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RESPONSE OF WHEAT PLANTS TO EM (EFFECTIVE MICROORGANISMS) APPLICATION AND/OR CYANOBACTERIA INOCULATION UNDER SANDY SOIL CONDITION

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ABSTRACT

During the successive seasons (2008-09 and 2009 -10) wheat field experiments were carried out at EL- Ismailia Agriculture Research Station Agric. Res. Center (ARC) to study the effect of cyanobacteria inoculation and/or effective microorganisms and mineral nitrogen fertilizer on wheat plants (Sakha 69). In this work, cyanobacteria inoculation and the effective microorganisms (EM) were applied to wheat each alone or both in combination under different levels of nitrogen, i.e., $^1/_4$, $^1/_2$ and full N recommended dose). Results revealed that cyanobacteria inoculation combined with EM application exhibited an economical view that it can save about 50 % was noticed when cyanobacteria 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, wheat grains technology, dehyderogenase activity and Co_2 evolution as index of soil fertility.

INTRODUCTION

Wheat is the most important cereal crop in Egypt and is the staple food of the people and thus occupies a central position in forming agricultural policies and dominates all crops in production attention in an attempt to meet the gap arises between the consumption and production. However, 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 microorganism (EM) in order to improve soil fertility and productivity. The use of nitrogen fixing cyanobacteria ensures entirely or partially 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). There is a great deal of interest in creating novel association between agronimically important plants, particularly 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 nitrogen 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).

A recent introduction namely "Effective Micro-organisms (EM) technology" is reported to increase soil fertility through enhanced release of the plant nutrients and is getting popularity among the farming community due to low capital cost. Majority of the farmers still have concerns about the significance of this material over the chemical fertilizers and organic manures. Effectiv-emicroorganisms have paid much attention lately in the entire world.

Higa and Parr (1994) reported that the use of EM will supply the following benefits for plants, i.e., grow high quality and seedlings., quickly establishes a beneficial micro-ecology that promotes extremely stable growing conditions and safeguards against common stress-related problems, such as over watering, helps maintain plant health with antioxidants (vitamins and trace minerals), enzymes, and organic acids, reduce problems associated with soils drying out and being re-hydrated due to improved texture and structure. They added that EM contains more than 70 beneficial organisms, more importantly lactic acid bacteria, photosynthetic bacteria (*Rhodopseudomonas palustris*) and yeast. Surprisingly, use of EM helps in augmenting the photosynthesis by about 30 % in all the crops. Further, it controls viruses and fungal damage to crops by inoculating lactic acid bacteria and actinomycetes.

Therefore, the aim of this work is to evaluate the effect of Effective-microorganisms solution and cyanobacteria inoculation each applied alone or both in combination on wheat cultivated in sandy soil in terms of wheat yield and its components, NPk uptake for both wheat grains and straw, soil available NPK and soil biological activity after wheat harvesting.

MATERIALS AND METHODS

Wheat field experiments were carried out at EL- Ismailia Agriculture Research Station, ARC, during two winter successive seasons of 2008/2009 and 2009/2010 to study the effect of both cyanobacteria inoculation and effective microorganisms (EM) application each 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), 50 % (FRD) (40 kg N) and 25 % (FRD) (20 kg N). The physio-chemical analyses (Page *et al*., 1982) of the experimental soil are 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 3.5 m each) to represent 12 treatments in three replicates arranged in statistical split plot design. Nitrogen fertilizer represents the main plot in three treatments, while cyanobacteria inoculation and/or effective microorganisms (EM), their combination and the control treatment without inoculation or (EM) application to represent the sub plot. Uniform application of phosphate at the rate of 200 kg fed^{-1} as superphosphate (15.5 % P_2O_5) and potassium in the form of potassium sulfate (48% K_2O) at the rate of 50 kg fed^{-1} were done as basal to each plot. Nitrogen as urea was applied in three split equal doses according to the treatment. Cyanobacteria inoculation at the rate of 10 kg dried *Nostoc* sp. was executed 7 days after sowing of wheat seeds, while (EM) was foliar sprayed two weeks after sowing at the rate of 40 L fed^{-1} in three split doses monthly starting from two weeks after sowing. Irrigation was carried out every three days using the sprinkler system.

At harvest for both seasons, wheat plants were cut gust above the soil surface to determine the wheat yield, its components and NPK uptake by wheat grains and straw (Chapman and Pratt, 1961). The remained soil was sampled and subjected to evaluate the available NPK (Page *et al*., 1982), as well as to determine the counts of bacteria (Allen, 1959), *Actinomycetes* (Williams and Davis, 1965), total fungi (Martin, 1950), *Azotobacter* & *Azospirillum* (Cochran, 1950) and cyanobacteria (Allen and Stanier, 1968) CO_2 evolution (Pramer and Schmidt, 1964) and dehydrogenase (Casida *et al*., 1964) activity as a soil fertility index.

Statistical analysis:

All obtained results in both seasons were statistically analyzed as mean values for both seasons which compared for the least significant difference (L. S. D.) as described by Gomez and Gomez (1984).

Cyanobacteria Inoculum:

The cyanobacteria inoculums containing the cyanobacterium *Nostoc* sp., was taken from Soils, Water & Environ. Res. Inst., ARC., Giza, Eygpt. The isolated *Nostoc* sp. was used to prepare the dried soil based cyanobacteria inoculum as described by Venkataraman (1972).

What is the effective microorganisms:

The effective microorganisms (commonly termed EM) is an organic biofertilizer containing a mixture of Lactica acid bacteria (*Lactobacillus plantarum and Lactobacillus casei*), photosynthetic bacteria (*Rhodopseudomonas palustris* and *Radobacter sphaeraides*), yeasts (*Saccharomyces cerevisia* and *Candida utilis*), ray fungi (*Streptomyces albus* and *Streptomyces griseus*) and fungi (*Aspergillus oryzae*). These microorganisms were blended in molasses or sugar medium, maintained at low pH under ambient conditions (kato *et al*., 1999) and used.

RESULTS AND DISCUSSION

Wheat yield components:

Data in Table (2) indicated the effect of cyanobacteria inoculation and/or EM application 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 EM treatments combined with full dose of mineral N (80 kg N fed $^{-1}$) followed by 1274.77 kg fed^{-1} for cyanobacteria + EM combined with ½ full mineral nitrogen (40 kg N fed^{-1}) dose treatment. However, there was no significant difference between these two treatments. The application of full nitrogen dose gave significantly the highest mean wheat grain yield (1043.91 kg fed^{-1}) compared with the other two levels of nitrogen (¼ and ½ 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 with cyanobacteria plus EM under full nitrogen dose and followed by (2.97 tons fed $^{-1}$) for cyanobacteria plus EM under ½ N level treatment without significant different between each others.

Towards nitrogen application also full dose recorded the highest significant mean 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 correlated drastically with the grain yield.

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 resulted from 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. Also, in the present study, inoculation with *Nostoc* sp. Inoculation increased significantly wheat yield and its components especially when combined with EM plus $^{1}/_{2}$ N dose. In this concern, Ragab *et al.* (2010) explained the increase in faba bean yield due to inoculation with *Azotobacter* plus EM may be attributed to that the role of *Rhizobium leguminosarum* that increased the growth and N_2-fixation by faba bean plants. While the high response of bean seed yield to EM application can be explained on the basis that EM increases germination, stimulates the photosynthetic process and enhances the enzymes activities. Microorganisms of EM and N-fixers have a beneficial role in speedy emergence of seedlings, leaf photosynthesis, diseases and herbs resistance which consequently, produces healthy growth and wealthy yield. Also, they reported that the use of EM enhanced yield of bean due to greater rates of photosynthesis and dry matter accumulation. Significant increase was found in all wheat yield parameter with application of EM was applied along with ½ recommended nitrogen dose producing (2831

kg grains ha^{-1}) very clause to full-recommended nitrogen dose (3017 kg grains ha^{-1}) (Hussain *et al*., 1999). These observations are in parallel with the results in this study.

Table (2): Effect of cyanobacteria inoculation, effective micro-organisms (EM) application and N-fertilization on wheat yield components (Data are a mean of two seasons)

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria +EM	Means
	Grain yield kg fed^{-1}				
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 dose N	872.10	993.48	1014.70	1295.34	1043.91
Means	727.49	881.34	908.88	1177.45	
L.S.Dat 5%					
N:			96.77		
Treatment:			72.26		
Interaction:			144.00		
	Straw yield ton fed^{-1}				
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 dose N	2.69	2.49	2.21	3.31	2.68
Means	2.57	2.62	2.41	2.85	
L.S.Dat 5%					
N:			0.31		
Treatment:			0.27		
Interaction:			0.53		
	1000 grains weight (g)				
1/4 N	45.87	53.30	48.77	49.00	49.24
1/2 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.Dat 5%					
N:			2.26		
Treatment:			3.92		
Interaction:			4.44		

NPK uptake by wheat grains:

Results in Table (3) revealed that inoculation with cyanobacteria and EM application under full nitrogen dose gave the highest N uptake amount (22.03 kg N fed^{-1}) with no significant difference with that recorded by cyanobacteria and EM treatment under ½ 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 ½ or full N dose treatments. Their respective mean N uptake values were 15.71 and 16.87 kg N fed^{-1}.

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 differ from 2.07 kg P

fed $^{-1}$ for cyanobacteria plus EM plus ½ N dose and cyanobacteria plus EM plus full N dose, treatments, respectively. While with K uptake, the highest value of 6.63 kg K fed $^{-1}$ was not significantly differed from that of 6.35 kg K fed $^{-1}$ for cyanobacteria plus EM + ½ N dose and cyanobacteria + EM + Full dose treatments, respectively.

Table (3): Effect of cyanobacteria inoculation, effective micro-organisms (EM) application and N-fertilization on NPK uptake by wheat grains (Data are a mean of two seasons)

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria +EM	Means
	N-uptake kg fed^{-1}				
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 dose N	18.31	11.92	15.22	22.03	16.87
Means	12.95	12.28	14.76	20.02	
L.S.Dat 5%					
N:			1.45		
Treatment:			1.20		
Interaction:			2.29		
	P-uptake kg fed^{-1}				
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 dose N	1.35	1.49	1.93	2.07	1.71
Means	1.09	1.47	1.50	1.86	
L.S.Dat 5%					
N:			0.16		
Treatment:			0.13		
Interaction:			0.24		
	K-uptake kg fed^{-1}				
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 dose N	4.45	5.17	5.38	6.35	5.34
Means	3.81	4.60	4.53	5.87	
L.S.Dat 5%					
N:			0.52		
Treatment:			0.38		
Interaction:			0.76		

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

Data in Table (4) indicated that N uptake value recorded by the cyanobacteria + EM + ½ N dose (14.83 kg N fed $^{-1}$) was not significantly differed from that recorded by Cyanobacteria + EM + full N dose treatment (16.55 kg N fed $^{-1}$). In contrast, P uptake value of 4.15 kg P fed $^{-1}$ (cyanobacteria + EM + ½ N dose) was significantly higher than that recorded by cyanobacteria + EM + Full N dose treatment (2.98 kg P fed $^{-1}$).

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 $^{-1}$) was significantly higher than that recorded by cyanobacteria + EM + ½ N dose treatment (16.91 kg k fed $^{-1}$) .

Table (4): Effect of cyanobacteria inoculation, effective micro-organisms (EM) application and N-fertilization on NPK uptake by wheat straw (Data are a mean of two seasons)

N-fertilization	Treatments				
	Control	Cyanobacteria	EM	Cyanobacteria +EM	Means
	N-uptake kg fed^{-1}				
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 dose N	16.16	14.94	13.38	16.55	15.26
Means	17.80	24.38	12.76	14.44	
L.S.Dat 5%					
N:	2.65				
Treatment:	2.15				
Interaction:	4.14				
	P-uptake kg fed^{-1}				
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 dose N	2.24	1.24	4.01	2.98	2.62
Means	2.63	3.09	3.09	2.83	
L.S.Dat 5%					
N:	0.59				
Treatment:	0.43				
Interaction:	0.87				
	K-uptake kg fed^{-1}				
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 dose N	17.77	19.67	15.84	22.18	18.87
Means	19.76	17.29	14.36	18.88	
L.S.Dat 5%					
N:	3.15				
Treatment:	2.32				
Interaction:	4.66				

Increasing the nutrient uptake by wheat grain and straw in response to the use of both EM and cyanobacteria as biofertilizer separately was confirmed by those of Abd EL- Rasoul *et al*. (2004) and Mussa *et al*. (2003) who indicated that spraying both EM and nitrogen fixing biofertilizers individually had significantly increased N P K uptake by grains and straw over the control treatments. The use of cyanobacteria along with EM plus full-N dose had achieved grains and straw yields, NPK uptake by grains and straw which were very close to and not significantly different from those achieved by same treatments under the influence of ½ N dose. 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