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Cyanobacteria and N-Fertilization Enhance the Efficiency of Rice Plants Grown Under Saline Soil Conditions

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CYANOBACTERIA play an important role in the maintenance and development of soil fertility and can improve nutrient uptake and growth of plants in saline soils. The effect of cyanobacteria applied with or without Nfertilizer on enhancing rice growth and yield quality as well as nutrient content and uptake under saline soil conditions was investigated. Cyanobacteria (Nostoccalcicolasp.) were applied in different application methods, i.e., seed soaking, seed coatingand foliar spray, and singly or in combination with different N-fertilizer rates during two rice field experiments. The experiments were conducted on a clay loam (saline soil) during 2017 and 2018 in the Sharkia Governorate, Egypt. Applied cyanobacteria as a foliar spray in combination with N fertilizer slightly decreased the soil pH and EC values and increased available N, P, K, Fe, Mn and Zn following rice harvest. Cyanobacterial foliar spray+N at a rate of 75 kg fed.$^{-1}$ was superior to the other treatments inincreasing straw and grain yield as well as N, P, K, Fe, Mn and Zn contents and uptake of rice straw and grains. Foliar cyanobacteria + N at a rate of 100 kg fed.$^{-1}$ was superior to the other treatments and increased the 1000-grain weight and plant weight as well as the straw and grain weight plant^{-1}. The highest carbohydrate and total chlorophyll contents were obtained with a treatment of 100 kg N fed.$^{-1}$+ spraying with cyanobacteria. The maximum protein content and yield were observed with 75 kg N fed.$^{-1}$+ spraying with cyanobacteria. The application of cyanobacterial inoculation combined with an N fertilizer application of 75 kg N fed.$^{-1}$ improved the soil properties of saline soils. Furthermore, improved availability and uptake of macro-and micro-nutrients were reflected in the rice grain yield and quality.

Key words: Cyanobacteria,rice, N-fixation, Saline soil, Sahl El-Hossania

Introduction

Saline soils are found throughout Egypt as salts form the natural weathering of minerals and salt deposits. Salts accumulate in the soil of arid climates as irrigation water or groundwater seepage evaporates, depositing minerals. Plant growth in saline soils can be improved using biofertilizers and biochar (Bassouny and Abbas, 2019, Elhusieny et al., 2020 and Youssef et al., 2020).

Biofertilizers are economic and environmentally friendly supplementary sources and can play a role in improving plant nutrient uptake and growth. Cyanobacteria play an important role in the maintenance and development of soil fertility, consequently allowing an increase in rice growth and yield as a natural biofertilizer (Song *e*t al., 2005). Rodriguez et al. (2006), Saadatnia and Riahi (2009) and Palaniappan et al. (2010) reported that cyanobacteria favorably contribute by (1) increasing soil porespace and adhesive substance production; (2) increasing excretion of growth-promoting substances such as hormones,e.g.,auxin, gibberellins, vitamins and amino acids); (3) increasing soil water holding capacity through their jelly structure; (4) increasing soil biomass after their death and decomposition; (5) decreasing soil salinity and preventing weeds growth; and (6) increasing available P via organic acid excretion.

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Cyanobacteria may further contribute to saline soil reclamation, by enhancing soil fertility, and may provide up to 25–35% of the N requirement of rice and improve the soil environment (Abul-Hashem, 2001). Hu et al. (2003) reported that cyanobacteria stabilize soil surfaces primarily through their production of extra cellular polysaccharides, which are prominent agents in the process of aggregate formation and stabilization. Certain cyanobacteria scavenge sodium from saline-sodic soils and increase soil fertility (Pandey et al., 2005). Probu and Dayasoorian (2007) showed that cyanobacteria (*Westiellopsi* sp.) application with amendments decreased soil pH from 8.05 to 7.71. Rodriguez et al. (2006) reported that cyanobacteria synthesize and liberate plant growth regulators such as gibberellins and can have a beneficial effect on salt-stressed rice plants.

The present study aimed to evaluate the effect of cyanobacteria applied in different modes with or without N fertilizer at different rates on enhancing rice growth and yield quality. Their effect on plant nutrient uptake and content under saline soil conditions was also evaluated.

Materials and Methods

Inoculation with N-fixing cyanobacteria (*Nostoc calcicola* sp.) as a biofertilizer was completed in different modes (seed soaking, seed coating and foliar spray) singly or in combination with different N-fertilizer rates applied to rice plants grown in saline soil. Two field experiments were conducted using rice (*Oriza sativa* L., cv. Giza 178) during two successive summer seasons in 2017 and 2018 in El-Rowad village, Sahl El-Hossinia, Sharkia Governorate, Egypt. Cyanobacterial strains were isolated from saline soil in El-Rowad village, Sahl El-Hossinia, Sharkia Governorate. Isolation and culturing techniques were conducted in a BG-11 culture medium (Stanier et al., 1971). The strain, *Nostoc calcicola*, was identified according to Venkataraman (1972) and used for the inoculation process. The main soil properties (Table 1) were determined before sowing according to Page et al. (1982) and Cottenie et al. (1982). The cyanobacterial treatments were as follows: Grains were soaked with a liquid cyanobacteria strain for 4 hr in 50 L/30 kg seeds before sowing. 2) Seeds were coated with gum media carrying the cyanobacteria immediately before sowing. 3) Foliar application of liquid cyanobacteria on soil and plants was complete data rate of 50 L of liquid cyanobacteria mixed with 400 L of water fed.$^{-1}$. The treatment was repeated after 21, 45, and 60 days of sowing.

The experimental design was a split-plot with three replicates. The plot was 12 × 13m in the area. Each plot was sown with rice on the 20th and 25th of May and harvested on the 2nd and 5th of September 2017 and 2018, respectively. Treatments included cyanobacteria singly or incombination with different levels of N fertilizer; urea (460 g N kg^{-1}) was applied at a rate of 0, 50, 75 and 100 kg N fed.$^{-1}$ at three equal doses after 21, 45 and 60 days of sowing. Phosphorus fertilizer was added to all plots before plowing and sowing at a rate of 15 kg P fed.$^{-1}$ as single superphosphate (66.0g P kg^{-1}). Potassium sulphate (400 g K kg^{-1}) was applied at a rate of 25.0 kg K fed.$^{-1}$ in two equal splits 30 and 45 days after sowing.

Soil analysis

Surface samples were collected from the top soil layer (0–30 cm) and prepared to estimate soil pH, EC, cations, anions, and available macro and micronutrients according to Jackson (1973), Richards (1958) and Soltanpour and Schwab (1977).

Plant analysis

Straw and grain yields were recorded at harvest in Mgha^{-1} (Mg = 10^6 g = 1000 kg = 1 ton). Ten plant samples were collected from each plot one day before harvesting, divided into grains and straw, air-dried at 70 °C in an oven, and weighed to obtain the dry matter of grain and straw per plant. The plant samples were thereafter digested using a concentrated H_2SO_4-$HClO_4$ mixture and analyzed for N, P, K, Fe, Mn and Zn according to the methods described by Chapman and Pratt (1961). Grain protein content was obtained by multiplying the grain N concentration by 5.95 according to the method given in AACC (2000). Protein yield (kg fed.$^{-1}$) was calculated as the protein percentage × grain yield (Mgfed.$^{-1}$) × 10.

TABLE 1. Physical and chemical soil properties

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture		O.M (g kg^{-1})		$CaCO_3$ (g kg^{-1})
5.30	33.37	23.04	38.29	Clay loam		5.71		46.71
pH §	EC, dS m^{-1} (soil paste extract)	Cations (mmol$_c$ l^{-1})				Anions (mmol$_c$ l^{-1})		
		Ca^{++}	Mg^{++}	Na^+	K^+	HCO_3^-	Cl^-	SO_4^{--}
8.41	7.84	12.53	18.67	90.41	0.89	8.29	78.90	35.31
Macronutrients (mg kg^{-1})			Micronutrients (mg kg^{-1})					
N	P	K	Fe	Mn		Zn		Cu
35	4.22	180	2.15	1.30		0.48		0.026

§ in (1:2.5) soil:water suspension

Results and Discussion

Yield components

Table 2 shows that cyanobacteria inoculation with and without nitrogen fertilization and their combinations significantly increased the straw and grain weight plant^{-1}, plant weight and 1000-grain weight, reflecting the positive effects of such treatments. Therefore, there was likely an increase in the accumulation of carbohydrates because of enhanced plant growth. Bioinoculation increases nutrient uptake and enhances plant growth and yield (Omran et al. 2009). Increasing available nutrients for enzymes contributes to the accumulation of different organic compounds and consequently improved rice plant growth. The data also show that cyanobacteria application as a foliar spray was superior to the other application methods, followed by coating > soaking > without biofertilizer addition.

Regarding the N-addition rate, the trend followed the order N100 >N75 >N50 > N0 for all yield and yield component values. The N100 + foliar spray of cyanobacteria treatment provided the highest values and increased the straw weight plant^{-1}, grain weight plant^{-1} and 1000-grain weight by 112%, 261% and 84.1% for the combined data compared to the control, respectively. Jan et al. (2017) found that adding cyanobacteria enhanced the rice yield grown under saline soil conditions. These results agree with those obtained by Aziz and Hashem (2004) and Pimratch et al. (2015). The data show that the interaction effect between N fertilizer rates ×biofertilizer addition methods exerted significant effectson straw weight plant^{-1}, grain weight plant^{-1} and 1000-grain weight. The positive effect of increased N application was more pronounced when soaking or foliar application was used.

TABLE 2. Yield and yield component (average of two seasons) of rice plants as affected by N-rate and addition method of cyanobacteria

N-rate (kg fed.$^{-1}$)	Bio addition method	Straw weight plant^{-1}	Grains weight plant^{-1}	Plant weight (g)	1000-grain weight	Yield (Mg fed.$^{-1}$)	
						Straw	Grains
0	Without	23.28	11.01	34.29	34.50	3.599	2.659
50		33.86	14.08	47.94	36.50	4.746	3.679
75		35.56	18.11	53.67	40.00	4.830	3.839
100		38.70	23.14	61.84	42.50	4.788	3.860
Bio addition Mean		32.85 d	16.59 d	49.44	38.38 d	4.494	3.511
0	Soaking	22.90	12.57	35.47	39.00	3.730	2.759
50		35.01	19.14	54.15	45.00	4.150	3.788
75		40.27	24.18	64.45	47.00	4.830	4.200
100		40.24	27.65	67.89	56.00	4.704	4.061
Bio addition Mean		34.61 c	20.89 c	55.49	46.75 c	4.368	3.709
0	Coating	25.91	14.10	40.01	43.50	3.742	2.890
50		37.16	27.17	64.33	51.50	4.578	3.998
75		41.81	33.70	75.51	55.00	4.914	4.284
100		45.32	36.68	82.00	60.00	4.788	4.112
Bio addition Mean		37.55 b	27.91 b	65.46	52.50 b	4.494	3.830
0	Foliar spray	32.22	17.63	49.85	44.50	3.742	3.091
50		42.83	30.73	73.56	56.00	5.166	4.284
75		48.40	36.26	84.66	60.50	5.334	4.326
100		49.29	39.73	89.02	63.50	5.208	4.284
Bio addition Mean		43.19 a	31.09 a	74.27	56.13 a	4.872	3.998
Mean of N-rate	N0:	26.08 d	13.83 d	39.91	40.38 d	3.709	2.852 b
	N50:	37.22 c	22.78 c	60.00	47.25 c	4.662	3.940 a
	N75:	41.51 b	28.06 b	69.57	50.63 b	4.956	4.171 a
	N100:	43.39 a	31.80 a	75.19	55.50 a	4.872	4.078 a
F-test	N-rate:	**	**	ns	**	ns	**
	Bio:	**	**	ns	**	ns	ns
	NxBio:	**	**	ns	**	ns	ns

Straw and grain yield

Straw and grain yields increased as a result of N addition and increasing rates singly or in combination with cyanobacteria, particularly for straw yield (Table 2). The increased N-addition rate increased the straw and grain yield by 34.0% and 46.3%, respectively. This result likely indicates the promotive effect of nitrogen. The order of response to the N rate was as follows:N75 >N100>N50 >N0. The positive effect of N was particularly evident for the foliar spray with cyanobacteria treatment. Sharma et al. (2012) reported that biofertilization using cyanobacteria is recommended for increasing the seed germination rate and growth parameters. Eletr et al. (2013) stated that cyanobacteria application results in a more favorable soil environment for root growth under saline conditions by producing more biomass as a result of enhanced organic matter. These results agree with those of Prabu and Udayasoorian (2007) and Paudel et al. (2012).

The highest straw and grain yields (5.334 and 4.326 Mg fed.$^{-1}$, respectively) were obtained under the N75 + foliar spray with cyanobacteria treatment which resulted in yield increases of 48.1 % and 62.0% for straw and grains, respectively.

Effect of N-addition rate and cyanobacteria application method on rice quality

Data presented in Table 3 show the effect of N addition and cyanobacteria application on protein content and yield as well as proline, carbohydrate and total chlorophyll contents of the rice plants. Plants receiving fertilizer showed increases in the quality parameters reflecting the role of the nitrogen-fixing capacity of microorganisms (Son et al. 2001). These results are similar to those reported by Mostasso et al. (2002) and Hungria et al. (2003).

TABLE 3. Rice quality (average of two seasons) as affected by N-rate and addition method of cyanobacteria

N-rate (kg fed.$^{-1}$)	Bio addition method	Protein content (g kg^{-1})	Protein yield (kg fed.$^{-1}$)	Proline (mg g^{-1}f.w)	Total Chlorophyll (mg g^{-1}f.w)	Carbohydrate (%)
0	Without	60.1	160	3.83	1.53	66.2
50		64.9	239	3.08	1.60	68.2
75		69.0	265	3.00	1.62	69.7
100		71.4	276	2.95	1.66	72.0
Bio addition, Mean		66.6 c	235 b	3.22	1.60	69.1 d
0	Soaking	70.8	195	3.49	1.56	68.6
50		76.8	291	2.98	1.63	70.5
75		78.5	331	2.92	1.67	73.5
100		77.4	314	2.71	1.71	76.8
Bio addition, Mean		76.2 b	283 b	3.03	1.64	72.3 c
0	Coating	94.0	272	3.61	1.57	71.0
50		97.0	388	2.52	1.65	74.6
75		100.0	430	2.40	1.69	77.1
100		99.4	408	2.32	1.73	78.5
Bio addition, Mean		97.6 a	374 a	2.71	1.66	75.3 b
0	Foliar spray	96.4	298	3.56	1.61	71.6
50		99.4	427	2.61	1.67	76.3
75		102.3	441	2.34	1.74	79.3
100		100.1	429	2.26	1.79	79.9
Bio addition, Mean		99.5 a	398 a	2.69	1.70	76.8 a
Mean of N-rate	N0:	80.3 b	381 b	3.62	1.57	69.4 d
	N50:	84.5 a	336 a	2.80	1.64	72.4 c
	N75:	87.5 a	367 a	2.67	1.68	74.9 b
	N100:	87.0 a	357 a	2.56	1.72	76.8 a
F-test	N-rate:	**	**	ns	ns	**
	Bio:	**	**	ns	ns	**
	NxBio:	ns	ns	ns	ns	**

Protein content and protein yield

The results listed in Table 3 show that rice grain protein content and yield increased as a result of the applied treatments. The pattern of response to N-addition rates was as follows: N75 ≥ N100 ≥ N50 > N0. The main effect of the biofertilizer addition in terms of protein content and yield followed the order foliar spray ≥ coating > soaking > without biofertilizer for protein content and foliar spray ≥ coating > soaking ≥ without biofertilizer for protein yield. The favorable effect of cyanobacteria in fixing nitrogen aids rice plants in increasing yield components with the best seed quality. These findings agree with those obtained by El-Shimy et al. (2006) and Hussein (2007). The favorable effect of N fertilization is a result of N being essential for plant growth. As the level of N increases, more protein is produced. Therefore, the increase in N fertilization increases metabolic processes and physiological activities, resulting in good grain quality (Russel, 1973).

The N75 + foliar spray with cyanobacteria treatment was superior to the other treatments and provided the highest values (102.3 g kg^{-1} and 441 kg ha^{-1}, respectively) for protein content and yield, with increases of 70.2% and 176%, respectively.

Proline and total chlorophyll content

Regarding proline and total chlorophyll contents concerning fresh leaf weight (Table 3), the data show decreased proline and increased total chlorophyll content upon application of biofertilizer singly or combined with N. Proline may be the major source of energy and nitrogen during immediate post-stress metabolism and accumulated proline supplies energy for growth and survival, thereby inducing salinity tolerance (Gad, 2005).

The non-treated plants that received neither cyanobacteria nor N increased proline showed the highest value (3.83 mg g^{-1} fresh weight). The lowest proline contentof 2.26 mg g^{-1} fresh weight was obtained under an N238 and foliar spray with cyanobacteria treatment.

The highest chlorophyll content of 1.79 mg g^{-1} fresh weight was obtained using the N238 plus foliar spray of cyanobacteria treatment showing an increase of 17.0%. Bakry et al. (2005) stated that applying *Azospirillium* to soil influenced the biological activity in the soil leading to improved plant growth, photosynthesis and dry matter accumulation. The increase in plant pigment as a result of bioinoculation may be attributed to increases in nitrogen fixed by plants *via* an increase inbacteria nitrogenase enzymatic activity, in which nitrogen is a major component of chlorophyll (Pimratcha et al., 2015).

Carbohydrates

Rice grain carbohydrate contents increased as a result of the application of N and/or cyanobacteria. The pattern of response to N addition was as follows: N100 >N75 >N50> N0. Regarding the bioaddition effect, the pattern of response followed the order foliar > coating > soaking > without biofertilizer. Sharma et al. (2012) reported that total soluble sugar and protein contents increased in germinating seeds following treatment with green algae exudates. The greatest carbohydrate value (79.9%) was found following the addition of treatment including 100 kg N $fed.^{-1}$ when the plants were sprayed with cyanobacteria, showing an increase of 20.7%.

Macronutrient uptake

The data listed in Tables 4 and 5 show that N, P and K contents in rice straw and grains increased as a result of the addition of N and/or cyanobacteria, except for the K uptake by rice grains which showed no significant effect.

The N75 +foliar cyanobacteria treatment was superior in increasing the uptake of all nutrients and showed the highest contents as well as uptake of NPK in straw and grains. The greatest increases in N, P, and K uptake were 65.7%, 203% and 61.0% in straw and 175%, 183% and 86.9% in grains, respectively.

The biofertilization methods were ranked as follows: foliar > coating ≥ soaking ≥ without biofertilizer for N and Kuptake and foliar > coating ≥ soaking > without biofertilizer for P uptake in straw.

Regardingthe effects of treatment on grains, the pattern was as follows: foliar ≥ coating > soaking ≥ without biofertilizer for N uptake and foliar ≥ coating ≥ soaking ≥ without biofertilizer for P uptake.

Regarding the N-fertilization rates, the data showed the following order: N75 ≥ N100 ≥ N50 > N0 for N, P and K uptake by grains as well as N and K uptake by straw while the order was N75 ≥ N100 >N50 > N0 for P uptake by straw.

Micronutrients uptake

Tables 6 and 7 show that the addition of nitrogen increased Fe, Mn and Zn uptake by rice straw and grains while inoculation with cyanobacteria showed increases in Mn and Zn uptake by straw; slight responses in Fe uptake by rice straw; and little effect on Fe, Mn and Zn uptake by grains.

TABLE 4. Macronutrients content and uptake (average of two seasons) in rice straw as affected by N-rate and addition method of cyanobacteria

N-rate (kg fed.$^{-1}$)	Bio addition method	Macronutrients (g kg^{-1})			Macronutrients uptake (kg fed.$^{-1}$)		
		N	P	K	N	P	K
0	Without	28.3	2.41	23.0	102	8.69	82.7
50		29.2	3.03	23.6	139	14.4	112
75		29.7	3.22	23.9	143	15.5	115
100		30.4	3.64	24.5	146	17.5	118
Bio addition Mean		29.4 b	3.08 c	23.8	132 b	13.9 c	107 b
0	Soaking	29.4	3.44	23.5	110	12.8	87.8
50		29.7	3.72	24.0	123	15.4	100
75		30.0	4.13	24.3	144	19.9	117
100		30.7	4.11	24.0	144	19.3	113
Bio addition Mean		30.0 b	3.85 b	24.0	131 b	16.7 b	105 b
0	Coating	30.7	3.53	23.8	115	13.2	89.0
50		31.1	3.92	24.4	143	18.0	112
75		31.4	4.54	24.7	154	22.3	121
100		30.9	4.21	24.3	148	20.2	117
Bio addition Mean		31.0 a	4.05 b	24.3	140 ab	18.3 b	110 b
0	Foliar spray	30.9	3.81	24.1	117	14.4	91.1
50		31.3	4.32	24.7	162	22.3	128
75		31.7	4.93	25.0	169	26.3	133
100		31.1	4.84	24.2	162	25.3	126
Bio addition Mean		31.3 a	4.48 a	24.5	153 a	21.9 a	120 a
Mean of N-rate	N0:	29.8	3.30 c	23.6	111 b	12.2 c	87.8 b
	N50:	30.3	3.75 b	24.2	142 a	17.5 b	113 a
	N75:	30.7	4.21 a	24.5	153 a	20.9 a	122 a
	N100:	30.8	4.20 a	24.3	150 a	20.5 a	118 a
F-test	N-rate:	ns	**	ns	**	**	**
	Bio:	**	**	ns	**	**	**
	NxBio:	ns	ns	ns	ns	ns	ns

TABLE 5. Macronutrients content and uptake (average of two seasons) in rice grains as affected by N-rate and addition method of cyanobacteria

N-rate (kg fed.$^{-1}$)	Bio addition method	Macronutrients (g kg^{-1})			Macronutrients uptake (kg fed.$^{-1}$)		
		N	P	K	N	P	K
0	Without	10.1	3.91	21.3	26.9	10.4	56.7
50		10.9	4.42	21.7	40.1	16.3	79.8
75		11.6	4.73	22.3	44.5	18.2	85.7
100		12.0	5.14	22.7	60.1	19.8	87.8
Bio addition Mean		11.2 c	4.55 d	22.0 d	39.3 b	16.0 c	77.3
0	Soaking	11.9	4.84	22.5	32.8	13.4	62.2
50		12.9	5.22	22.8	48.7	19.8	86.5
75		13.2	5.83	23.3	55.4	24.5	97.9
100		13.0	5.21	22.8	52.9	21.2	92.4
Bio addition Mean		12.8 b	5.28 c	22.9 c	47.5 b	19.6bc	84.8
0	Coating	15.8	5.04	23.2	45.8	14.6	67.2
50		16.3	5.92	23.7	65.1	23.7	94.9
75		16.8	6.31	24.2	72.2	27.1	104
100		16.7	5.83	23.9	68.5	24.0	98.3
Bio addition Mean		16.4 a	5.78 b	23.8 b	63.0 a	22.1 ab	91.1
0	Foliar spray	16.2	5.94	24.0	50.0	18.4	74.3
50		16.7	6.51	24.3	71.8	28.0	104
75		17.2	6.82	24.6	73.9	29.4	106
100		16.8	5.93	24.0	72.2	25.4	103
Bio addition Mean		16.7 a	6.30 a	24.2 a	66.8 a	25.2 a	96.8
Mean of N-rate	N0:	13.5 b	4.93 b	22.8 b	38.5 b	14.1 b	65.1 b
	N50:	14.2 a	5.52 a	23.1 ab	55.9 a	21.7 a	91.1 a
	N75:	14.7 a	5.92 a	23.6 a	61.3 a	24.7 a	98.2 a
	N100:	14.6 a	5.53 a	23.4 a	59.6 a	22.6 a	95.3 a
F-test	N-rate:	**	**	**	**	**	**
	Bio:	**	**	**	**	**	ns
	NxBio:	ns	ns	ns	ns	ns	ns

TABLE 6. Micronutrients content and uptake (average of two seasons) in rice straw as affected by N-rate and addition method of cyanobacteria.

N-rate (kg fed.$^{-1}$)	Bio addition method	Micronutrient content (mg kg^{-1})			Micronutrient uptake (g fed.$^{-1}$)		
		Fe	Mn	Zn	Fe	Mn	Zn
0	Without	128.6	48.4	28.3	463	174	102
50		128.7	51.5	28.4	613	245	135
75		129.1	51.5	28.5	622	248	137
100		129.2	51.4	28.6	620	247	137
Bio addition Mean		128.9 b	50.7 b	28.5 c	580	228 ab	128 b
0	Soaking	130.5	51.2	29.2	487	191	109
50		130.6	51.3	29.3	542	213	122
75		130.7	51.5	29.4	629	248	141
100		130.6	51.5	29.3	614	242	138
Bio addition Mean		130.6 a	51.4 a	29.3 b	568	224 b	127 b
0	Coating	130.9	51.5	29.4	490	193	110
50		130.8	51.6	29.4	600	237	135
75		130.9	51.8	29.4	643	254	144
100		130.8	51.6	29.3	628	248	141
Bio addition Mean		130.8 a	51.6 a	29.4 b	590	233 ab	133 b
0	Foliar spray	131.2	51.6	30.1	496	195	114
50		131.3	51.7	30.2	679	267	156
75		131.4	51.8	30.3	700	276	161
100		131.2	51.7	30.2	685	270	158
Bio addition Mean		131.3 a	51.7 a	30.2 a	641	252 a	147 a
Mean of N-rate	N0:	130.2	50.7 b	29.2	483 b	188 b	108 b
	N50:	130.4	51.5 a	29.3	609 a	241 a	137 a
	N75:	130.5	51.6 a	29.4	649 a	256 a	146 a
	N100:	130.5	51.6 a	29.3	637 a	252 a	143 a
F-test	N-rate:	ns	*	ns	**	**	**
	Bio:	**	*	**	ns	*	*
	NxBio:	ns	*	ns	ns	ns	ns

TABLE 7. Micronutrients content and uptake (average of two seasons) in rice grains as affected by N-rate and addition method of cyanobacteria

N rate (kg fed.$^{-1}$)	Bio addition method	Micronutrients content (mg kg^{-1})			Micronutrients uptake (g fed.$^{-1}$)		
		Fe	Mn	Zn	Fe	Mn	Zn
0	Without	74.4	31.4	37.6	180	83.6	100
50		74.5	31.5	37.6	274	116	139
75		74.5	31.6	37.7	286	121	145
100		74.6	31.6	37.7	288	122	145
Bio addition Mean		74.5 b	31.5	37.7	261	111	132
0	Soaking	75.2	31.7	38.2	208	87.4	105
50		75.3	31.7	38.2	285	120	145
75		75.3	31.8	38.3	317	134	161
100		75.3	31.7	38.2	306	129	155
Bio addition Mean		75.3 ab	31.7	38.2	279	118	142
0	Coating	75.3	31.7	38.2	218	91.6	110
50		75.4	31.8	38.3	301	127	153
75		75.5	31.8	38.3	325	137	165
100		75.5	31.7	38.2	310	130	157
Bio addition Mean		75.4 ab	31.8	38.3	289	122	147
0	Foliar spray	75.6	31.8	38.3	234	98.3	118
50		75.7	31.9	38.4	325	137	165
75		75.8	32.0	38.5	326	138	166
100		75.7	31.9	38.4	325	137	165
Bio addition Mean		75.7 a	31.9	38.4	303	128	154
Mean of N-rate	N0:	75.1	31.7	38.1	214 b	90.2 b	108 b
	N50:	75.2	31.7	38.1	296 a	125 a	150 a
	N75:	75.3	31.8	38.2	314 a	133 a	159 a
	N100:	75.3	31.7	38.1	307 a	129 a	156 a
F-test	N-rate:	ns	ns	ns	**	**	**
	Bio:	*	ns	ns	ns	ns	ns
	NxBio:	ns	ns	ns	ns	ns	ns

Regarding the effect of Nrates on Fe, Mn and Zn uptake by rice straw and grains, an increase via increased N rates was evident, particularly at N75 kg fed.$^{-1}$. The pattern of response was as follows: N75 ≥N100 ≥N50 >N0. The pattern of response to the cyanobacteria application method was as follows: foliar ≥ coating ≥ soaking ≥ without biofertilizer. The highest Fe, Mn and Zn uptake values (700, 276 and 161 g fed.$^{-1}$) by rice straw and rice grains (326, 138 and 166 g fed.$^{-1}$) were observed for plants treated with N75 kg fed.$^{-1}$ + foliar spray with cyanobacteria, resulting in increases of 51.1%, 58.6% and 57.8%, respectively, by straw and 81.1%, 65.1% and 66.2%, respectively, by grains.

Effect of cyanobacteria and N rates on soil properties

Soil pH and soil salinity (ECe)

All treatments receiving biofertilization with or without N showed a slight decrease in soil pH in the rhizosphere of rice plants (Fig. 1). The greatest decrease in pH value (8.02) was achieved by treating the soil with 75 kg N fed.$^{-1}$ + foliar spray with cyanobacteria, causing a decrease of 3.26% as compared to the pH of the native soil (8.29). This may have been a result of active microorganisms, biological activity in particular, and organic acid production. The positive effects of biofertilizers on decreasing soil salinity hazards have been reported (Rashed, 2006). These results are similar to those obtained by Abdel Lattif (2007), Poraas et al. (2009) and Attia et al. (2014).

Concerning the effect of the treatments on soil salinity, data in Fig. 1 show that the EC values decrease as a result of cyanobacteria + N. The effect is more pronounced in soil with the N75 + foliar cyanobacteria treatment, which caused a 35.8% decrease.

Eletr et al. (2013) reported that inoculation with cyanobacteria decreased soil EC. Molnar and Ordog (2005) noted that cyanobacteria released some plant growth-promoting regulators (PGPRs) as defense systems to counteract the salt stress through a decrease inEC. Cyanobacteria can excrete extra cellular compounds, such as peptides, organic acids, lipids, and polysaccharides, leading to improved soil conditions (El Ayouty et al., 2004).

Available macro-and micro-nutrients in soil following harvest

Data in Fig. 2 and 3 show that available N, P, K, Fe, Mn and Zn in the soil following harvest increased in response to the treatments. The maximum values of 53.6, 6.63 and 243 mg kg^{-1} for available N, P and K, respectively, as well as 3.86, 2.56 and 1.11mg kg^{-1} for available Fe, Mn and Zn, respectively, were a result of the N75 plus foliar spray of cyanobacteria treatment.

This treatment resulted in increases of 46.4%, 32.9%, 26.6%, 31.7%, 56.1% and 35.4% for available N, P, K, Fe, Mn and Zn, respectively. These results show the beneficial role of the biofertilizer and the microorganisms and their biological activity, in particular in aiding the development of soil microflora. Application of the mineral N improves the microorganism activities responsible for N transformation (Shaban et al. 2008). Biofertilizer showing greater positive effects in soil nutrient availability possibly indicates the release of active organic acids during microbial activity, thus enhancing nutrient solubilization from native and added sources (Ewees and Abdel Hafeez, 2010). Nitrogen-fixing bacteria and cyanobacteria improved the soil chemical properties through decreasing EC and pH compared to that of the control; therefore, the dissolved CO_2 in the soil led to a reduced soil pH. Molnar and Ordog (2005) noted that plant growth-promoting regulators (PGPRs) are released by cyanobacteria, leading to alleviation of high soil EC and an increase in soil available N, P, K, Fe, Mn and Zn. Similar findings were observed by Strik and Staden (2003), who noticed that incorporation of Azolla (fresh or dry) into soil succeeded in significantly increased soil organic matter, which in turn upon its decomposition by soil microorganisms released macro-and micro-nutrients into the soil. Singh et al. (2008) reported that cyanobacteria led to increases in soil biological activity, which consequently increased soil fertility and nutrient availability under a salt-stressed condition. Sahu et al. (2012) reported that cyanobacteria play an important role in enhancing soil fertility.

Conclusions

Cyanobacteria applied as a foliar spray extract, coating, or seed soaking significantly increased the nutrient content of rice plants. Application of 75% of the recommended dose of Nin addition to cyanobacteria decreased the recommended dose of N by 25%. Cyanobacteria contributed to a more efficient N availability for rice by increasing available N, P, K, Fe, Mn and Zn yields; yield components; and macro-and micro-nutrient contents of both grains and straw.

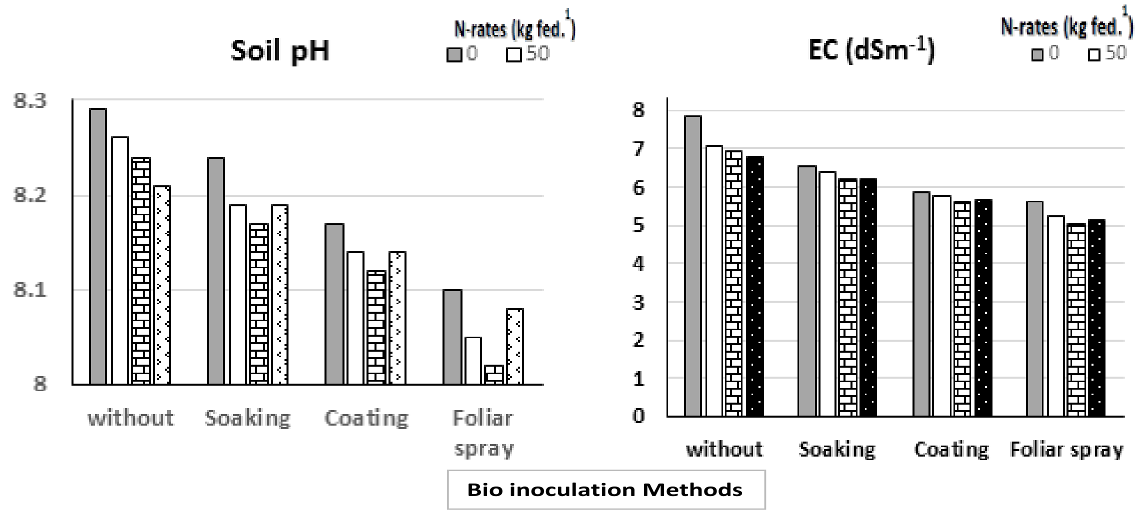

Fig. 1. Soil pH and EC (dSm-1) following rice harvest as affected by N addition rate and inoculation with cyanobacteria under saline soil conditions

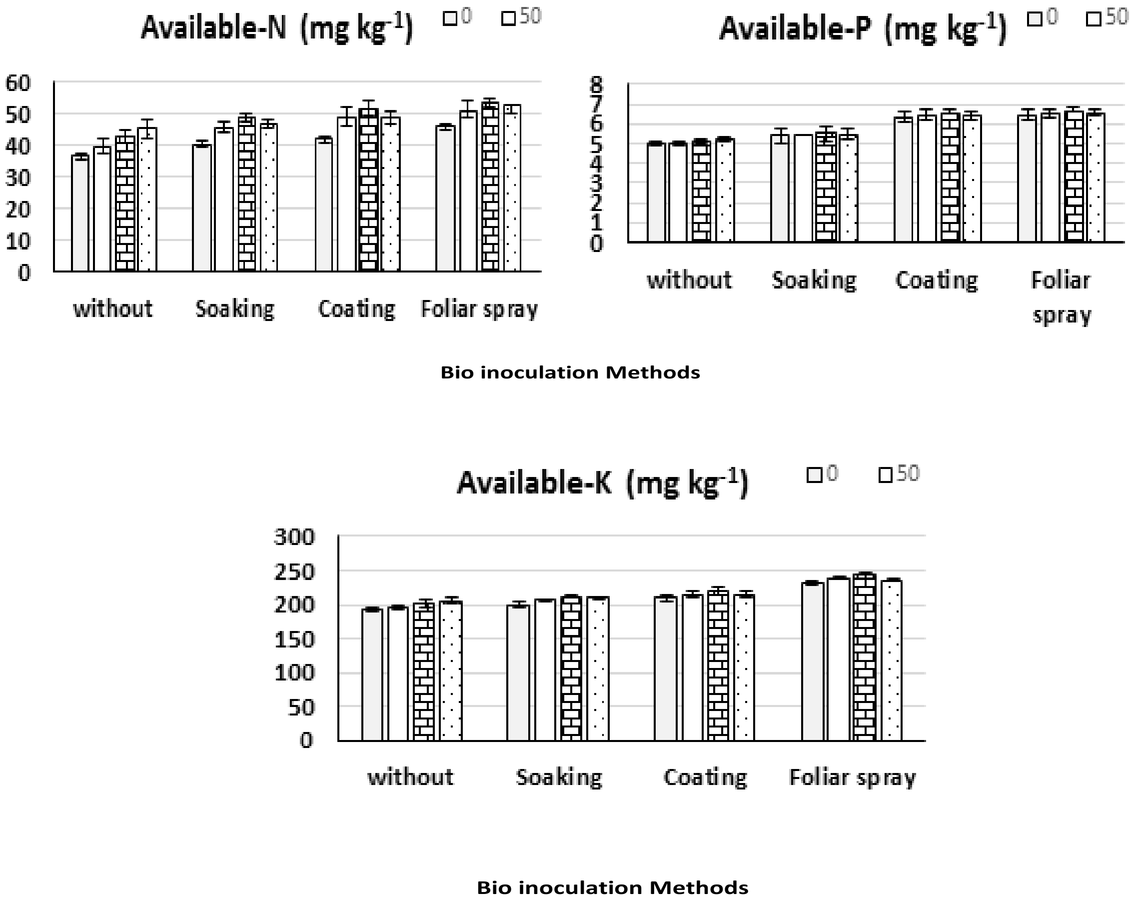

Fig. 2. Available macronutrients (mg kg-1) following rice harvest as affected by N addition rates and inoculation with cyanobacteria under saline soil conditions

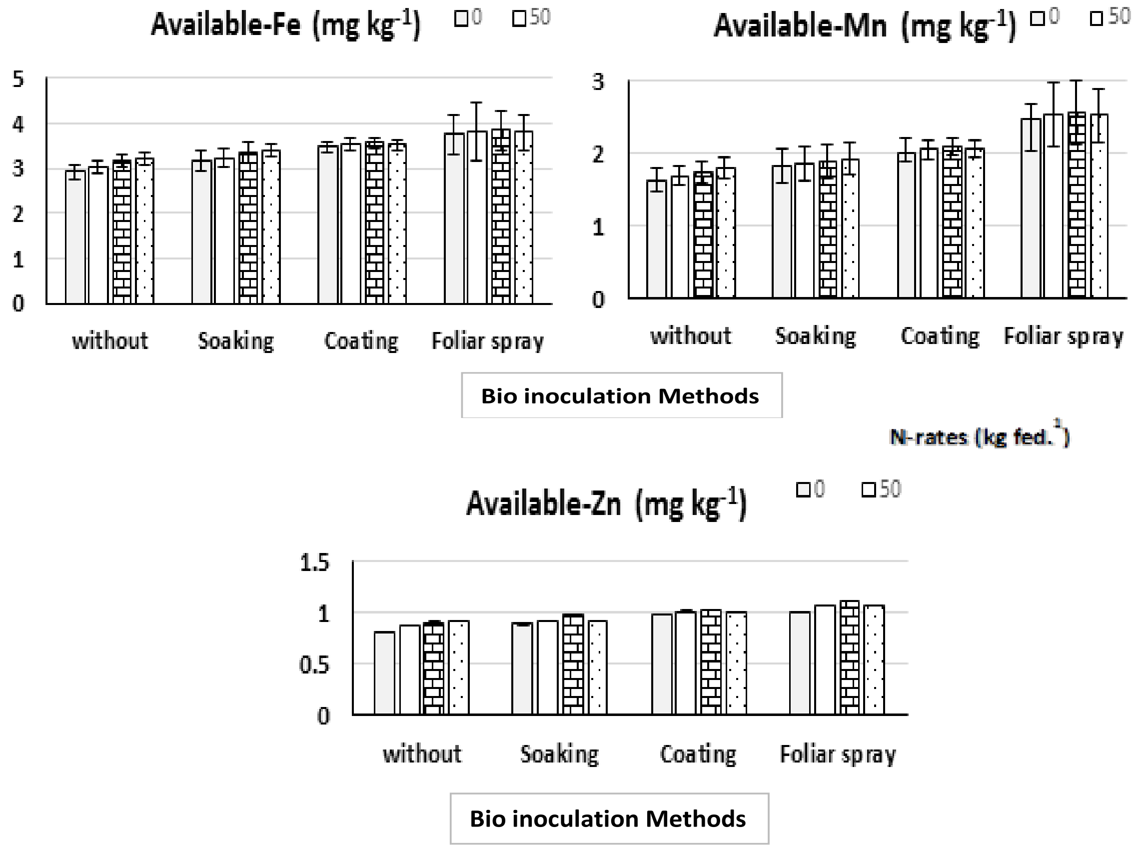

Fig. 3. Available micronutrients (mg kg-1) following rice harvest as affected by N addition rate and inoculation with cyanobacteria under saline soil conditions

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السيانوباكتريا و التسميد النيتروجيني وتحسين كفاءة نباتات الأرز النامية تحت ظروف التربة الملحية

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لدراسة دور التلقيح الحيوي بالسيانوباكتريا المثبتة للنيتروجين (*Nostoccalcicola* sp.) كسماد حيوي بإضافتة بعدة طرق (نقع البذور – تغليف البذرة – الرش الورقي) بمفردها أو بالتداخل مع التسميد النيتروجيني بمعدلات مختلفة علي رفع كفاءة إنتاجية الأرز النامي تحت ظروف التربة الملحية، تم إجراء تجربتين حقليتين خلال موسمي صيف ٢٠١٧ و ٢٠١٨ بمنطقة سهل الحسينية - محافظة الشرقية – مصر. وقد خلصت النتائج إلي أن التلقيح بالسيانوباكتريا من خلال الرش الورقي بالتداخل مع التسميد النيتروجيني أدي إلي أنخفاض طفيف في قيم الحموضة pH و ملوحة التربة EC و أزدادت قيم العناصر الكبري (ن ، فو و بو) و العناصر الصغري (حديد ، منجنيز و زنك) الميسرة بالتربة بعد الحصاد. أيضاً التلقيح بالسيانوباكتريا من خلال الرش الورقي بالتداخل مع التسميد النيتروجيني بمعدل ٧٥ كجم ن فدان-١ كانت هي المعاملة المفضلة والتي تفوقت علي باقي المعاملات في زيادة محصول الأرز (القش و الحبوب) وكذلك محتوي العناصر الكبري و الصغري والممتص منها بواسطة قش وحبوب الأرز. من ناحية أخري، التلقيح بالسيانوباكتريا من خلال الرش الورقي بالتداخل مع التسميد النيتروجيني بمعدل ١٠٠ كجم ن فدان-١ كانت هي المعاملة المفضلة والتي تفوقت علي باقي المعاملات في زيادة وزن الألف حبة ، وزن النبات ، وزن القش نبات-١ ووزن الحبوب للنبات-١. أعلي محتوي للكربوهيدرات و المحتوي الكلي للكلوروفيل تم التوصل إليها نتيجة لمعاملة الإضافة التسميد النيتروجيني بمعدل ١٠٠ كجم ن فدان-١ معالرش الورقي بالسيانوباكتريا بينما، كانت أعلي قيمة لمحتوي ومحصول البروتين للحبوب قد تم التحصل عليها نتيجة لمعاملة الإضافة التسميد النيتروجيني بمعدل ٧٥ كجم ن فدان-١ معالرش الورقي بالسيانوباكتريا.