

## **CYANOBACTERIA AND EFFECTIVE MICROORGANISMS (EM) AS POSSIBLE BIOFERTILIZERS IN WHEAT PRODUCTION**

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### **ABSTRACT**

Recently, a great attention is paid in establishing the concept of the associations between wheat plants and a variety of N<sub>2</sub>-fixing microorganisms. In this work, the soil based cyanobacteria inoculum (SBI) and the effective microorganisms (EM) were applied to wheat in a field experiment with different rates under different levels of nitrogen. Results revealed that cyanobacteria inoculation (SBI) combined with EM application exhibited an economical view that it can save about 50 % of the mineral nitrogen dose required for wheat crop production especially. The trend was noticed when SBI combined with EM which recorded a grain yield not significantly different from that obtained by the full recommended nitrogen dose in wheat cultivation. Cyanobacteria inoculation to wheat crop along with EM have also enhanced the NPK- uptake by wheat plant, the soil microbial community, dehydrogenase activity and CO<sub>2</sub> evolution as indices of soil fertility.

**Keywords:** Cyanobacteria, Effective microorganisms (EM), biofertilizers, wheat production.

### **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 leave 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. Two of the several approaches to achieve this goal are using the nitrogen fixing cyanobacteria and the effective microorganisms (EM) in order to improve soil fertility and productivity. The application of nitrogen fixing cyanobacteria likely ensures a partial replacement of the mineral nitrogen, while EM is expected to enhance the

availability of soil nutrients and humus formation and to control certain plant diseases and pathogens (Myint, 1999).

Recently, there is a great deal of interest in creating novel association between agronomically important plants, particularly cereals such wheat and N<sub>2</sub>-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<sub>2</sub>- fixed by *Nostoc* sp. in association with wheat is taken up by the plant and supports its growth, improving grain yields and grain quality (Gantar *et al.*, 1995).

The aim of this study is to apply cyanobacteria and EM as biofertilizers as a trial to improve wheat yield production and to save the environment by reducing the amounts of inorganic nitrogen fertilizer applied in wheat production, as well as to sustain soil degradation. Also to explore their effects on soil fertility status through the measuring of the soil microbial counts, dehydrogenase activity and CO<sub>2</sub> evolution after wheat harvesting.

## MATERIALS AND METHODS

A field trial was conducted at El Ismailia Research station, Agricultural Research Center (2001 / 2002) to study the effect of both cyanobacteria inoculation cyanobacteria inoculum (SBI) was prepared as dried algae flakes containing *Nostoc* sp. (local cyanobacteria isolate) as described by Venkataraman, 1981}and effective microorganisms (EM) application each alone or 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/fed.), 50 % (FRD) (40 kg N/fed.) and 25 % (FRD) (20 kg N/fed.). 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 <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
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 <sub>3</sub> %	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 (SBI) and effective microorganisms (EM), their combination and the control

treatment without inoculation or (EM) application to represent the sub plots. Uniform application of phosphate @ 30 kg P<sub>2</sub>O<sub>5</sub> as super-phosphate(15 % P<sub>2</sub>O<sub>5</sub>) and potassium, 48 kg as K<sub>2</sub>O<sub>5</sub> as potassium sulphate were done as basal to each plot. Nitrogen as urea was applied in five split doses. Cyanobacteria inoculation (SBI) at the rate of 15 kg fed<sup>-1</sup> dried alga flakes of *Nostoc* sp. local isolate was applied 7 days after sowing of wheat seeds, While (EM) was sprayed on the plants two weeks after sowing at the rate of 40 L fed<sup>-1</sup> in three split doses monthly starting from cultivation. Irrigation was carried out every three days using the sprinkler system.

At harvest, wheat plants were cut just above the soil surface to determine the wheat yield components, NPK uptake by wheat grains and straw (Chapman and Pratt, 1961). The remained soil after wheat harvesting was sampled to estimate available NPK (Jackson, 1973) as well as to compute the total microbial count (Allen, 1959), total count of Actinomycetes (Williams and Davis, 1965), total count of fungi (Martin, 1950), total count of *Azotobacter* and *Azospirillum* (Cochran, 1950), total count of cyanobacteria (Allen and Stanier, 1968) CO<sub>2</sub> evolution (Pramer and Schmidt, 1964) and dehydrogenase activity (Casida *et al.*, 1964) as index for soil fertility. All obtained results were then subjected to the statistical analysis according to Gomes and Gomes (1984).

What is the effective microorganisms (EM)

The technology of effective microorganisms (commonly termed EM) was developed in the 1970s at the university of Ryukyus, Okinawa, Japan. The inception of the technology was based on blending multitude of microbes, and was subsequently refined to include the principal types of organisms commonly found in all ecosystems, namely lactic acid bacteria, photosynthetic bacteria, yeasts, actinomycetes and fungi (Table 2). These were blended in molasses or sugar medium and maintained at low pH under ambient conditions (kato *et al.*, 1999).

**Table (2): The main species included in EM.**

Effective microorganisms(EM)	
<u>Lactica acid bacteria:</u>	<u>Yeasts:</u>
<i>Lactobacillus plantarum</i>	<i>Saccharomyces cerevisiae</i>
<i>Lactobacillus casei</i>	<i>Candida ulitis</i>
<i>Streptococcus lactis</i>	<u>Actinomycetes</u>
<u>Photosynthetic bacteria:</u>	<i>Streptomyces albus</i>
<i>Rhodopseudomonas palustris</i>	<i>Streptomyces griseus</i>
<i>Radobacter sphaeraides</i>	<u>Fungi:</u>
	<i>Aspergillus oryze</i>

## RESULTS AND DISCUSSION

The nitrogen fixing cyanobacteria inoculation to wheat is recently established to substitute partially or entirely the mineral nitrogen utilization (Mussa *et al.*, 2003), while effective micro organisms is recommended to enhance soil characteristics degraded due to conventional farming methods (Sangakkara, 1999). Herein , a field experiment is laid out to detect the influence of both cyanobacteria wheat association and EM application either

each alone or in combination under different levels of mineral nitrogen on wheat yield components, available NPK in soil, soil microbial community, CO<sub>2</sub> evolution and dehydrogenase activity as soil fertility indices.

**Wheat yield components:**

Data in Table (3) indicate the effect of cyanobacteria association, EM 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<sup>-1</sup>) was recorded in the treatment of cyanobacteria plus EM treatments combined with full dose of mineral N (80 kg N fed<sup>-1</sup>) followed by 1274.77 kg fed<sup>-1</sup> for cyanobacteria + EM combined with ½ full mineral nitrogen dose (40 kg N fed<sup>-1</sup>) treatment. However, there was no significant difference between these two treatments.

**Table (3): Effect of Cyanobacteria inoculation, effective micro-organisms (EM) and N-fertilization on wheat yield components.**

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria+EM	Means
<b>Grain yield kg fed<sup>-1</sup></b>					
1/4 N	574.28	780.96	845.49	962.25	790.75
1/2 N	736.09	869.57	866.46	1274.77	936.72
Full N dose	872.10	993.48	1014.70	1295.34	1043.91
Means	727.49	881.34	908.88	1177.45	
LSD at 5%					
N:	96.77				
Treatments:	72.26				
Interaction:	144.00				
<b>Straw yield ton fed<sup>-1</sup></b>					
1/4 N	2.61	2.33	2.59	2.28	2.45
1/2 N	2.41	3.03	2.42	2.97	2.71
Full N dose	2.69	2.49	2.21	3.31	2.68
Means	2.57	2.62	2.41	2.85	
LSD at 5%					
N:	0.31				
Treatments:	0.28				
Interaction:	0.53				
<b>1000 grains weight (g)</b>					
1/4 N	45.87	53.30	48.77	49.00	49.24
1/2 N	46.97	53.83	45.50	48.30	50.90
Full N dose	42.63	49.97	40.10	50.90	45.90
Means	45.16	52.37	47.79	49.40	
LSD at 5%					
N:	2.26				
Treatments:	3.92				
Interaction:	4.44				

On the average application of full nitrogen dose gave significantly the highest wheat grain yield ( $1043.91 \text{ kg fed}^{-1}$ ) compared with the other two levels of nitrogen ( $\frac{1}{4}$  and  $\frac{1}{2}$  N dose).

Same behavior exhibited by grain yield was observed for straw yield indicating the highest straw yield ( $3.31 \text{ tons fed}^{-1}$ ) for the treatment cyanobacteria plus EM under full nitrogen dose and followed by ( $2.97 \text{ tons fed}^{-1}$ ) for cyanobacteria plus EM under  $\frac{1}{2}$  N level treatment without significant different between each others.

Full dose of nitrogen gave a significant increase in the mean of straw yield ( $2.68 \text{ tons fed}^{-1}$ ) compared with the other two nitrogen levels.

1000-grain weight showed variable trends in response to the tested treatments. Due to nitrogen doses application,  $\frac{1}{2}$  N dose treatment gave the highest mean value of 1000-grain weight ( $50.90 \text{ g}$ ) without significant difference with those recorded for  $\frac{1}{4}$  and full N doses. Cyanobacteria treatments showed the highest mean value ( $52.37 \text{ g}$ ) which exceeded significantly the mean values recorded for either control or EM treatments but not with E M + cyanobacteria treatments.

#### **NPK uptake by wheat grains:**

Table (4) showed that, inoculation with cyanobacteria plus EM application under full nitrogen dose gave the highest N uptake value ( $22.03 \text{ kg N fed}^{-1}$ ) with no significant difference with that recorded by cyanobacteria plus EM treatment under  $\frac{1}{2}$  N dose ( $21.67 \text{ kg N fed}^{-1}$ ). Due to nitrogen application alone, there was no significant difference in N uptake values obtained by either  $\frac{1}{2}$  or full N dose treatments. Their respective mean N uptake values were  $15.71$  and  $16.87 \text{ kg N fed}^{-1}$ , respectively.

Phosphorus 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  $2.17 \text{ kg P fed}^{-1}$  ( $\frac{1}{2}$  N dose + cyanobacteria + EM) was not significantly different from  $2.07 \text{ kg P fed}^{-1}$  recorded for cyanobacteria plus EM plus full N dose, treatments, respectively. While with K uptake, the highest value of  $6.63 \text{ kg K fed}^{-1}$  which recorded for cyanobacteria plus EM +  $\frac{1}{2}$  N dose was not significantly different from that of  $6.35 \text{ kg K fed}^{-1}$  for cyanobacteria + EM + Full dose treatment.

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

**Table (4): Effect of cyanobacteria inoculation, effective micro-organisms (EM) and N-fertilization on NPK uptake by wheat grains.**

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria+EM	Means
<b>N- uptake kg fed<sup>-1</sup></b>					
1/4 N	8.04	10.15	15.20	16.36	12.44
1/2 N	12.51	14.78	13.86	21.67	15.71
Full N dose	18.31	11.92	15.22	22.03	16.87
Means	12.95	12.28	14.76	20.02	
<b>LSD at 5%</b>					
N:	1.451				
Treatments:	1.197				
Interaction:	2.290				
<b>P- uptake kg fed<sup>-1</sup></b>					
1/4 N	0.75	1.17	1.18	1.35	1.11
1/2 N	1.18	1.74	1.39	2.17	1.62
Full N dose	1.35	1.49	1.93	2.07	1.71
Means	1.09	1.47	1.50	1.86	
<b>LSD at 5%</b>					
N:	0.160				
Treatments:	0.125				
Interaction:	0.244				
<b>K- uptake kg fed<sup>-1</sup></b>					
1/4 N	2.99	3.75	4.22	4.62	3.90
1/2 N	3.98	4.87	3.99	6.63	4.87
Full N dose	4.45	5.17	5.38	6.35	5.34
Means	3.81	4.60	4.53	5.87	
<b>LSD at 5%</b>					
N:	0.516				
Treatments:	0.379				
Interaction:	0.762				

**NPK uptake by wheat straw:**

Table (5) indicated that N uptake value recorded by the cyanobacteria + EM + 1/2 N dose (14.83 kg N fed<sup>-1</sup>) was not significantly different from that recorded by cyanobacteria + EM + full N dose treatment (16.55 kg N fed<sup>-1</sup>). In contrast, P uptake value of 4.15 kg P fed<sup>-1</sup> (cyanobacteria + EM + 1/2 N dose) was significantly higher than that recorded by cyanobacteria + EM + Full N dose treatment (2.98 kg P fed<sup>-1</sup>). This increase in phosphorus could be attributed to that the increase of nitrogen fertilizer from 1/2 to full N dose which may suppress the nitrogen fixation and solubilizing phosphate processes by cyanobacteria (Myint, 1999).

Same observations were noticed by K -uptake, that the K- uptake value recorded by the treatment of cyanobacteria + EM + Full N dose (22.18 kg K fed<sup>-1</sup>) was significantly higher than that recorded by cyanobacteria + EM + 1/2 N dose treatment (16.91 kg k fed<sup>-1</sup>).

**Table (5): Effect of cyanobacteria inoculation, effective micro-organisms (EM) and N-fertilization on NPK uptake by wheat straw.**

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria+EM	Means
<b>N- uptake kg fed<sup>-1</sup></b>					
1/4 N	13.05	27.94	10.37	11.95	15.83
1/2 N	24.18	30.27	14.54	14.83	20.96
Full N dose	16.16	14.94	13.38	16.55	15.26
Means	17.80	24.38	12.76	14.44	
LSD at 5%					
N:	2.65				
Treatments:	2.15				
Interaction:	4.14				
<b>P- uptake kg fed<sup>-1</sup></b>					
1/4 N	1.55	3.51	2.34	1.36	2.19
1/2 N	4.10	4.52	2.91	4.15	3.92
Full N dose	2.24	1.24	4.01	2.98	2.62
Means	2.63	3.09	3.09	2.83	
LSD at 5%					
N:	0.59				
Treatments:	0.43				
Interaction:	0.87				
<b>K- uptake kg fed<sup>-1</sup></b>					
1/4 N	16.18	12.82	14.65	17.55	15.30
1/2 N	25.34	19.37	12.60	16.91	18.56
Full N dose	17.77	19.67	15.84	22.18	18.87
Means	19.76	17.29	14.36	18.88	
LSD at 5%					
N:	3.15				
Treatments:	2.32				
Interaction:	4.66				

**Available NPK in soil:**

In respect to soil available NPK amounts (Table 6), results indicated that available-N significantly increased in response to increasing nitrogen fertilizer doses over 1/2 N dose with priority to 1/2 N-dose treatment, which recorded 126.25 ppm available N.

Apart from nitrogen doses, cyanobacteria inoculation combined with EM application had achieved the highest available-N amounts (116.6 ppm). This high available-N amount was significantly different from that recorded by either cyanobacteria (105.00 ppm-N) or EM (103.33 ppm-N) treatments each applied alone.

Both cyanobacteria, EM each alone or in combination when being affected with the different fertilizer-N dose showed the highest available-N amount (140.00 ppm) for the treatment received cyanobacteria + EM + 1/2 N dose. However, this high amount was significantly exceeded all other interacted treatments.

**Table (6): Effect of cyanobacteria inoculation, effective micro-organisms (EM) and N-fertilization on nitrogen, phosphorus and potassium after wheat harvesting.**

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria+EM	Means
<b>Available nitrogen (ppm)</b>					
1/4 N	90	100	80	100	92.50
1/2 N	120	125	120	140	126.25
Full N dose	80	90	100	110	95.00
Means	96.66	105	103.33	116.6	
LSD at 5%					
N:	2.65				
Treatments:	2.14				
Interaction:	4.14				
<b>Available phosphorus (ppm)</b>					
1/4 N	7.4	9.0	11.4	5.90	8.43
1/2 N	4.16	8.3	6.9	7.07	6.61
Full N dose	6.34	6.5	7.6	7.50	6.99
Means	5.97	7.93	8.63	6.82	
LSD at 5%					
N:	1.40				
Treatments:	1.16				
Interaction:	2.50				
<b>Available potassium (ppm)</b>					
1/4 N	103	110	86	58	89.25
1/2 N	70	79	81	76	76.50
Full N dose	100	79	118	70	91.75
Means	91	89.33	95	68	
LSD at 5%					
N:	3.54				
Treatments:	3.09				
Interaction:	6.93				

Available phosphorus amounts decreased significantly in response to nitrogen fertilizer doses, since they recorded less amounts of 6.61 and 6.99 ppm than that of 8.43 ppm for 1/2, full and 1/4 N-dose treatments, respectively.

Due to cyanobacteria inoculation, and EM each alone or in combination, results revealed that the highest significant available phosphorus amount of 8.63 ppm (EM treatment alone) was higher than those of 6.82 and 7.93 ppm for cyanobacteria + EM and EM treatments, respectively. However, the nitrogen, cyanobacteria and EM relation gave the highest significant available-P amount (11.4 ppm) for EM combined with 1/4 N-dose. Compared to the other interaction treatments except for cyanobacteria combined with 1/4 N-dose (9.00 ppm-P) treatment.

Available potassium amounts had fluctuated between decrease in response to 1/2 and full nitrogen dose compared to 1/4 N-dose application. Nevertheless, the highest available-K amount of 91.75 ppm (full-N dose) was significantly higher than that recorded by 1/2 N dose (76.50ppm-K) but did not than that of 89.25 ppm-K for 1/4 N-dose treatment. On the other hand, EM

applied alone had achieved the available-K amount (95.00 ppm) being significantly higher than those of cyanobacteria (89.33ppm-K) and cyanobacteria + EM (68.00 ppm-K) treatments. Nitrogen, cyanobacteria and EM interactions + resulted in the highest significant available-K amount of 118.00 ppm more than those recorded by the other interaction relations.

**Soil Micro-organisms Counts:**

Total fungi in soil after wheat harvesting (Table 7) exhibited no significant in response to nitrogen fertilizer doses. However, the highest total fungi count ( $32.90 \times 10^2$  cfu g<sup>-1</sup> soil) obtained by 1/2 N-dose treatment.

Apart from nitrogen doses, cyanobacteria + EM treatment gave the highest insignificant total fungi of  $36.33 \times 10^2$  cfu g<sup>-1</sup> soil compared to those recorded by the other treatments.

**Table (7): Effect of cyanobacteria inoculation, effective micro-organisms (EM) and N-fertilization on total fungi, actinomycetes and total bacterial counts after wheat harvesting.**

N-fertilization	Treatments				Means
	Control	Cyanobacteria	EM	Cyanobacteria+EM	
<b>Total fungi x 10<sup>2</sup></b>					
1/4 N	7.20	29.10	53.70	38.90	32.23
1/2 N	22.60	36.00	15.70	57.30	32.90
Full N dose	15.60	25.50	33.20	12.80	21.77
Means	15.13	30.20	34.20	36.33	
LSD at 5%					
N:	n.s				
Treatments:	6.49				
Interaction:	14.19				
<b>Actinomycetes x 10<sup>3</sup></b>					
1/4 N	1.00	38.20	58.10	31.80	32.275
1/2 N	18.70	46.70	18.60	84.10	42.025
Full N dose	8.30	31.60	38.80	7.10	21.450
Means	9.30	38.83	38.50	71.00	
LSD at 5%					
N:	5.22				
Treatments:	9.01				
Interaction:	14.14				
<b>Total bacteria x 10<sup>4</sup></b>					
1/4 N	26.10	38.70	273.2	45.10	106.42
1/2 N	38.20	77.60	32.0	131.40	69.80
Full N dose	28.50	34.70	47.8	17.70	32.18
Means	30.93	50.30	117.66	64.73	
LSD at 5%					
N:	25.26				
Treatments:	15.72				
Interaction:	24.20				

Interaction effect due to the treatments under the influence of N-doses resulted in higher significant total fungi count ( $57.3 \times 10^2$  cfu g<sup>-1</sup>soil) compared to the other interaction influences except for those recorded by cyanobacteria + EM + 1/4 N-dose ( $38.90 \times 10^2$  cfu g<sup>-1</sup> soil), and EM + 1/4 N dose ( $53.70 \times 10^2$  cfu g<sup>-1</sup> soil).

Actinomycetes count indicate significant response due to N-dose application, when the highest count ( $42.03 \times 10^3$ ) cfu g<sup>-1</sup> soil) recorded by the use of ½ N- dose compared with the other applied N-doses.

Cyanobacteria treatment + EM had recorded the highest significant actinomycetes count ( $71.00 \times 10^3$  cfu g<sup>-1</sup>soil) compared with those recorded by either cyanobacteria or EM each alone.

On the other hand, when cyanobacteria combined with EM under the effect of ½ N-dose gave the highest actinomycetes count of ( $84.10 \times 10^3$  cfu g<sup>-1</sup>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 ¼ N-dose, EM treatment, and cyanobacteria combined with EM under the effect of ½ N-dose application. The corresponding total bacterial count were 106.42, 117.66 and  $131.40 \times 10^6$  cfu g<sup>-1</sup> soil.

*Azotobacter* gave its highest count ( $6.30 \times 10^5$  cfu g<sup>-1</sup> soil) in response to cyanobacteria + EM + ½ N dose treatment (Table 8). This high count was significantly higher than those given by all the other treatments except for that recorded by cyanobacteria + EM + full- N dose treatment ( $7.00 \times 10^5$  cfu g<sup>-1</sup> soil).

*Azospirillum* had recorded its favorite count number of  $7.9 \times 10^5$  cfu g<sup>-1</sup> soil with the use of cyanobacteria + EM + full- N dose treatment which, was significantly different from that attained by cyanobacteria + EM + ½ N dose treatment ( $6.00 \times 10^5$  cfu g<sup>-1</sup> 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 + EM + ½ N dose had achieved the highest significant cyanobacteria count ( $43.30 \times 10^3$  cfu g<sup>-1</sup> soil) in soil compared to the other treatments.

### **CO<sub>2</sub> evolution:**

Carbon dioxide evolution by soil (Table 9) calculated after wheat harvesting showed its highest significant amount of 391.33, 394.30 and 470.00 mg CO<sub>2</sub> 100 g<sup>-1</sup> soil due to the application of EM treatment alone, ½ N-dose alone and cyanobacteria combined with EM under the influence of ¼ N-dose, respectively in comparison to their related treatment without nitrogen.

### **Dehydrogenase activity ( DHA) :**

Dehydrogenase activity in soil after wheat harvesting (Table 9) expressed its highest significant values of 47.30, 26.00 and 22.90 mg TPF 100 g<sup>-1</sup> soil by EM combined with ¼ N-dose, EM treatment alone and full-N dose alone, respectively. These DAH values were significantly higher than those recorded by the other related treatment.

The aforementioned results are in agreement with those described by (Abd-Alla *et al.*, 1994 and Mussa, *et al.*, 2003) they attributed the increases in wheat yield components in the cyanobacteria inoculated treatments to the substantial increases in N<sub>2</sub> 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.

**Table (8): Effect of cyanobacteria inoculation, effective micro-organisms (EM) and N-fertilization on N<sub>2</sub>-fixers (*Azotobacter*, *Azospirillum* and total cyanobacteria) counts in soil after wheat harvest.**

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria+EM	Means
<b><i>Azotobacter</i> x 10<sup>5</sup></b>					
1/4 N	5.60	4.40	9.10	2.50	5.40
1/2 N	2.00	5.40	2.90	6.30	4.15
Full N dose	5.40	2.30	3.10	7.00	4.45
Means	4.33	4.03	5.03	5.27	
LSD at 5%					
N:	0.23				
Treatments:	0.23				
Interaction:	0.40				
<b><i>Azospirillum</i> x 10<sup>5</sup></b>					
1/4 N	5.20	1.20	6.30	1.70	3.60
1/2 N	1.10	4.80	2.20	6.00	3.53
Full N dose	4.60	1.60	2.20	7.90	4.08
Means	3.63	2.53	3.57	5.2	
LSD at 5%					
N:	0.23				
Treatments:	0.16				
Interaction:	0.35				
<b>N<sub>2</sub>-fixing cyanobacteria x 10<sup>3</sup></b>					
1/4 N	1.70	11.70	2.00	27.70	10.775
1/2 N	6.00	4.00	19.00	43.30	18.075
Full N dose	0.63	3.30	1.00	1.00	5.93
Means	2.77	6.33	7.33	24.00	
LSD at 5%					
N:	7.19				
Treatments:	6.49				
Interaction:	9.34				

Significant increases were found in all wheat yield parameters with application of EM combined with ½ recommended nitrogen dose producing (2831 kg grains ha<sup>-1</sup>) very close to full-recommended nitrogen dose (3017 kg grains ha<sup>-1</sup>) (Hussain *et al.*, 1999). These observations are in parallel with the results in this study. On the other respect, increasing the nutrient uptake by wheat grains and straw in response to the application of either EM or cyanobacteria as biofertilizer, was confirmed by those of Abd El Rasoul *et al.*, (2003) and Mussa *et al.*, (2003) they indicated that spraying both EM and nitrogen fixing biofertilizers individually had significantly increased N, P and K uptake by grains and straw over the control treatments. Cyanobacteria combined with EM plus full-N dose gave yields of grains and straw and NPK

contents of grains and straw in very close amounts to those achieved by same treatments under the influence of ½ N dose. This trend is similar to that revealed by El Mancy *et al.*, (1997) they reported 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. Nitrogen fixing *Azospirillum* inoculation to wheat as biofertilizer combined with ½ recommended N dose, significantly increased grains and straw yields and NPK- uptake by grains and straw compared to the control treatment without inoculation (EL- Kasas, 2002).

**Table (9): Effect of cyanobacteria inoculation, effective micro-organisms (EM) and N-fertilization on CO<sub>2</sub>-evolution and Dehydrogenase activity in soil after wheat harvesting.**

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria+EM	Means
<b>CO<sub>2</sub>-evolution (mg CO<sub>2</sub>/100g soil)</b>					
1/4 N	199	368	470	372	352.2
1/2 N	370	431	315	461	394.3
Full N dose	257	358	389	249	313.3
Means	280.33	358.66	391.33	360.66	
<b>LSD at 5%</b>					
N:	2.75				
Treatments:	2.94				
Interaction:	5.17				
<b>Dehydrogenase activity (? g TPF/ 100 g soil/ day)</b>					
1/4 N	9.2	15.0	47.3	18.9	22.6
1/2 N	13.8	9.30	18.3	16.2	14.4
Full N dose	26.1	30.8	14.8	19.9	22.9
Means	16.36	18.36	26.8	18.33	
<b>LSD at 5%</b>					
N:	2.69				
Treatments:	2.60				
Interaction:	4.70				

The use of cyanobacteria and EM 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. EL- Kasas, (2002) Reported that inoculation of *Azospirillum* to wheat increased the soil *Azospirilla* and other microbial population including fungi, actinomycetes and *Azotobacter* , and consequently increased both the dehydrogenase activity and CO<sub>2</sub> evolution, which are considered as indices for biological activity and soil fertility (Abd EL-Aal *et al.*, 1982).

Due to The use of EM, Frighetto, *et al.*, (1999), confirmed that EM application enhanced the soil biological and chemical properties which ensures not only organic and biological sources of the essential nutrients

supply but also show some positive interaction with chemical fertilizers through increasing their efficiency and thereby reducing the environmental hazards.

Generally, It could be concluded that application of cyanobacteria inoculation combined with EM in addition to one half of the recommended nitrogen dose in wheat production (especially in the newly reclaimed soil) can enhance grains and straw yields, NPK-uptake by grains and straw, and improve the soil nutrient status after wheat harvesting. However, this study need to be confirmed through its execution for several seasons in different locations in Egypt, with special concern with effective microorganisms application.

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## السيانوبكتريا والميكروبات الفعالة كسماد حيوى ممكن فى انتاج القمح شعبان عبد الرسول - منى ميخائيل حنا - الهام محمد عارف - فكرى محمد غزال معهد بحوث الأراضى والمياه والبيئة - مركز البحوث الزراعية - الجيزة - مصر

أجريت تجربة حقلية بمحطة البحوث الزراعية بالاسماعيلية والتابعة لمركز البحوث الزراعية وذلك فى موسم ٢٠٠١/٢٠٠٢ لتقييم امكانية استخدام كل من السيانوبكتريا ومحلول الميكروبات الفعالة كسماد حيوى يوفر جزء من السماد النيتروجينى اللازم لعملية انتاج القمح وتأثير ذلك على محصول القمح من الحبوب والقش، المحتوى النيتروجينى والفوسفورى والبوتاسيومى لكل من الحبوب والقش، النيتروجين المتاح بالتربة و المحتوى الميكروبى بالتربة بعد حصاد القمح. فى هذه التجربة تم تقيح القمح بالسيانوبكتريا جافة بمعدل ١٥ كجم / للقدان وكذلك رش محلول الميكروبات الفعالة بمعدل ٤٠ لتر للقدان بحيث تم استخدام أى منهما منفردا أو مجتمعين تحت تأثير مستويات مختلفة من النيتروجين هى المستوى الموصى به و ١/٤ المستوى الموصى به و ١/٢ المستوى الموصى به. وكانت أهم النتائج المتحصل عليها كما يلى:

أولا: مكونات محصول القمح (الحبوب والقش):

١- تحقق أعلى محصول للحبوب (١٢٩٥ و ٣٤ كجم/ فدان) عند استخدام السيانوبكتريا + الميكروبات الفعالة + المستوى النيتروجينى الكامل الا أن هذا المحصول كان غير مختلف معنوياً مع ذلك المتحصل عليه عند استخدام نفس المعاملة + ١/٢ المستوى النيتروجينى الكامل (١٢٧٤ و ٧٧ كجم/ فدان).

٢- أعطى استخدام المستوى النيتروجينى الكامل منفردا أعلى متوسط محصول للحبوب بالمقارنة مع استخدام مستويات النيتروجين الأخرى منفردة.

٣- أظهر محصول القش نفس الاتجاه المتحقق مع محصول الحبوب.

٤- لم يكن هناك اتجاه محدد لتأثير المعاملات تحت الدراسة على وزن ال ١٠٠٠ حبة.

ثانياً : محتوى العناصر (نيتروجين - فوسفور - بوتاسيوم) لكل من الحبوب والقش:

١- لقد تحقق أعلى محتوى نيتروجينى للحبوب (٣ و ٢٢ كجم نيتروجين/ فدان) عند استخدام السيانوبكتريا + الميكروبات الفعالة + المستوى النيتروجينى الكامل الا أن هذا المحتوى النيتروجينى كان غير مختلف معنوياً مع ذلك المتحصل عليه عند استخدام نفس المعاملة + ١/٢ المستوى النيتروجينى الكامل (٦٧ و ٢١ كجم نيتروجين / فدان).

٢- أظهر محتوى الحبوب من الفوسفور والبوتاسيوم نفس الاتجاه المتحقق مع المحتوى النيتروجينى.

٣- أظهر محتوى القش من النيتروجين نفس الاتجاه المتحقق مع المحتوى النيتروجينى للحبوب.

٤- كان محتوى القش من الفوسفور عند استخدام نفس المعاملة + ١/٢ المستوى النيتروجينى الكامل (١٥ و ٤ فوسفور/ فدان) أعلى معنوياً من ذلك المتحصل عليه عند استخدام نفس المعاملة + المستوى النيتروجينى الكامل (١٨ و ٢ فوسفور / فدان) هذا وعلى العكس من ذلك كان محتوى القش من البوتاسيوم أعلى معنوياً لمعاملة السيانوبكتريا + الميكروبات الفعالة + المستوى النيتروجينى الكامل من معاملة السيانوبكتريا + الميكروبات الفعالة + ١/٢ المستوى النيتروجينى الكامل.

ثالثاً: العناصر المتاحة بالتربة بعد حصاد القمح:

أدى التقيح بالسيانوبكتريا أو الرش بمحلول الميكروبات الفعالة السى زيادة كل من النيتروجين والفوسفور المتاح بالتربة بعد حصاد القمح بالمقارنة مع المعاملات الأخرى. ومن ناحية أخرى فقد تراجح تركيز البوتاسيوم المتاح بالتربة بعد حصاد القمح بين الزيادة والنقصان نتيجة لأثر المعاملات تحت الدراسة. رابعاً : أعداد الميكروبات بالتربة بعد حصاد القمح:

كان عدد الميكروبات بالتربة بعد حصاد القمح نتيجة المعاملات المختلفة متأثراً بأنواعها حيث أدى التقيح بالسيانوبكتريا أو الرش بمحلول الميكروبات الفعالة الى زيادة غير معنوية فى أعدادها كمانى أعداد الفطريات أو زيادة معنوية فى أعدادها كما فى أعداد الأكتينوميسيتات.

خامساً : تركيز غاز ثانى أكسيد الكربون ونشاط انزيم الديهيدروجينيز بالتربة بعد حصاد القمح:

١- حققت المعاملة السيانوبكتريا + الميكروبات الفعالة + ١/٤ المستوى النيتروجينى الكامل أعلى تركيز لغاز ثانى أكسيد الكربون بالتربة بالمقارنة مع المعاملات الأخرى.

٢- حققت المعاملة بالميكروبات الفعالة + ١/٤ المستوى النيتروجينى الكامل أعلى نشاط لانزيم الديهيدروجينيز بالتربة بالمقارنة مع المعاملات الأخرى.