J.Soil Sci. and Agric. Eng., Mansoura Univ., Vol. 6(2): 233 - 242 , 2015

RESPONSE OF WHEAT YIELD AND COMPONENTS TO HUMIC SUBSTANCES AND CYANOBACTERIA APPLICATION

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ABSTRACT

Two field experiments were conducted at the Experimental farm of Sakha Agric. Res. Station during 2012/2013 and 2013/2014 growing seasons to evaluate the potential effect of addition of humic substances and cyanobacteria (mixture strain of *Anabeana oryzae* and *Nostoc muscorum*), on wheat (Giza 168) yield and its components. The experiment layout was split plot design. The main plots were assigned by application methods where three application treatments (soaking, foliar and soaking with foliar) were used. The subplots were assigned by four treatments of cyanobacteria, H.S and without. Results indicated that inoculation with cyanobacteria and H.S significantly increased grain and straw yields. The yield and yield components, chlorophyll content, N uptake and protein % in grain were increased over their values with the untreated plants in both growing seasons.

The obtained results indicated also that treatment of (soaking + foliar) with cyanobacteria + H.S significantly increased the yield compared with other application method, the highest values of grain yield 23.79 and 23.43 ardab/fed., straw 4.82 and 4.84 ton/fed., protein 11.73 and 11.6%, total chlorophyll 43.65 and 43.48 mg/kg and plant height 89 and 87 cm with (S.+F.) treatment.

Keywords: (S.+F.) = wheat, humic substance, cyanobacteria

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important to the million of farmers who grow this crop, to many landless workers who derive income from working on these farms and to the billion of consumers of this crop around the world. Rapid increase in world population indicates the need of increasing production in the same time. According to Food and Agricultural Organization (FAO) World Cereal Production in 2008 was forecasted to increase 2.6% to a record 2.164 million ton. However, with increasing in cultivation of cereal, use of chemical fertilizers is also increasing simultaneously as it can rapidly give more reliable boost to crop yield (Amer, 2009).

High cost of nitrogen fertilizer, the widening gap between supply and demand, the low punching power of small marginal farmers and their adverse effect on environmental had led agricultural scientists to look to alternate strategies of crop productions. Organic substance and biofertilizer are alternate source to meet the nutrient or stimulating requirement of crop and to bridge the future gap. Further, knowing show the deleterious effect of using only the chemical fertilizer, use of soil microorganisms which can fix atmospheric nitrogen or solubilizing phosphorus or stimulate plant through synthetic plant growth promoting substances will be environmentally benign approach for nutrient management and ecosystem function (Prasanna *et al.*, 2010).

Cyanobacteria (formally called blue-green algae) are photoautotrophic prokaryotes including a large variety of species of widespread occurrence and with diverse morphological, physiological and biochemical properties.

An important of many cyanobacrteria is their ability to fix atmospheric nitrogen under free-living. Nitrogen fixation carried out by large, thick-walled called known as heterocysts. They appeared to be a rich source for many useful products known to produce number of bioactive compounds (Carmicheal, 2001).

Effect of humic substances (H.S) on plant growth has been observed and extensively documented in many research and review articles (Nardi *et al.*, 2002). The effect of H.S on plants were often conducted under conditions of deficient mineral supply. This could have resulted in a positive plant growth response directly related to the addition of macro-nutrients (N, P, K) with the (H.S). Solution, were not always purified before the plant growth trail. Because, the essential role of major nutritional elements in plant growth is widely recognized, an effort has made in this review to focus on the synergistic effects of this (H.S) and nutritional elements in the presence of complete and considered to be optimal nutrient supply. Antoun *et al.* (2010) found that addition of humic substances markedly increased wheat plant height, panicle length, 1000-grain weight, grain, straw yield/fed., protein content in grain and NPK uptake of both grain and straw.

The objective of the current work is to obtain the best foliar, soaking method individually or mixed. Also study the effect of inoculation with cyanobacteria and humic substance on wheat growth and its yield.

MATERIALS AND METHODS

Two field experiments were carried out at Sakha Agricultural Research Station at Kafr El-Sheikh Governorate during the two successive winter growing seasons of 2012/2013 and 2013/2014 to investigate the effect of humic substances (H.S) and cyanobacteria application on yield of wheat (Giza 168) and its components.

The experimental site represents circumstances and condition of north middle Nile Delta region which allocated at $31^{o}5$N latitude, $30^{o}57$E longitude with an elevation of about 6meters above mean sea level. Split plot design was used with four replicates. The main plots were assigned by three treatments for addition methods of [soaking (A_1), foliar (A_2) and soaking + foliar (A_3)]. The subplots were assigned by four treatments, without (B_1), cyanobacteria (B_2), H.S.(B_3) and cyano + H.S. (B_4). The used humic substance (H.S was extracted and prepared from mature compost by Soil Fertility and Plant Nutrition Department, Soil Water and Environment Res. Inst., Sakha Station and Cyanobacteria (mixture strains of *Anabeana oryzae* and *Nostoc muscorum*), was from Soil Microbiology Department, Sakha Agric. Res. Station. Soil characteristics for different depths at the experimental field were determined at each 15 cm up to 30 cm for some physical and chemical properties which were shown in Table (1) according to Jackson (1967) and Black *et al.* (1965).

Table 1:Some physical and chemical properties of the soils used in the two seasons of study.

Years	Depth	pH	Particle size distribution				O.M %	EC dSm^{-1}	Available nutrients mg/kg			Soluble cations meq/L				Soluble anions meq/L			
			Sand%	Silt%	Clay%	Texture			N	P	K	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
2012/2013	0-15	7.5	21.1	32.6	46.3	Clay	1.60	2.20	36.0	5.69	235	4.7	4.9	14.2	0.55	0.0	9.0	3.7	11.65
	15-30	7.5	20.7	31.5	47.8	Clay	1.30	2.25	32.0	2.42	244	4.0	5.2	15.3	0.38	0.0	9.1	8.0	7.78
2013/2014	0-15	7.6	18.42	25.53	56.05	Clay	1.01	2.27	37.0	6.43	239	4.5	5.8	16.4	0.28	0.0	10.2	8.5	8.28
	15-30	7.7	22.54	26.16	51.3	Clay	0.65	2.70	33.0	2.64	246	5.5	4.5	18.9	0.19	0.0	10.0	11.0	8.09

Statistical analysis for data was done using MSTATE computer program according to Snedecor and Cochran (1990) using LSD test to compare the means of each treatment. The soil texture in the experimental field is clayey. All cultural practices were performed as recommended for the crop and the studied area except the studied treatments. Humic substances was added at the concentration (2 L/fed.). Soaking, foliar and (soaking + foliar) at the time of tillering stage.

The dry inoculum, (cyanobacteria) was used at the rate of 500 g/fed. The dry inoculatin were produced by the biological nitrogen fixation unit at Sakha Agricultural Research Station.

Nitrogen content in grain and straw were determined using micro-kjeldahl technique according to Jackson (1967)Protein % = N % x 5.7).

Leaf total chlorophyll content (SPAD unit) values was determined by using portable Minolta chlorophyll meter (Model SPAD-5011). Leaf sample collected in mid-June and the reading was taken at the middle of leaf blade according to Murquard and Timpton, 1987).

RESULTS AND DISCUSSION

Effect of humic substances and cyanobacteria application:
Vegetative growth:

Data presented in Table (2) indicated that wheat treated with [soaking (A_1), foliar (A_2) and S+F (A_3)] significantly varied in their plant height, total chlorophyll content and number of tillers. The differences between application methods [(A_1), (A_2) and (A_3)] may be attributed to differences in leaf area similar results were found by Knany *et al.* (2009b).

It is clear from the data that significant increases were obtained in the above mentioned characters over the control due to algalization, (H.S) and (cyanobacteria + H.S) in both seasons.

The highest values were obtained by using (B_4) treatment. The corresponding the average increase values of both seasons as compared with control 17.4, 45.17% and 33.77%for plant height, total chlorophyll content and number of tillers, respectively. The beneficial effect of algalization to wheat yield component could be attributed to the ability of cyanobacteria to fix N_2 and producing phytohormons which stimulate growth. These results came in accordance with those presented by Omar (2001) and Abdo (2005), Song *et al.* (2005) and Osman *et al.* (2010).

The beneficial effects of humic substance (H.S) on plant growth are usually exhibited by easily measurable parameters, such as leaf chlorophyll concentration, shoot and root fresh and dry weight, the number of root initial and the number of flower buds (Chen, 1996; Knany *et al.*, 2009b and Nardi *et al.*, 2002).

Table (2): Effect of fertilizer application method on total chlorophyll, plant height and number of tillers/plant.

Treatments	Total chlorophyll SPAD unit		Plant height, cm		No. of tillers/plant	
	1st	2nd	1st	2nd	1st	2nd
Soaking (A_1)	30.45	30.80	82.0	80.0	19.60	19.59
Foliar (A_2)	34.89	35.69	84.0	83.0	21.27	21.53
S+F (A_3)	43.65	43.48	89.0	87.0	22.75	22.96
LSD 0.05	0.156	0.332	0.260	0.280	0.215	0.175
Without (B_1)	29.34	30.16	80.0	81.0	18.27	18.26
Cyano (B_2)	34.56	34.76	83.0	84.0	20.53	20.40
(B) H.A (B_3)	38.50	38.23	90.0	89.0	21.89	22.05
Cyano + H (B_4)	42.94	43.47	95.0	94.0	24.14	24.74
LSD 0.05	0.338	0.384	0.029	0.031	0.174	0.179
Interaction						
A_1B_1	24.16	25.55	72.0	71.0	16.32	16.40
A_1B_2	29.25	30.40	75.0	73.0	19.43	19.23
A_1B_3	32.64	30.95	79.0	76.0	20.14	20.22
A_1B_4	35.77	36.31	82.0	78.0	22.51	22.55
A_2B_1	28.36	29.25	84.0	83.0	18.93	18.95
A_2B_2	33.58	33.94	87.0	86.0	20.70	20.72
A_2B_3	37.44	38.08	90.0	89.0	22.18	22.72
A_2B_4	40.19	41.49	93.0	92.0	23.25	23.76
A_3B_1	35.49	35.68	96.0	94.0	19.56	19.43
A_3B_2	40.86	39.96	98.0	97.0	21.44	21.27
A_3B_3	45.42	45.67	97.0	99.0	23.36	23.21
A_3B_4	52.84	52.61	99.0	100.0	26.65	27.93
LSD 0.05	0.585	0.664	0.045	0.043	0.302	0.312

Straw yield (ton/fed.)

Data presented in Table (3) clearly showed that the values of wheat straw yield were affected by fertilizer application methods. The highest values during the two seasons were recorded under application methods A_3 (soaking seeds + foliar application on plants) in comparison with other application methods A_2 and A_1. The mean values for wheat straw yield can be descended in order A_3 (S+F)>A_2 (foliar) >A_1 soaking and the mean values are 4.82, 4.74 and4.63 ton/fed.. in 1st season and 4.84, 4.76 and 4.61 ton/fed. in the 2nd season, respectively. This may be due to soaking + foliar treatment had the highest chance for absorption from the root (soaking) and from the leave (foliar application). On the other hand, soaking had the lowest values because the soil in the rhizosphere made retention some soaked materials which decreased the absorption.

Table 3: Effect of fertilizer application method on grain yield, and straw yield.

Treatments	Grain yield (ardab/fed.)		Straw yield (ton/fed.)	
	1st	2nd	1st	2nd
Soaking (A_1)	19.88	21.18	4.63	4.61
Foliar (A_2)	21.65	22.39	4.74	4.76
S+F (A_3)	23.79	23.43	4.82	4.84
LSD 0.05	0.1037	0.0585	0.087	0.072
Without (B_1)	19.08	19.75	4.24	4.30
Cyano (B_2)	20.94	21.27	4.79	4.73
(B) H.A (B_3)	22.55	23.27	4.85	4.80
Cyano + H (B_4)	24.50	25.03	5.04	5.00
LSD 0.05	0.088	0.085	0.054	0.031
Interaction				
A_1B_1	17.35	18.84	4.01	4.13
A_1B_2	19.43	20.46	4.74	4.62
A_1B_3	20.17	22.27	4.84	4.83
A_1B_4	22.57	23.14	4.91	4.94
A_2B_1	18.94	19.93	4.29	4.28
A_2B_2	20.75	21.25	4.81	4.74
A_2B_3	22.79	23.28	4.80	4.93
A_2B_4	24.09	25.00	5.03	5.01
A_3B_1	20.95	20.49	4.41	4.49
A_3B_2	22.66	22.09	4.80	4.82
A_3B_3	24.69	24.15	4.89	4.95
A_3B_4	26.83	26.96	5.17	5.07
LSD 0.05	0.1525	0.1475	0.093	0.054

These results are in a great harmony with those obtained by Hussain *et al.* (2009) and Knany *et al.* (2009b) who found that spraying with humic substances (H.S) extracted and separated by Soil Fertility and Plant Nutrition Department, Soils, Water and Environment Institute, Agric. Res. Station, increased faba bean yield, biological yield, 100 seeds weight, N% and P%.

Concerning with the effect of biofertilizers application in the two growing seasons, the highest mean values were recorded under biofertilizer application treatment B_4 (application of cyano + H.S) under all methods of application and the mean values are 5.04 and 5.00 ton/fed. in the 1st and 2nd seasons, respectively comparing with other biofertilizer types. The highest mean values can be descended order $B_3>B_2>B_1$ and the mean values through season, this,may be due to that microorganisms produced materials (enzymes, acids, gas) that enhanced the root and shoot growth, increased the availability of some nutrients which reflected on the crop growth and yield. Abd-Alla (1994) stated that the increase in growth parameters could be attributed to the substantial increase of N_2-fixation due to nitrogen activity of the cyanobacteria (Osman *et al.*, 2010). The promotive effect of cyanobacteria inoculant, especially on growth, hold promise for use of such inoculants tolerance nitrogen status of irrigate plantation crops.

Grain yield (ardab/fed.):

Data presented in Table (3) clearly illustrated that the mean values of wheat grain yield were affected by application methods. The highest mean values during the two seasons were recorded under application method (soaking seeds + foliar application on plants) in comparison with other application methods A_2 and A_1. The mean values for wheat grain yield can be descended in order (soaking + foliar) (A_3) > foliar (A_2) > soaking (A_1) and the mean values are 23.79, 21.65and 19.88 ardab/fed. in the 1st season and23.43, 22.39 and 21.18 ardab/fed. in the 2nd season. These results are in great harmony with those obtained by Rai *et al.* (2000) and Aziz, Manal *et al.* (2008) and Seod *et al.* (2008).

Scientists are now interested in creating artificial symbiosis between higher plants and N_2-fixing microorganisms with a view to introducing nitrogen fixing ability into the plants. Novel association including those between Rhizobium and Rice (All-Mallah *et al.*, 2002) and anabaena and tobacco (Gusev *et al.*, 2002).

Grain and straw nitrogen (%), protein percent (%) and N uptake in wheat grains and straw in the two seasons:

Tabulated data in Table (4) clearly indicated that the mean values of the abovementioned studied parameters were affected by application method and cyanobaceria +humic substances application.

Table 4 :Effect of fertilizer application method on N% in grain and straw and N content in grain and straw at wheat yield and grain protein percent.

Treatments	Seed N%		Straw N%		Protein %		grain N uptake		Straw N uptake	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Soaking (A_1)	1.73	1.72	0.38	0.40	9.86	9.80	51.58	54.64	17.5	18.4
Foliar (A_2)	1.82	1.79	0.49	0.45	10.37	10.20	59.1	59.44	23.2	21.4
S+F (A_3)	1.87	1.85	0.52	0.52	10.66	10.55	66.73	65.01	25.2	25.3
LSD 0.05	0.0055	0.0238	0.004	0.0113	0.0376	0.1479	0.473	0.58	0.62	1.68
Without (B_1)	1.69	1.71	0.33	0.34	9.6	9.75	48.36	50.65	13.9	14.6
Cyano (B_2)	1.78	1.77	0.42	0.42	10.14	10.09	55.9	56.47	20.1	19.87
(B) H.A (B_3)	1.85	1.81	0.49	0.49	10.55	10.32	62.57	63.17	23.76	23.5
Cyano + H (B_4)	1.90	1.86	0.55	0.55	10.83	10.60	59.82	69.83	27.66.7	27.5
LSD 0.05	0.01088	0.0166	0.006	0.0099	0.0716	0.1041	0.651	0.67	0.64	0.70
Interaction										
A_1B_1	1.64	1.66	0.22	0.24	19.3	9.46	42.68	46.91	8.70	10.0
A_1B_2	1.67	1.69	0.35	0.36	9.5	9.6	48.67	51.86	16.70	16.6
A_1B_3	1.77	1.71	0.42	0.43	10.09	9.75	53.53	57.12	20.5	20.3
A_1B_4	1.82	1.81	0.52	0.51	10.37	10.32	61.6	62.82	25.40	25.3
A_2B_1	1.69	1.71	0.32	0.35	9.6	9.75	48.61	51.12	13.90	15.0
A_2B_2	1.81	1.77	0.41	0.42	10.32	90.09	56.33	56.41	19.50	19.7
A_2B_3	1.87	1.81	0.51	0.48	10.66	10.32	62.92	63.2	25.00	23.7
A_2B_4	1.91	1.86	.55	0.54	10.89	10.60	69.01	70.13	27.50	26.1
A_3B_1	1.74	1.76	0.44	0.43	9.92	10.03	54.69	54.09	19.60	19.5
A_3B_2	1.86	1.85	0.49	0.47	10.60	10.55	69.22	61.29	23.70	22.8
A_3B_3	1.92	1.89	0.558	0.56	10.94	10.77	71.1	68.46	26.80	27.9
A_3B_4	1.98	1.93	0.59	0.61	11.29	11.00	79.68	70.13	30.50	30.9
LSD 0.05	0.0188	0.0289	0.00159	0.0171	0.1240	0.1803	1.26	1.24	1.08	1.21

Regarding the effect of application method, A_3 (soaking + foliar) application had the highest values. On the contrary, the lowest mean values for abovementioned studied parameters were recorded with soaking. Generally, the mean values for the studied parameters can be descended in order A_3>A_3>A_1. For nitrogen percent in grain values are 1.87, 1.82 and 1.73% in the 1st season and 1.86, 1.79 and 1.72%in the 2nd season. While for protein percent the mean values are 11.73, 11.36, and 10.77% in the 1st season and 11.6, 11.18 and 10.74 in the 2nd season. The value for nitrogen uptake in grain are 51.58, 59.1 and 66.73 in the 1st season and 54.64, 59.44 and 65.01 in 2nd season under A_3, A_2 and A_1, respectively. The promotive effect of cyanobacteria inoculant, especially on growth, hold promise, for use such inoculants to enhance the nitrogen status of irrigated plantation crops concerning with effect of biofertilizer and humic substances) application highest mean values were recorded under application of (cyano.+ humic substances) under all studied parameters and application method in the two seasons. These results are in a great harmony with those obtained by El-Sheref *et al.* (2004), Prasanna *et al.* (2009) and Chen *et al.* (2010).

Conclusion and recommendation:

This study recommended that application of (soaking + foliar) application of (cyano + H.S) for wheat crop in the North Middle Nile Delta region to give the best yield and yield components

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إستجابة محصول القمح ومكوناته لإضافة المواد الهيومية (الدبالية)والسيانوبكتيريا

منال عادل عزيز و سحر حسن راشد
معهد بحوث الأراضى والمياه والبيئة ، مركز البحوث الزراعية بالجيزة- مصر.

أقيمت تجربتان حقليتان على محصول القمح بمحطة البحوث الزراعية بسخا فى موسمى ٢٠١٢/٢٠١٣ ، ٢٠١٣/٢٠١٤ وذلك بهدف دراسة أثر إضافة السيانوباكتيريا والمواد الهيومية على محصول القمح ومكوناته وذلك باستخدام مستخلص الهيومات والسيانوبكتيريا وأثرها على زيادة المحصول. وكانت المعاملات الرئيسية هى (طرق الاضافة) النقع والرش و(النقع + الرش)

أما المعاملات التحت رئيسية هى بدون وإضافة السيانوبكتيريا وإضافة المواد الدبالية بالاضافة للمعامة المزدوجة الواد الهيومية + السيانوبكتيريا.

أما النتائج المتحصل عليها فكانت كالآتى:

١) أوضحت النتائج المتحصل عليها أن المعاملة المزدوجة من كل من السيانوبكتيريا + المواد الهيومية أعطت أفضل النتائج فى المحصول ومكوناته وكذلك الكلوروفيل والبروتين % بالمقارنة بالكنترول.
٢) كما أشارت النتائج أن أفضل طريقة من طرق الأضافة هى كانت المعاملة المزدوجة (النقع + الرش) كانت عندها أيضا أفضل النتائج بالنسبة للمحصول ومكوناته.
٣) بالنسبة للمعاملة المزدوجة السيانوبكتيريا +المواد الهيومية(المواد العضوية) كانت أعلى القيم فى الحبوب ٢٤.٥ ، ٢٥.٠٣ أردب /فدان وفى القش ٥.٠٤ ، ٥.٠٠ طن /فدان فى كلا الموسمين على التوالى.