معاملة السيانوبكتريا + ريزوبيا الراديوباكتر + 75% المستوى النيتروجينى الكامل اعلى فاعلية لتحلل الفلورسين بالتربة والذى كان غير مختلف معنوياً مع المتحصل عليه من المعاملة السيانوبكتريا + ريزوبيا الراديوباكتر + المستوى النيتروجينى الكامل.

قام بتحكيم البحث

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