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EFFECT OF CYANOBACTERIA, HUMIC SUBSTANCES AND MINERAL NITROGEN FERTILIZER ON RICE YIELD AND ITS COMPONENTS

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ABSTRACT

Two field trails were conducted at Sakha Agric. Res.. Station in the two seasons of 2012 and 2013 to study the effect of cyanobacteria (mixture strains of *Anabaena oryzae* and *nostoc muscorum*), humic substance (HS) and mineral nitrogen (urea 46.5%N) on yield of some rice varieties (Sakha 101 and Sakha 105).

It could be observed from the results that the variety Sakha 101 was superior to Sakha 105 in both growing seasons in regard to grain yield and harvest index.

Results indicated that inoculation with cyanobacteria, application of humic substances and nitrogen fertilization individually significantly increased grain and straw yields, the yield components as well as chlorophyll content and N uptake in plant over their values with the untreated plants in both growing seasons.

Also, the obtained results indicated that the application of 50% cyanobacteria + 50% HS + 50% N fertilizer of the recommended dose in the 1^{st} and 2^{nd} seasons achieved the highest values of grain yield (3.89 and 3.68 ton/fed, respectively) ,straw yield (6.57 and 5.66 ton/fed, respectively) ,total chlorophyll (40.14 and 41.6 mg/kg, respectively) ,number of tillers (30.0 and 30.88/plant, respectively),plant height (103 and 101 cm, respectively) and panicle length (26.1 and 24.8 cm ,respectively). While the lowest values of these parameters were obtained with the untreated plants in both growing seasons.

The interaction between rice cultivars and biofertilizer and nitrogen application was found to be not significant.

INTRODUCTION

Rice (*Oryza sativa*, L.) is considered as one of the most important cereal crops and it is a source of nutrition for half of the world's population. In Egypt, rice area ranged annually between 1.0 to 1.5 million feddan. Average rice productivity is 4.2 ton/fed. and the annual production is 6.74 million tons, which is sufficient for the local consumption and export. Thus, rice plays a main role for the Egyptian economic (Economical Sector, Ministry of agriculture and Soil Reclamation, 2006).

Yield of rice depends on its genetic potential, agroclimatic condition and various management practices (Singh and Singh, 1998). Beside, nitrogen is the key element in rice plant nutrition to promoting growth and yield. However, under direct-seeding, in the early establishment stage alternate welting and drying of soil causing losses of basally nitrogen applied , so nitrogen supply especially at late season affects the growth, yield and quality of drill-rice (Sahoo *et al.*, 1990).

In addition, split application of nitrogen fertilizer commensurable with crop growth stages is the most common approach for increasing nitrogen use efficiency and produced maximum yield (Nageswari and Balasubramanian, 2004).

Cyanobacteria (blue green algae) are photoautotrophic prokaryotes including a large variety of species of widespread occurrence and with diverse morphological, physiological and biochemical properties. An importance of many cyanobacteria strains is their ability to fix atmospheric nitrogen both under free living and symbiotic conditions. They appeared to be a rich source for many useful products known to produce number of bioactive compounds (Osman,*et al*.2010).

Effect of humic acid on plant growth are usually exhibited by easily measurable parameters, such as leaf chlorophyll content, shoot and root fresh and dry weights, the number of initial roots and the number of flower buds (Chen, 1996). Humic substances are organic compounds that resulted from the decomposition of plant and animal materials. Humic fraction of the soil organic matter that are responsible for the generic improvement of soil fertility, improved productivity and change physical properties of soil, promote the chelation of many elements to be available to plants. Antoun *et al.* (2010) found that addition of humic acid markedly increased wheat plant height, panicle length, 1000-grain weight, grain and straw yields/fed., protein content in grain, and NPK uptake of both grain and straw.

MATERIALS AND METHODS

To achieve the above mentioned objectives two field trials were conducted at Sakha Agricultural Research Station Farm during the two successive summer seasons of 2012 and 2013 using rice varieties Sakha 101 and Sakha 105.

The soil of the experimental sites (as an average of two seasons) is clayey and non-saline as shown in Table (1).

Table (1): Some physical and chemical properties of soil of the experimental field in seasons 2012 and 2013.

Season	Depth (cm)	pH 1:2.5	EC dSm^{-1}	Cation (meq/L)				Anion (meq/L)			Particle size distribution (%)			Available nutrient			OM	SAR
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	$SO_4^{=}$	Clay	Silt	Sand	N	P	K		
2012	0-20	8.0	2.20	4.7	4.9	14.2	0.6	9.0	3.7	11.6	56.1	25.4	18.5	28.5	9.4	316	1.2	6.48
	20-40	8.01	2.25	4.0	5.2	15.3	0.4	9.1	8.0	7.78	51.2	26.4	22.4	30.58	9.3	310	0.94	7.13
2013	0-20	7.9	2.58	5.5	2.2	17.9	1.1	4.5	9.5	12.7	57.4	24.2	18.4	31.44	9.6	320	1.21	9.12
	20-40	8.01	2.75	8.6	2.5	17.2	1.0	6.1	11.4	11.8	59.4	26.0	14.6	31.74	9.5	317	0.89	7.30

SAR = Sodium adsorption ratio = $\dfrac{Na}{\sqrt{\dfrac{Ca+mg}{2}}}$

OM% = Organic matter%

The soil analysis was done according to Black (1965) and Page *et al.* (1982)

A split plot design with four replicates was used. The main plots were devoted for rice varieties, while the sub plots were designed for algalization with cyanobacteria, application of HS and nitrogen fertilizer separately or together.

The algalization treatments were inoculated 5 days after transplanting using a mixture of cyanobacteria strains. The inoculum was used at the rate of 500 gm/fed. The dry inoculum, were produced by the Biological Nitrogen Fixation Unit at Sakha Agricultural Research Station.

The experimental treatments were applied as follow:

F-0: Control (without treatments).
F-1: Algalization with 500 g cyanobacteria/fed (recommended dose)
F-2: The recommended dose of humic substances, HS (2 L/fed.).
F-3: The recommended dose of N (150 kg N /fed).
F-4: 50 % recommended dose of cyanobacteria and 50% HS.
F-5: 50 % recommended dose of cyanobacteria and 50% N.
F-6: 50 % recommended dose of HS and 50% N.
F-7: 50 % recommended dose of Cyanobacteria, 50% HS and 50% N.

Nurseries were established after wheat in May while, transplanting was in June. The plot area was 12 m^2 and all plots received the same amount of P_2O_5 as superphosphate (15 % P_2O_5) at the rate of 100 kg/fed before transplanting. Nitrogen fertilizer (Urea, 46.5 % N) was splitted into two half doses .The first half dose was added on dry soil before transplanting and incorporated into the soil by plowing, and the other half dose was added one month later. Humic acids was added by foliar application at rate of 2 L/fed. at the time of tillering stage.

At maturity the central area of each plot were harvested, dried and threshed to estimate the grain and straw yields and the biological yields (grain and straw) and the harvest index were calculated.

After 120 days from transplanting, the plant height, panicle length, number of productive tiller/hill, total chlorophyll, harvest index, were recorded. Nitrogen content in grain and straw were determined using micro-kjeldahl technique. All the collected data were statistically analyzed according to Snedecor and Cochran (1990) using LSD test to compare the means of each treatment.

RESULTS AND DISCUSSION

Grain, straw yield and harvest index:

Data presented in Table (2) showed significant differences between rice cultivars regarding the grain yield , straw yield and harvest index in both seasons. The superiority was to Sakha 101 for the grain yield and harvest index in both seasons, while Sakha 105 is superior in regard to straw yield in both seasons. The obtained results are in harmony with those recorded by El-Refaee (2002), El-Refaee *et al.* (2005) and Saito *et al.* (2006).

In regard to the effect of algalization , humic substance and nitrogen application on rice yield, the data in Table (2) indicated that the grain and straw yields as well as harvest index in both growing seasons were affected significantly by different treatments. The control treatment recorded significantly the lowest yield value. Irrespective of rice variety , results showed significant increases of rice yield due to algalization, humic substance and nitrogen application individually or in combination. The highest yield values were obtained by using the 50% recommended dose of cyanobacteria + 50% H.S + 50% N fertilizer (F-7) followed by 50 % recommended dose of both HS and N (F-6), while the lowest values were obtained with the control (F-0) in both growing seasons. Therefore, the grain yield was increased over the control in the 1st and 2nd seasons by 31.0 and 19.5 %, respectively with F-7 treatment and by 29.3 and 17.5 %, respectively, with F-6 treatment. Also, the straw yield was increased over the control in the 1st and 2nd seasons by 20.1 and 13.4 %, respectively with F-7 treatment and by 19.4 and 12.0 %, respectively, with F-6 treatment.

With respect to the harvest index, data in Table (2) show that algalization, humic substance and N fertilizer individually or with their combination caused significant effects on harvest index. The highest value of harvest index was achieved with 50 % recommended dose of both 50% cyanobacteria + 50% H.S + 50% N fertilizer in both seasons (39.88 and 37.33 %, respectively).

Table(2): Rice grain and straw yield and harvest index as affected by different treatments and their interaction

Varieties	Grain yield (ton/fed.)		Straw yield (ton/fed.)		Harvest index %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Sakha 101	3.84	3.77	5.16	5.89	42.66	39.02
Sakha 105	3.38	3.13	5.64	6.42	37.47	32.74
LSD 0.05	0.072	0.055	0.126	0.072	0.655	0.786
Fertilizers (F)						
F-0 (control)	2.97	3.08	5.47	4.99	35.21	38.13
F-1	3.29	3.18	5.54	5.11	37.26	38.33
F-2	3.49	3.31	5.88	5.26	37.33	38.55
F-3	3.80	3.63	6.44	5.57	37.14	38.44
F-4	3.77	3.51	6.33	5.51	37.33	39.88
F-5	3.81	3.60	6.49	5.55	36.97	39.34
F-6	3.84	3.62	6.53	5.59	37.01	39.30
F-7	3.89	3.68	6.57	5.66	37.30	39.31
LSD 0.05	0.096	0.099	0.184	0.108	1.116	0.85
Interactions VXF	NS	NS	NS	NS	NS	NS

F-0: Control (without treatments).
F-1: Algalization with 500 g cyanobacteria/fed (recommended dose)
F-2: The recommended dose of humic substances, HS (2 L/fed.).
F-3: The recommended dose of N (150 kg N /fed).
F-4: 50 % recommended dose of cyanobacteria and 50% HS.
F-5: 50 % recommended dose of cyanobacteria and 50% N.
F-6: 50 % recommended dose of HS and 50% N.
F-7: 50 % recommended dose of Cyanobacteria, 50% HS and 50% N.

An important feature of cyanobcteria is their ability to fix atmospheric nitrogen under both free living and symbiotic conditions (Rajaniemi *et al.*, 2005). They can enhance the plant growth directly and/or indirectly. The direct ways include the biological production of various growth promoting active substances including phytohormones, such as auxin (Prasanna *et al.*, 2010), gibberellins (Rodriguez *et al.*, 2006) and cytokinin (Hussain *et al.*, 2009). The indirect promotion of plant growth occurs with fixing atmospheric nitrogen by cyanobacteria (Osman *et al.*, 2010). Also, they can improve the plant growth by improving the soil structure as they have potential to secret extracellular polysaccharides that help in soil aggregation and water retention (Maqubela *et al.*, 2009). The growth enhancement in the presence of HS may be attributed to the increase of the micronutrient availability, Fe and Zn in particular, either via complexing or through binding of colloidal conformations of the metal hydroxide of the HS. Also, the beneficial effect of HS on plant growth are usually exhibited by easily measurable parameters, such as chlorophyll content in leaves, shoots and roots fresh and dry weights, the number of root initials, and the number of flower buds (Chen, 1996).

Respecting nitrogen application, data revealed that, grain and straw yield increased with N application individually or combined with other materials .The positive effect of N might be due to increasing total chlorophyll content and flag leaf area which improve the net photosynthesis and accumulate more photosynthetic production. Such results are in line with those obtained by Clark *et al.* (2001), Hassain *et al.* (2002) and Abo Khalifa and El-Rewiny. (2005).

The data presented in Table (2) indicate that there is no significant interaction effect of the treatment on grain and straw yield as well as harvest index during both seasons. Maximum yield of grain and straw and harvest index was produced when rice cultivars were treated with adding 50% of cyanobacteria + 50% H.S + 50% N fertilizer, while the minimum was produced by the control in both seasons.

Vegetative growth :

Data presented in Table (3) indicated that rice cultivars significantly varied in their plant height, number of productive tiller, panicle length and total chlorophyll content in both growing except panicle length in 2^{nd} season. The differences between the two cultivars may be attributed to differences in leaf area and genetic background between them. Such results were found by Badawy, Shimaa (2002) and Chopra and Nisha-Chopra (2004).

It is clear from the data that significant increase were obtained in the above mentioned characters over the control due to algalization, humic substance (HS) and N fertilizer in both seasons. The highest values were obtained by using F-7 treatment. The corresponding the average increase values of both seasons as compared with control were 39.68, 28.49. 22.00 and 39.61% for total chlorophyll content, number of tiller, plant height and panicle length, respectively. The beneficial effect of algalization to rice yield components could be attributed to the ability of cyanobacteria to in N_2-fixation as well as producing the growth promoting substances such as ascorbic acid, auxins and vitamin B_{12}. Such results came in accordance with those presented by Yani *et al.* (1999), Omar (2001) and Song *et al.* (2005).

Table (3):Total chlorophyll, No. of tillers, plant height and panicle length as affected by different treatments

Varieties (V)	Plant height (cm)		No. of tillers/hill		Panicle length (cm)		Total chl. (mg/kg)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Sakha 101	94.56	94.75	23.56	24.39	22.78	22.47	34.81	35.39
Sakha 105	96.96	96.81	53.44	24.67	22.44	22.45	34.74	35.43
LSD 0.05	0.15	0.13	1.31	1.34	0.106	NS	1.86	1.89
Fertilizers (F)								
F-0	83.38	83.63	21.13	22.13	17.75	18.75	28.96	29.55
F-1	95.00	96.25	25.13	26.00	21.38	22.00	33.10	33.74
F-2	91.75	94.13	25.16	25.13	20.75	21.00	34.10	33.19
F-3	98.75	98.00	28.75	28.00	23.50	24.13	38.30	36.30
F-4	95.88	96.13	27.50	27.00	21.88	21.75	32.64	35.46
F-5	98.88	97.75	28.88	28.25	24.25	24.13	34.30	34.92
F-6	99.38	99.38	28.50	29.00	24.13	24.38	36.69	37.52
F-7	103.00	101.00	30.00	30.88	26.13	24.75	40.14	41.59
LSD 0.05	0.028	0.026	0.048	0.046	0.005	0.045	0.066	0.064
Interaction(VXF)	NS	NS	NS	NS	NS	NS		

F-0: Control (without treatments).
F-1: Algalization with 500 g cyanobacteria/fed (recommended dose)
F-2: The recommended dose of humic substances, HS (2 L/fed.).
F-3: The recommended dose of N (150 kg N /fed).
F-4: 50 % recommended dose of cyanobacteria and 50% HS.
F-5: 50 % recommended dose of cyanobacteria and 50% N.
F-6: 50 % recommended dose of HS and 50% N.
F-7: 50 % recommended dose of Cyanobacteria, 50% HS and 50% N.

In this respect, nitrogen fertilizer had an early effect on plant nutrition at the time of tiller initiation and vegetative growth. The beneficial effect of N application on panicle length could be attributed mainly to the effect of N in encouraged rice growth and subsequently excretion of its panicle (El-Sheref *et al.*, 2004 and Abdo, 2005).

The beneficial effects of HS 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).

Also, data clearly indicated that all above mentioned parameters are not affected significantly by the interaction between different treatments in both growing seasons.

Nitrogen percentage and uptake:

Data in Table (4) show that rice cultivars significantly varied in their grain and straw N% and N-uptake during the two seasons. N% and N-uptake values in grain for Sakha 105 cultivar were higher than that for Sakha 101 rice cultivar in both growing seasons. While the superiority was to Sakha 101 in respect to N% and N-uptake values in straw for both seasons. These varietal variation might be due to its genetic makeup. Such results are in harmony with those of Singh and Singh (1998) and Ntanos and Koutroubas (2002).

Respective the algalization, humic substances and N-fertilizers, data clearly revealed that grain N% and N uptake values were significantly increased by using all types of fertilizer, in both seasons. These findings mainly due to N-fertilizer which in turn increased nitrogen absorption and translocation, consequently increased grain N % and N uptake. These findings are in harmony with those recorded by Koutroubas and Ntanos (2003) and Ebaid (2005a).

Results indicated also that the nitrogen uptake in grains obtained from both mineral nitrogen fertilized or biofertilized plants were significantly higher than those obtained from control. Among the different types of biofertilizers , the treatment F-7 was superior to all other treatments in increasing N % and N uptake in 1st and 2nd seasons.

The highest values of grain N % (1.29 and 1.23 % in 1st and 2nd seasons, respectively), grain N uptake (49.9 and 44.8 kg/fed in 1st and 2nd seasons, respectively) , straw N % (0.70 and 0.69 % in 1st and 2nd seasons, respectively) and straw N uptake (45.5 and 38.3 in 1st and 2nd seasons, respectively). While the lowest amount was recorded by the untreated control for the abovementioned characters in the two seasons.

Many reports have been published on the beneficial effect of cyanobacteria on rice plants, Begum *et al*., (2011) proposed that inoculation with cyanobacteria enhanced microbial proliferation and increased organic matter content and available N nutrient in soil surface. They appeared to be a rich source for many useful products and are known to produce a number of bioactive compounds (Carmicheal, 2001).

Also, inoculation with cyanobacteria produced some growth promoting such as phosphate solubilization, nitrogen fixation, hydrogen cyanid and IAA production (Prasanna *et al.*, 2009). In these respect , nitrogen had an early effect on plant nutrition at the time of tiller initiation and vegetative growth ,this in turn led to increases in straw yield as well as their total nitrogen content. Abd El-Fattah, Faiza *et al.* (1994) and El-Kholy *et al.* (1999-b) reported high nitrogenase activity associated with exhaustion of nitrogen fertilizer at the end of vegetative growth and this in turn could supply the inoculated plants with extra nitrogen enabling photosynthesis to extended longer time into the grain filling period. Also, an additive criteria of the applied biofertilizer in secretion of phytophormones. Similar results were reported by Ebaid (2005b), Begum *et al.* (2008), and Prasanna *et al.* (2009).

Table (4): N% in rice grain, straw yield and N uptake in grain and straw as affected by different treatments

Varieties (V)	N %				N uptake (kg/fed)			
	Grain		Straw		Grain		Straw	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Sakha 101	0.97	0.89	0.56	0.62	37.40	33.90	33.50	32.60
Sakha 105	1.12	1.09	0.44	0.37	38.20	34.50	28.50	21.20
LSD 0.05	0.01	0.02	0.01	0.01	0.53	0.94	0.52	0.61
Fertilizers (F)								
F-0	0.84	0.79	0.36	0.35	24.70	24.20	19.20	16.90
F-1	0.94	0.88	0.39	0.38	30.90	27.80	21.20	19.10
F-2	0.96	0.91	0.41	0.39	32.30	29.80	33.60	20.50
F-3	1.20	1.13	0.56	0.57	45.40	40.80	36.50	31.40
F-4	1.05	1.00	0.51	0.52	39.20	35.00	31.60	28.60
F-5	1.03	0.99	0.52	0.54	39.10	35.30	35.60	29.50
F-6	1.05	1.00	0.57	0.55	40.00	36.10	37.20	30.50
F-7	1.29	1.23	0.70	0.69	49.90	44.80	45.20	38.30
LSD 0.05	0.0192	0.0215	0.022	0.020	0.94	1.14	1.64	1.13
Interaction	*	*	NS	NS	NS	NS	NS	NS

F-0: Control (without treatments).
F-1: Algalization with 500 g cyanobacteria/fed (recommended dose)
F-2: The recommended dose of humic substances, HS (2 L/fed.).
F-3: The recommended dose of N (150 kg N /fed).
F-4: 50 % recommended dose of cyanobacteria and 50% HS.
F-5: 50 % recommended dose of cyanobacteria and 50% N.
F-6: 50 % recommended dose of HS and 50% N.
F-7: 50 % recommended dose of Cyanobacteria, 50% HS and 50% N.

In these respect, the beneficial effect of humic substances on N % and N uptake due to supply of minerals, mostly N, P, K and micronutrients to the roots and increase the soil microbial population and increase soil cation exchange capacity. Similar results were reported by Chen *and Aviad* (1990) . In addition, HS activity of various enzymes and on membrane permeability have also been suggested (Pinton *et al.*, 1992). These results are in harmony with those obtained by Nardi *et al.* (2002).

Regarding the interaction between rice cultivar and nitrogen fertilizers (mineral or biofertilizer) have significant effect only on grain N % in 1st and 2nd seasons.

CONCLUSION

It could be concluded that application of N-biofertilizers contained the major N_2-fixer group (cyanobacteria) and humic substance beside chemical nitrogen fertilizer to rice, can save about 30 % of its total nitrogen requirements. Also, they left in soil sufficient amount of nitrogen, enough to increase grain yield of the following crop. Such result is very important from the economical point of view beside their role in conservation the environment by reducing pollution hazards due to application of the mineral N.

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تأثير التسميد بالسيانوبكتريا والمواد الهيومية والتسميد النتروجيني المعدنى على محصول الارز ومكوناته

منال عادل عزيز ، فايزة كمال عبد الفتاح و فاطمة الشريف
معهد بحوث الاراضى والمياة والبيئة – مركز البحوث الزراعية

أقيمت تجربتان حقليتان بمحطة البحوث الزراعية بسخا فى الموسمين الزراعيين 2012 ، 2013 على محصول الارز (صنف سخا 101 وسخا 105) وتم تلقيح الارز بالسيانوبكتريا (خليط من أنابينا أوريزا ونوستوك ماسكورم) والتسميد بالمواد الهيومية والنتروجين المعدنى (اليوريا) سواء بمفردها أو كمعاملات مزدوجة لدراسة أثرها على المحصول ومكوناته.

أوضحت النتائج الأتى:

1. أوضحت النتائج أن صنف سخا 101 كان متفوق على الصنف سخا 105 في كل من الموسمين فيما يتعلق محصول الحبوب ودليل الحصاد. كما أشارت النتائج إلى أن التلقيح بالسيانو بكتيريا ، وإضافة المواد الهيومية والتسميد النيتروجيني كل على حدة أدى إلى زيادة كبيرة للمحصول ومكوناته وكذلك الكلوروفيل ومعدل إمتصاص النتروجين فى النبات بالمقارنة بالغير معاملة (الكنترول) في كلا الموسمين .كما أشارت النتائج المتحصل عليها أن المعاملة 50% من السيانوبكتيريا +50% المواد الهيومية + 50% من السماد المعدنى النتروجينى من الجرعة الموصى بها في الموسم الأول والثانى حققت أعلى قيم محصول حبوب فى الموسمين 3.89 ، 3.68 طن للفدان على التوالى أما محصول القش فكان 6.57 ، 5.66 طن /فدان على التوالى لكلا الموسمين أما الكلوروفيل الكلى فكان 40.14 ، 41.6 ملجم/كجم لكلا الموسمين على التوالى أما عدد الأفرع فكان 41.00 ، 41.9 على التوالى لكل نبات لكلا الموسمين على التوالى أما طول النبات فكان 103 ، 101 سم لكلا الموسمين على التوالى وطول السنبلة كان 26.1 ، 24.8 على التوالى لكلا الموسمين بينما كانت أقل القيم مع معاملة المقارنة (الكنترول) فى كلا الموسمين
2. التفاعل بين أصناف الأرز والتسميد الحيوي، والتسميد النتروجينى المعدنى كانت غير معنوية.