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Effect of Inoculation with Cyanobacterial Strains and Nitrogen Fertilization on Yield and Component of Rice Plant

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ABSTRACT

The nitrogen fixed by blue-green algae could supply rice plants with their needs of nitrogen. Cyanobacteria can fix atmospheric nitrogen in submerged rice fields. A field experiment was carried out at Rice Research and Training Center, Sakha Research Station, Agricultural Research Center (ARC) Kafer EL-Sheikh, Egypt during the summer season 2016 to investigate the effect of biofertilizers as inoculants *(Anabaena oryzae* D and *Nostoc muscorum* K) . The both cyanobacterial strains were selected according to the amount of indole acetic acid and nitrogenase activities. The results indicated that highly significant increase in plant height, panicle length, number of tillers/plant, number of panicles/plant, biomass and grain yield t/ha. On the other hand , there were no significant for 1000 grain weight and harvest index. The cyanobacteria have been saved the inorganic nitrogen application by 20-25% compare with recommended nitrogen application.

Keywords : Cyanobacteria ; Rice plant ; Inorganic nitrogen fertilizer; IAA ; Nitrogenase.

INTRODUCTION

The amount of fixed nitrogen by blue- green algae could supply rice plants with their needs of nitrogen. In this respect, the release of growth promoting substances during algal growth must not be neglected. It is well known that cyanobacteria are group of photosynthetic prokaryotes which, can fix atmospheric nitrogen in submerged rice fields. Biological N_2 fixation is essential for cyanobacterial process and is dependent upon an adequate population (El-Saadny, 2013). Cyanobacterial strains which do possess the character of nitrogen fixation adds up to value addition of these microbial strains mainly in the field of culture, food, fertilizer (Muthukumar *et al* ., 2007).Inoculation with cyanobacteria into paddy fields is practiced in many countries, resulting in reducing the use of inorganic high cost N fertilizers . Algal inoculation has been recommended for rice fields (Venkataraman, 1969). Algalization with fresh, field propagated, soil- based inoculums of blue-green algae in the Northern part of Nile Delta increased rice yields by about 33% (Yanni *et al*., 1988) .Increased use of mineral fertilizers, especially nitrogen fertilizers, in enhancement of agricultural crop productivity accompanied with soil and water pollution which rise in crop production costs and adverse environmental with consequences on human and animal health. To fix these complicated problems, agricultural activities must be directed to successful management of resources to meet human needs while improve the environment and management of natural resources soundly to preserve their integrity. This can lead to reduce costs and avoid pollution of the environment due to excessive use of mineral fertilizers (El-Saadny, 2013). Therefore , aim of the present study was to determine the effect of inoculation with two cyanobacterial strains as individual or mixture with doses of inorganic nitrogen on the growth and yield of rice plant.

MATERIALS AND METHODS

Cyanobacterial strains

Anabaena oryzae D and *Nostoc muscorum* K were isolated and identified by Abou Elatta , 2018.

Soil sample

Soil samples were collected for mechanical analysis. The physical and chemical properties of the experimental soil in Table (1) were determined according to Piper (1950) and Jackson (1958) .

Table 1. Some physical and chemical properties of the experimental soil

Properties	Soil sample
Texture analysis	
Sand (%)	40.0
Silt (%)	30.0
Clay (%)	30.0
Soil texture	Clay loam
EC (ds/m $^{-1}$)	2.4
pH	7.7
Soluble cations(meq/100g)	
Ca++	6.5
Mg++	4.0
Na+	9.0
K+	2.1
Soluble anions(meq/ 100g)	
CO_3--	0.0
HCO_3^-	4.5
Cl-	9.0
SO_4--	8.1

Detection of IAA

Each cyanobacterial strains were grown in BG11 medium supplemented with 0.1% tryptophan according to Ahmad *et al.* (2005). The method was shown to be most sensitive and most specific (Glickmann and Dessaux, 1995).

Nitrogenase activity

The ability of cyanobacterial strains to fix nitrogen was assayed by acetylene reduction technique according to Hardy *et al*. (1973).

Inorganic fertilizers

Nitrogen as urea (64.5 % N) , super phosphate (15.5% P_2O_5) and zinc sulphate (22%Zn) were used and they were supplied by Rice Res. Institute , ARC , Sakha , Kafr El Sheikh, Egypt .

Field experiments

A field experiment was carried out at the Rice Research Institute, Agricultural research Center (ARC) Sakh, Kafer EL Sheikh, Egypt during the summer season of 2016 to study the effect of biofertilizers as inoculants from the two cyanobacterial strains .This experiment was carried out in a clay loam soil located at Kafr El- Sheikh Governorate. Sakha101 rice cultivar was used and obtained from Rice Research Institute, Agricultural Research Center (ARC) Sakh, Kafer EL Sheikh, Egypt . After thirty days from planting the seedlings were transplanted in the field experiment. The plot size was 4x5m², each plot was divided into rows and each row was five meters long and contained 20 hills each with 20 cm apart with three replications was adopted. The main plot represented cyanobacterial inoculation (*Anabaena oryzae* D and *Nostoc muscorum* K) . The cyanobacterial inoculants were applied just after 5 days from transplanting date at a rate of 1 Kg of sieved clay loam soil inoculated with the two cyanobacterial strains. The inoculum was applied in concentrate suspension of fresh biomass in distilled water to each try corresponded to about 0.18g / tray biomass .

Biomass determination was used to estimate the cyanobacteria biomass of the cyanobacterial culture suspension used for soil inoculation . Numbers of cyanonbacteria in soil based inoculants were determined using the colony formed unit per gram of soil (cfu).

Treatments were nitrogen fertilizer at the rates of (Zero, 23, 46and 60 kg N/fed), added as urea. Each dose was divided into two parts,(⅔ was added before transplanting) and the rest ⅓ was applied after 30 days from transplantion . The other practices such as phosphorus fertilizer in the form of super phosphate was added at the rate of 100 kg/fed. before the first tillage, zinc sulphate at the rate of 1 kg Zn/fed. was added before planting and after puddling. The characters studies were plant height(cm), panicle length(cm) , number of tillers/plant , number of panicles/hill, panicle weight(g) 1000-grain weight (g), grain and straw yields (tons/fed.) and harvest index were determined at harvest stage. The treatments were shown as following:

1.	60 units (Urea)	2.	46 units (Urea
3.	23 units (Urea)	4.	0 units (Urea)
5.	46 units (Urea) + *Nostoc muscorum*	6.	46 units(Urea)+ *Anabaena oryzae*
7.	23 units (Urea) + *Nostoc muscorum*	8.	23 units (Urea)+ *Anabaena oryzae*
9.	0 units (Urea) + *Nostoc muscorum*	10.	0 units (Urea)+ *Anabaena oryzae*

The type of design used in the experiment : Randomized Complete Block Design .

Total cyanobacterial count

N. muscorum k and *A. oryzae* D strains were counted on medium BG11 (Ellora Malakar,*et al.* 2012) using Most probable Number (MPN) technique as described by tubes were incubated at 30°C under continuous light of 120 cm long white fluorescent lamps intensity of 2500 lux .

Statistical analysis

Data were subjected to statistical analysis, according to the procedure outlined by Steel and Torrie (1980). Differences among treatment means were compared using the Revised L.S.D. and Duncan's at 5% and 1% levels of significant .

RESULTS AND DISCUSSION

Production of Indole Acetic Acid (IAA)

Data in Table (2) indicate that producation IAA was increasead with the incubation period of two cyanobacterial strains (*Nostoc muscorum* k and *Anabaena oryzae* D) . *Nostoc muscorum* K gave the highest value of IAA at all incubation periods 7,14 and 21 days (8.42, 21.96 and 37.01 µg/100 ml) respectively, while the lowest values with *Anabaena oryzae* D were (2.74, 11.15 and 11.74 µg/100 ml) respectively. The cyanabacterial filtrates in suspensions significantly increased the IAA reported that with El-Zawawy (2016) & Tantawy and Atef (2010) .

Table 2. production of IAA from the tested cyanobacterial strains (µg/100 ml)

Cyanobacterial strains	Incubation period (days)		
	7	14	21
Anabaena oryzae D	2.74	11.15	11.74
Nostoc muscorum K	8.42	21.96	37.01
LSD 0.05	1.6	1.64	1.79
LSD 0.01	2.3	2.34	2.38

Where: K=Kafr El Sheikh D = El-Dkahalia Governorates .

Nitrogenase activity

Efficiency of the two cyanobaterial strains in nitrogenase activity was tested and represented in Table (3) .The strains *Nostoc muscorum* k and *Anabaena oryzae* D were applied according to the amount of nitrogenase activities . The valeus were 170.88 and 99.60 nano mole C_2H_2 day^{-1} ml^{-1} respectively. These results are similar to those reported by El-Sawah, (2018) and Priya *et al*. (2015) who found that the ability of cyanobacteria fix N_2 was observed by the most active cyanobacteria induced nitrogenase activity of *Anabaena oryzae* and *Nostoc* spp.

Table 3. Nitrogenase activity of cyanobacterial strains

Cyanobacterial strains	Nitrogenase activity (nano mole C_2H_2 day^{-1} ml^{-1})
Anabaena oryzae D	99.60
Nostoc muscorum K	170.88
LSD 0.05	0.17
LSD 0.01	0.28

Where: K=Kafr El Sheikh D = El-Dkahalia Governorates .

Growth and yield parameters of rice

Plant height (cm), panicle length (cm), and number of tillers/plant were the main rice characteristics strongly affected by cyanobacterial inocula and nitrogen fertilizers as recorded in Table (4) . Plant height (cm) of rice plant as effected by inoculation with cyanobacterial strains, *Nostoc muscorum* K, *Anabaena oryzae* D and (46, 23 and zero N/fed. were significantly increased, where cyanobacteria were found to be in the order of 106.00, 105.00, 102.67, 101.00, 97.2 and 96.43 (cm) over the control, (non inoculated) and in the treatments without cyanobacteria (60, 46,23 and zero Nitrogen/fed) 110.67, 103, 98.33 and 90.00(cm) respectively. These results were in accordance with Chittapun *et al*. (2018), El-Sheekh *et al*. (2018), El-Saadny, (2013) and El-Zawawy (2006) who indicated that cyanobacteria gave significant increase plant height by adding cyanobacteria might be attributed to their production of plant growth regulators, which enhanced the ability of rice plants to take up the nutrients from soil, and increase the plant height.

In the case of mean panicle length (cm) as effected by inoculation with cyanobacterial strains, *Nostoc muscorum* k and *Anabaena oryzae* D (46, 23 and zero N/fed) indicated that inoculation with the two strains of cyanobacteria significantly increased, where cyanobacteria were found to be in the order of 25.33, 24.67, 24.67, 24.33, 22.43 and 22.31 (cm) in the control, (non inoculated) and in the treatments without cyanobacteria (60, 46,23 and zero

N/fed) 25.67, 24.38, 22.51 and 15.22 (cm) respectively. These results are in accordance with Chittapun *et al.* (2018), El-Saadny, (2013) El-Zawawy (2006), Basal and Zahran (2002) and Singh *et al.* (2002) indicated that cyanobacteria gave significant increase in the panicle length (cm). The increase in Panicle length (cm) by adding cyanobacteria might be attributed to their production of plant growth regulators, which enhanced the ability of rice plants to take up the nutrients from soil, and increase the Panicle length (cm). Nitrogen fertilization, gave in the same time positive results in this respect.

The same trend recorded with mean number of tillers/plant as effected by cyanobacterial inoculation and nitrogen fertilization was showed . Cyanobacteria inoculation as well as nitrogen fertilization significantly increased this parameter and agreement with Chittapun *et al*. (2018), El-Zawawy (2006), Basal and Zahran (2002).

Table 4. Effect of two cyanobacterial strains with the different doses of urea on plant height, panicle length and number of tillers/plant

Treatments	Plant height (cm)	Panicle length (cm)	Number of tillers/plant
60 N (Urea)	110.67 a	25.67 a	26.70 a
46 N(Urea)	103 c	24.38 a	19. 68 d
23 N (Urea)	98.33 e	22.51b	17. 25 e
0 N (Urea)	90.00 g	15.22 d	13.53 f
46 N(Urea)+ *Nostoc muscorum* k	106.00 b	25.33 a	26.45 b
46 N (Urea) + *Anabaena oryzae* D	105.00 c	24.67 a	25.70 c
23N(Urea) + *Nostoc muscorum* k	102.67 c	24.67 a	22.41 bc
23 N (Urea) + *Anabaena oryzae* D	101.00 d	24.33 a	19.81 d
0 N (Urea) + *Nostoc muscorum* k	97.2 ef	22.43 b	17.39 e
0 N (Urea) + *Anabaena oryzae* D	96.43 f	22.31 b	16.34 e

In the same column , means followed by the same letter are not significantly different at 5% level .

On the other hand , number of panicles/plant , panicle weight (g) and weight of 1000 grains as effected by inoculation with cyanobacterial strains, *Nostoc muscorum* K and *Anabaena oryzae* D (46, 23 and zero N/fed) is presented in Table (5). Number of panicles/plant indicated that inoculation with the both inoculants of cyanobacteria strains significantly increased growth of rice, where cyanobacteria were found to be in the order of 22.49, 21.81, 20.62, 18.23, 17.22 and 17.20. While , in the treatments inoculated with nitrogen fertilization (60, 46, 23 and zero N/fed) were 26.41, 19. 68, 17. 25 and 13.53 respectively. These results are in accordance with Aziz *et al*. (2014) and El-Zawawy (2006) who studied that cyanobacteria gave significant increase in the number of panicles/plant by adding cyanobacteria might be attributed to their production of plant growth regulators, which enhanced the ability of rice plants to take up the nutrients from soil, and increase the number of panicles/plant.

Also , Panicle weight (g) as effected by inoculation with two strains of cyanobacteria , *Nostoc muscorum* K and *Anabaena oryzae* D (46, 43 and zero N /fed) is presented in Table (5) and indicated that inoculation with the both inoculants of cyanobacteria significantly increased growth of rice, where cyanobacteria were found to be in the order of 5.18, 4.88, 4.63, 4.20, 3.98 and 3.91 and in the treatments with different doses of urea (60, 46,23 and zero N/fed) were 6.12, 4.63, 3.98 and 2.00 respectively. These results are in accordance with Prasanna *et al*. (2012) and El-Zawawy (2006) indicated that cyanobacteria gave significant increase in the panicle weight (g) by adding cyanobacteria might be attributed to their production of plant growth regulators, which enhanced the ability of rice plants to take up the nutrients from soil, and increase the panicle weight (g) .

While , weight of 1000 grain (g) of rice showed no response to cyanobacteria inoculation. The same result was also recorded with the effect of nitrogen fertilization Table (5) indicated that this is expected, where the most important benefit expected from this process is the net yield and not the weight of 1000 grains. However this parameter is important from the view of marketing point. These results are in accordance within Aziz *et al*.(2014) and El-Zawawy (2006).

Table 5. Effect of two cyanobacterial strains with the different doses of urea on panicles/plant, panicle weight and 1000-grain weight

Treatments	Number of panicles/plant	Panicle weight (g)	1000-Grain weight (g)
60 N (Urea)	26.41 a	6.12 a	30.48 a
46 N (Urea)	19. 68 d	4.63 c	29.39 b
23 N (Urea)	17. 25 ef	3.98 f	28.06 c
0 N (Urea)	13.53 g	2.00 g	29.23 b
46N Urea) + *Nostoc muscorum* K	22.49 b	5.18 b	30.04 b
46 N (Urea) + *Anabaena oryzae* D	21.81 cd	4.88 d	29.53 b
23N(Urea) + *Nostoc muscorum*K	20.62 bc	4.63 c	27.50 c
23 N (Urea) + *Anabaena oryzae*D	18.23 e	4.20 e	27.12 c
0 N (Urea) + *Nostoc muscorum*K	17.22 f	3.98 f	26.06 d
0 N (Urea)+ *Anabaena oryzae*D	17.20 f	3.91 f	26.02 d

In the same column , means followed by the same letter are not significantly different at 5% level .

The same trend of biomass (t/ha) was significantly increased due to cyanobacterial inoculation. In the non-inoculated treatment and treatments inoculated with *Nostoc muscorum* K and *Anabaena oryzae* D (46, 43 and zero N/fed) respectively .Table 6 indicated that a gradual increase was also recorded with biomass t/ha as a result of nitrogen fertilization, where *Nostoc muscorum* K was recorded with 46 kg N/fed. The previous review cited with biomass t/ha could be used here, where cyanobacteria inoculation could be used instead of 23 kg N/fed or even 46 kg N/fed. for *Nostoc muscorum* K yield of rice biomass t/ha. These results are in accordance with in Prasanna *et al*. (2012) .

At the same time grain yield inoculation with two cyanobacterial strains inoculants increased the yield of rice plants (t/ha); Table 6 indicated that *Nostoc muscorum* K inoculant was found to be more effective than *Anabaena oryzae* D inoculant in this respect. Mean amounts of rice yield were found to be in the order 11.05, 10.73, 10.53, 9.03, 8.40 and 8.38 t/ha. It must be mentioned in this respect, that yield of rice resulted from cyanobacteria inoculation exceed that resulted from fertilization with 46 kg N/fed. It means that cyanobacteria inoculation is economic practice Nayak and Adnikary (2004), Pandiyarajan and Nagarajan (2004), Vaishampayan (2004), Singh (2004), Castro *et al*. (2003), Premi and Kalia (2003), Biswas (1995), Yanni (1992) indicated that the application of cyanobacteria gave significantly higher grain yield as compared with the other treatments. This increase in grain yield/fed. by adding cyanobacteria could be mainly due to the increase in the number of tiller and number of panicles /hill.

While , harvest index indicates the percentage ratio of grain yield to grain +straw yields Table (6) indicated that no significant was recorded in the interaction between algal inoculation and nitrogen fertilization. Inoculation with and *Anabaena oryzae* D as well as with *Nostoc muscorum* K under 23 and 46 kg N/fed. These results are in accordance with (Jan *et al*. (2018) and El-Saadny (2013)

Table 6 . Effect of two cyanobacterial strains on biomass, grain yield and harvest index traits with urea application

Treatments	Biomass t/ha	Grain yield t/ha	Harvest index
60 N (Urea)	19.53 a	12.05 a	61.69 a
46 N Urea	19.30 b	9.32 c	48.29 c
23 N Urea	18.62 b	8.48 c	45.54 b
0 N Urea	6.75 d	2.22 d	32.88 d
46 N Urea +*Nostoc muscorum* K	18.97 b	11.05 b	58.24 b
46 N Urea + *Anabaena oryzae* D	18.87 b	10.73 b	56.86 b
23 N Urea + *Nostoc muscorum* K	18.47 c	10.53b	57.01 b
23 N Urea + *Anabaena oryzae* D	18.33 c	9.03c	49.26 c
0 N Urea + *Nostoc muscorum* K	18.55 c	8.40 d	45.28 c
0 N Urea + *Anabaena oryzae* D	18.50 c	8.38 d	45.29 c

In the same column , means followed by the same letter are not significantly different at 5% level

Total cyanobacterial count in rice field under different levels of urea application

The changes of total both cyanobacterial strains (*N. muscorum* k and *A. oryzae* D) during three times after zero ,35 and 70 days in the rhizosphere of rice plant is presented in Table (7). Results revealed that the total cyanobacterial count in the rhizosphere of inoculated treatments were higher than those in the rhizosphere of nil cyanobacterial ones. So, it can say that the both cyanobacterial strains has apronounced increase count in all three time under different levels of doses urea comparison with the mineral fertilization nil cyanobacteria. Also, it was observed that the highest values of cyanobacterial numbers were obtained at 70 days under all applications, the counts were gradualy increased until 70 days with 46N being 0.229 x10^4 for *N.muscorum* k and 0.175x10^4cfu/g for *A.oryzae* D respectively .Results are in agreement to those obtained by Shatta *et al.* (2014) who noted that the total cyanobacterial count increasing in all stages of growth of rice plant nitrogen fertilization.

Table 7. Total cyanobacterial count (10^4 cfu/g dry soil) in the rhizosphere of rice plant at the three times after tansplanting

Treatments		Days after transplanting		
		0	35	70
Nil cyanobacterial strains	Zero	0.010	0.070	0.090
	23	0.010	0.070	0.090
	46	0.010	0.070	0.090
	60	0.010	0.070	0.090
Nostoc muscorum K	0	0.036	0.160	0.18
	23	0.064	0.175	0.215
	46	0.064	0.184	0.229
Anabaena oryzae D	0	0.055	0.130	0.190
	23	0.055	0.091	0.135
	46	0.055	0.125	0.175

CONCLUSION

Application of inoculation with two cyanobacterial strains (*N. muscorum* K and *A. oryzae* D) increased yield and component of rice plant .

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تأثير التلقيح بالطحالب الخضراء المزرقه مع الأسمده النتروجينه على محصول نبات الأرز ومكوناته

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تعتبرالطحالب الخضراء المزرقه من مثبتات النيتروجين الجوى حيث تقوم بتزويد نباتات الأرز باحتياجتها من النتروجين فى حقول الأرز المغموره. وفى هذه الدراسه أجريت تجربه حقليه فى مركز بحوث وتدريب الأرز ، بمحطة بحوث سخا مركز البحوث الزراعيه كفرالشيخ مصر خلال موسم الصيف 2016 بغرض دراسة تأثير الأسمده الحيويه (الطحالب الخضراء المزرقه) كمخصبات حيويه وبالتالى كان اكثر السلالات كفاءه والتى تم تحديدها واستخدامها سلالة النوستوك موسكورم (المعزوله من أراضى محافظة كفر الشيخ) وسلالة الأنابينا أوريزا (المعزوله من أراضى محافظة الدقهليه) وذلك وفقا لتقدير نشاط انزيم النتروجينيز و إندول حمض الخليك. وقد اجريت هذه التجربه فى تربه طينيه طمييه فى محافظة كفرالشيخ بإستخدام صنف أرز سخا101 . وتم التلقيح بسلالاتى الطحالب الخضراء المزرقه بعد خمسة أيام من الشتل بمعدل واحد كيلو جرام للفدان من التربه الطينيه الطمييه المنخوله حيث كانت المعاملات عباره عن سماد نتروجينى من اليوريا بمعدلات مختلفه (صفر- 23 – 46 – 60) وحدة نيتروجين للفدان وقد أشارت النتائج الى وجود فرق معنوى فى طول النبات وطول السنابل وعدد النباتات وعدد الفروع والكتله الحيويه ومحصول الحبوب ،ولكن من ناحيه أخرى لم يكن هناك فروق معنويه فى وزن الألف حبه ودليل الحصاد . وقد أدى التلقيح بالطحالب الخضراء المزرقه المثبته للنتروجين الجوى الى توفير 20-25% من المعدلات النتروجينيه الموصى بها .