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Comparative Evaluation of Biological Treatments and Mineral NPK on Rice Productivity in Alkaline-Saline Soil

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ABSTRACT

A field experiment was conducted at Sahl El-Hussinia Station, El-Sharkia Governorate, Egypt, during the summer seasons of 2012 to study the individual and combined effecs of N_2-fixing bacteria *(Azotobacter chroococcum, Klebsiella pneumonia* and *Colstridium pastereurianm*) and cyanobacteria (*Nostoc muscorum*, *Nostoc humifusum, Anabaena oryzae, Wollea* sp, *Phormedium* sp and *Spirulina platensis*) compared to Cyanobacterin (commercial bio-product for rice) on growth, yield and yield components of rice (cv-Giza 178). Inoculation with biological treatments positively affected the soil biofertility through enhancing soil biological activity in terms of dehydrogenase and nitrogenase activities which in turn supported the growth of rice plant. The treatment of 75% NPK + N_2-fixing bacteria + cyanobacteria recorded the highest plant height, number of panicles/plant, weight of 1000-grain and plant biomass (rice straw and grain yields) compared to the other tested treatments. Also, this treatment increased significantly the total contents of N, P and K in plants. Inoculation with N_2-fixing bacteria + cyanobacteria together with 50% mineral NPK level supported rice growth under saline conditions. While, significant enhance of nitrogen fixing potential was recorded by the biological treatments involving the combination of bacteria-cyanobacteria strains. The treatment of 75% NPK + N_2-fixing bacteria + cyanobacteria in increasing the growth and grain yield of rice and improving soil health, besides mineral NPK saved 25 to 50%. The study authenticated the positive effects of co-inoculation with bacterial and cyanobacterial strains on rice crop production.

Key words: PGPR, bacteria, cyanobacteria, Cyanobacterin, rice, saline soil

Introduction

In Egypt, climate change is manifested in drought and salinization of its soils and water bodies. Total cropped (cultivated) area has increased from 4.7 million ha in 1982 to 6.5 million ha in 2003. Of these, one million ha of irrigated area suffers from problems of salinization (FAO, 2005). The main problem at Sahl El-Hossynia soil is related to high salinity conditions. Soil degradation caused by salinization and sodication is a universal concern. Saline (EC > 4 dSm-1), or salt affected soil is a major environmental issue, as it limits plant growth and development, causing productivity losses (Qadir *et al.*, 2008). Improving the fertility of the saline soil is an utmost necessary from the agriculture point of view. Rice (*Oryza sativa* L.) is one of the most prominent food crops globally, and represents the staple diet for almost half of the human population of the world, which accounts for 23% of the world's calorie intake (Bernier *et al.* 2008). It is estimated that there will be about 8 million people by the year 2020, requiring 760 million tons of rice. This means that the production of rice needs to be increased by 2% per year to meet future demands. This will require double the amount of currently applied synthetic fertilizers, which is neither economically feasible nor environmentally desirable. In order to make rice cultivation sustainable and less dependent on chemical fertilizers, it is important to know how to use the plant growth promoting rhizobacteria (PGPR) that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that can contribute to the improvement of rice growth (Keyeo *et al.* 2011). Aerobic free living N_2-fixing microbes include *Azotobacter, Beijerinckia, Azonomas* etc., facultative anerobes include *Klebsiella,* anaerobic bacteria like *Clostridium acetobutylicum, C. pasteurianum*, photosynthetic bacteria like *Chlorobium, Rhodospirillium* etc. (Schlegel, 1991). Plant growth promoting activity has been reported in bacterial isolates belonging to several genera such as *Burkholderia*, *Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum* and *Acetobacter* (Khalid *et al.* 2004; Herman *et al.* 2008). It has been established that among cyanobacteria, both heterocystous and non heterocystous forms, *Anabaena, Nostoc, Trichodesmium, Lyngbya, Plectonema* etc. fix nitrogen (Rao and Burns, 1990; Schlegel, 1991). In cyanobacteria, a light and thick walled cell structure called heterocyst is the point where nitrogen fixation takes place. However, in members like *Lyngbya* and *Plectonema* where heterocyst is absent, nitrogen fixation occurs in internally organized cells (Schlegel, 1991). There are reports that some cyanobacteria can grow successfully on saline soil

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where most plants with the exception of halophytes fail to grow. Cyanobacteria are known by their ability to excrete growth-promoting substances such as hormones (auxin, gibberellins), vitamins and amino acids. They also increase the water- holding capacity through their jelly structure, increase soil biomass after their death and decomposition and preventing weeds growth (Alam *et al.*, 2014). The photoautotrophic nitrogen-fixing cyanobacteria exhibit considerable tolerance to salt or osmotic stress and reclamation of saline/sodic soils and stimulate the growth of plants, due to the presence of auxin, cytokinins, gibberellins and related growth regulators (Mia *et al.*, 2010). Soil based cyanobacterial inoculum that are prepared from saline soils and that are adapted to salt stresses may effectively used in N economy and improved fertility of these soils (Aziz and Hashem, 2003). Cyanobacteria are also known as a low-cost technology in rice fields (Roy and Srivastava, 2013) to increase soil fertility by enhancing the available N and P levels and exhibited an economical view that they can compensate about 50% of the recommended doses of N, P and K (Mahmoud *et al* ,2007). Studies have been undertaken either with PGP bacteria or cyanobacteria in rice but studies which deploy combinations of bacterial and cyanobacterial are scarce. Therefore, this study aims to investigate the promising combination of N-fixing/plant growth promoting bacteria and cyanobacteria for effective nutrient management of rice under different levels of mineral NPK fertilizers in alkaline-saline soil.

Materials and Methods

A field experiment was conducted at Sahl El-Hussinia Station, El-Sharkia Governorate, Egypt, during the summer season of 2012 to study the individual and combined effect of PGPR and N_2-fixing bacteria and cyanobacteria strains compared to Cyanobacterin (commercial algal product for rice) on growth, yield and yield components of rice (cv-Giza 178). The experimental layout comprised four levels of NPK (0, 50, 75 and 100% of the recommended dose) under saline-sodic soil conditions. The control was received the full recommended NPK doses as follows: 100 kg/fed of nitrogen as urea (46.5% N), 200 kg/fed of phosphorus as super phosphate (15.5 % P_2O_5) and 100 kg/fed of potassium as potassium sulphate (48 % K_2O). Some mechanical and chemical characteristics (Table,1) of the studied soil were determined according to the methods of Page *et al.* (1982).

Table 1: Mechanical and chemical properties of the studied soil

Coarse sand %	Fine sand %	Silt %	Clay %	Texture	O.M %	$CaCO_3$ %		
2.10	12.32	38.67	46.91	Clay	0.62	10.36		
pH (1:2.5)	EC in soil paste ($dS.m^{-1}$)	Cations ($meq.L^{-1}$)				Anions ($meq.L^{-1}$)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^{--}
8.50	24.37	14.22	27.12	201	1.34	9.93	186	47.75
Available nutrients ($mg.kg^{-1}$ soil)								
N	P	K	Fe	Mn	Zn	Cu		
31	3.77	197	1.70	2.92	0.52	0.063		

Halotolerant N_2-fixing strains *(Azotobacter chroococcum, Klebsiella pneumonia* and *Colstridium pastereurianm*) were isolated from Sahl El- Hessiania and tested for their activity as nitrogen fixers and PGPR in their specific media (Table, 2).

Table 2: Some characteristics of the N_2-fixing bacteria strains used in the experiment

Strains	Gram strain	Capsular	Counts*10^6 Cfu ml^{-1} culture	N_2-ase (µg mol C_2H_4 hr^{-1} ml^{-1} culture)	[1]IAA (ppm)	[2]GA (ppm)	[3]TAA (ppm)	[4]EPS (ppm)
Azotobacter chroococcum	-	+	0.28	98.9	12.8	12.2	0.49	3.85
Klebsiella pneumonia	-	+	0.22	42.9	14.6	18.9	0.21	2.92
Colstridium pastereurianm	+	+	0.11	60.9	13.5	11.7	0.29	1.18

[1]IAA: Indole acetic acid; [2]GA: Gibberellic acid; [3] TAA: Total amino acids; [4] EPS: Exo-polysaccharides

PGPR strains (*Bacillus. circulans* and *Bacillus* spp.) were isolated from Sahl el-Hessiania Research Station, North Sinai, Egypt and grown on nutrient broth medium (Difico Manual, 1984) at 30°C for 24 hr. These strains were purified and examined for their ability to produce growth regulators i.e. auxins and gibberellins besides their ability to produce exo-polysaccharide and total amino acids (Table, 3).

Table 3: Some bio-chemical activities of the tested PGPR strains

Strains	Total amino acids (ppm)	Growth regulators		Exo-polysaccharides g/L
		GA (ppm)	IAA (ppm)	
Bacillus circulans circulans	0.29	15.20	11.20	1.32
Bacillus spp.	0.33	13.60	12.10	0.96

**IAA, Indole acetic acid; GA, gibberellic acid*

Cyanobacteria inoculum is a mixture of N_2-fixing *Nostoc muscorum*, *Nostoc humifusum, Anabaena oryzae* and *Wollea* sp and non N_2-fixing *Phormedium* sp and *Spirulina platensis* obtained from Microbiology Department; Soils, Water and Environment Res. Inst. (SWERI), Agric. Res., Center (ARC), Egypt. N_2-fixing strains were maintained in N-free $BG11_0$ medium (Rippka *et al*., 1979) while, non- N_2-fixing *Phormedium* sp and *Spirulina platensis* strains were maintained in BG11 (Rippka *et al*., 1979) and Zarrouk medium (Zarrouk, 1966), respectively. Cyanobacteria inoculum was prepared by mixing equal aliquots of the algal culture suspensions at the log phase. The culture growth parameters are shown in Table (4).

Table 4: The growth parameters and phytohormone composition of cyanobacteria

	Nostoc muscorum	*Spirulina platensis*	*Anabaena oryzae*	*Wollea sp.*	*Nostoc humifusum*	*Phormedium sp.*
	Culture growth parameters					
pH	8.2	10.3	7.5	7.11	8.4	9.01
Optical density at 560 um	1.19	2.87	0.89	2.51	1.73	2.21
Chlorophyll-a ($mg.l^{-1}$)	5.76	12.02	5.29	10.15	8.88	4.5
Biomass dry weight ($mg.l^{-1}$)	765.2	1832	588.4	1580.5	1079.2	1290.4
	Phytohormone composition of cyanobacterial cultures ($\mu g.100ml^{-1}$culture filtrate)					
IAA *	0.182	0.350	0.112	0.115	0.111	0.119
ABA *	0.523	0.00	0.018	0.013	0.016	0.002
GA *	0.286	0.767	0.884	0.871	0.938	0.933

**IAA, Indole acetic acid; ABA: abscisic acid and GA : gibberellic acid*

Rice cv. Giza 178 grain bags were soaked in water for 24 hr. then, kept warm for another 24 hr. under a layer of decomposing manure, following the traditional habit. The germinated grains except for mineral and Cyanobacterin treatments were soaked overnight in large basins in contact with the PGPR-bacteria suspension (a mixture of liquid culture of *B. circulans* and *Bacillus* spp. approximately contains 10^9 cfu ml^{-1}). The Arabic gum was added as an adhesive agent to the bacterial suspension (5% w/v).

Cyanobacteria (at a rate of 20 $l.fed^{-1}$) and N_2-fixing bacteria (at a rate of 15 $l.fed^{-1}$) inoculums were applied as soil drench after 30 and 60 days from seed sowing followed by foliar spray after 90 days from seed sowing.

The experimental design

The experiment was laid out in a split plot design with three replicates as follows:

A) Main plots:
1. mineral fertilizers (without any biological treatments)
2. N_2-fixing bacteria mixed culture (15 $l.fed^{-1}$ as soil drench application and 5 $l.fed^{-1}$ as folir application).
3. cyanobacteria mixed cultures (30 $l.fed^{-1}$ as soil drench application and 10 $l.fed^{-1}$ as folir application).
4. mixed culture of N_2-fixing bacteria + cyanobacteria mixed cultures
5. Cyanobacterin (one $bag.fed^{-1}$).

B) Sub plots:
1. 0% mineral NPK fertilizers
2. 50% mineral NPK fertilizers
3. 57% mineral NPK fertilizers
4. 100% mineral NPK fertilizers (Control)

Rice rhizosphere soil was sampled after 90 days from sowing to determine pH, EC and total NPK (Page *et al*., 1982) of soil as well as the soil biological activity in terms of dehydrogenase activity (Casida *et al*., 1964) and nitrogenase activity (Hardy *et al*., 1973). At harvest, Straw and grains of rice crop were collected from each plot to evaluate the rice yield components in terms of straw and grain yields (tons.ha-1) as well as 1000-grain weight (g). Samples of straw and grains were oven dried at 70°C for 48 h, then weighed to constant weights, ground and prepared for digestion according to Page *et al*. (1982). The digests were exposed to estimation of N, P, K and Na (Cottenie *et al*., 1982).

Results

Biological activity of rice rhizosphere:

The effects of Cyanobacterin, cyanobacteria and bacteria inoculation combined with different NPK rates on soil rhizosphere biological activity after 90 days from sowing in terms of dehydrogenase and nitrogenase activities are illustrated (Figures 1-3). All the biological treatments increased the soil enzymes activity compared to un-inoculated treatments. Data of dehydrogenase and nitrogenase activities (Figures 1 and 2) as affected by different treatments under various NPK levels showed the same trend and could be ranked in the following order: bacteria+cyanobacteria>cyanobacteria>Cyanobacterin>bacteria>mineral. The co-inoculation of cyanobacteria and bacteria combined with 75% NPK led to the highest records of nitrogenase and dehydrogenase (907.0 µg mol C_2H_4 g^{-1} soil day^{-1} and 71.81 g TPF.g^{-1} dry soil. hr^{-1}, respectively) which positively reflected on the rice yield as shown in Table (5).

The highest records of dehydrogenase and nitrogenase activities (53.16 µg TPFg^{-1} dry soil hr.$^{-1}$ and 702.4 µg mol C_2H_4 g^{-1} soil. hr.$^{-}$1, respectively) were achieved when 75% of NPK was applied.

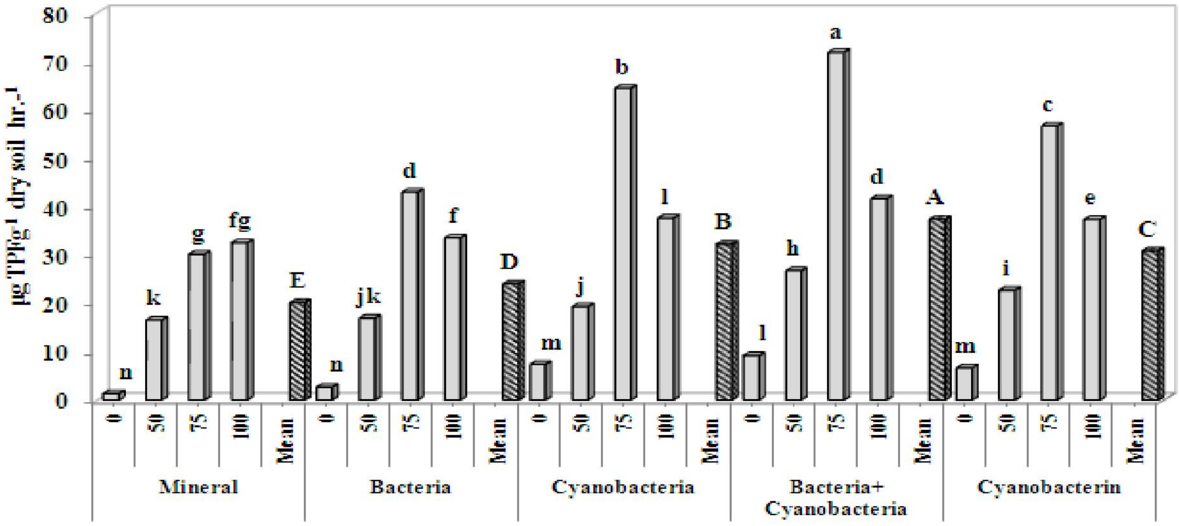

Fig. 1: Effects of Cyanobacterin, PGPR/N_2-fixing bacteria and cyanobacteria inoculation on dehydrogenase activity under different NPK rates in alkaline-saline soil .

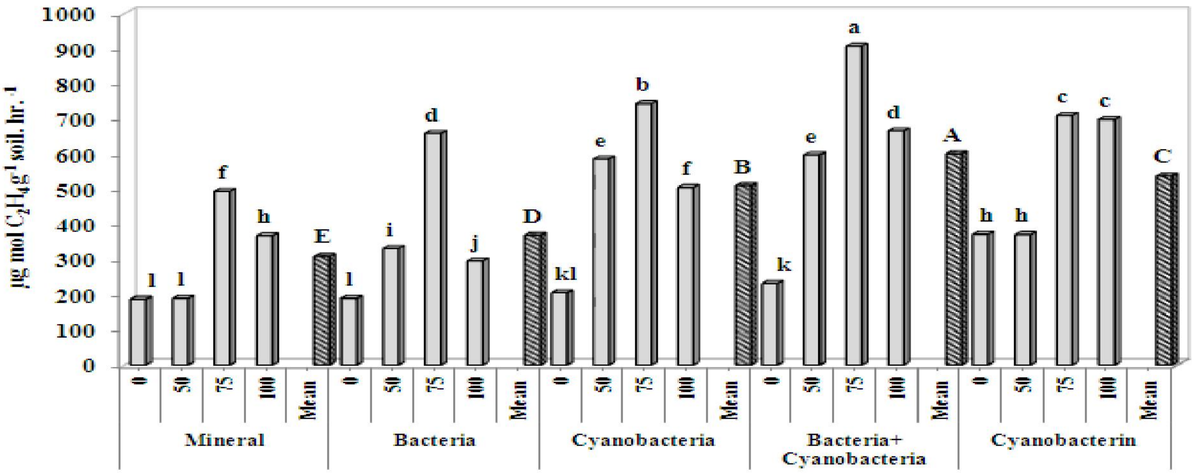

Fig. 2: Effects of Cyanobacterin, PGPR/N_2-fixing bacteria and cyanobacteria on nitrogenase activity under different NPK rates in alkaline-saline soil

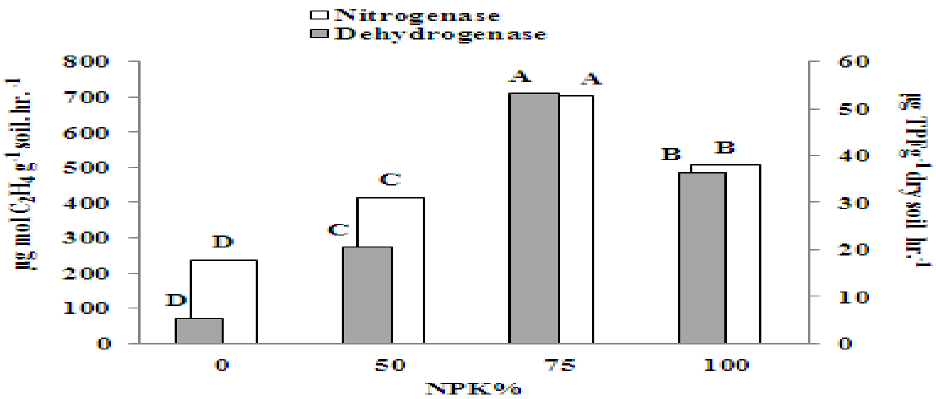

Fig. 3: Effect of different levels of NPK on dehydrogenase and nitrogenase activities under alkaline-saline soil conditions.

Rice yield:

Table (5) presents the effects of Cyanobacterin, PGPR/N_2-fixing bacteria and cyanobacteria inoculation combined with different NPK levels on rice yield components. The yield components of all biological treatments were significantly higher than those of mineral treatments alone. The highest mean values of plant height, branches number/plant, panicles number/plant, weight of 1000-grain, straw yield, grain yield and harvest index (%) were achieved by the integration of N_2-fixing bacteria + cyanobacteria. The highest significant effect of NPK level on plant height was observed with 100% NPK (69.40 cm), there were no significant differences in mean values of plant height between bacteria, bacteria+cyanobacteria and cyanobacteria regardless NPK levels. The tallest plant (77.67 cm) was due to combination of N_2-fixing bacteria and cyanobacteria with 75% NPK.

Table 5: Rice yield components as affected by biological inoculation under different NPK rates in alkaline-saline soil

Treatments	NPK%	Plant height (cm)	Branches number /plant	Panicles number /plant	Weight of 1000 - grain (g)	Straw yield (ton.ha^{-1})	Grain Yield (ton.ha^{-1})	Harvest index (%)
Mineral	0	53.33m	5.00i	0.67jk	9.67k	4.10ef	1.87i	31.28j
	50	65.00g-i	6.67e-h	3.00gh	23.76h	6.86d	4.07g	37.22hi
	75	63.00jk	7.33c-f	3.33fg	27.09g	7.83cd	4.77f	37.83hi
	100	70.67c	8.00b-d	3.00gh	29.79ef	8.10c	5.42f	40.07fg
Mean		63.00B	6.75BC	2.50C	22.58D	6.72C	4.03E	36.60E
Bacteria	0	65.33gh	5.67hi	1.67ij	11.00j	3.67f	2.15i	36.96i
	50	67.00ef	6.00g-i	3.67fg	29.35de	7.47cd	5.12f	40.66e-g
	75	72.50bc	7.00d-g	4.00e-g	30.09d-f	8.27bc	6.36e	43.48bc
	100	71.00c	6.67e-h	3.70fg	30.35de	9.68a	6.82c-e	41.32d-f
Mean		68.96A	6.33C	3.26B	25.20C	7.27B	5.11D	40.61D
Cyanobacteria	0	63.67i-k	6.00g-i	5.00de	15.17i	4.32ef	3.27h	43.08cd
	50	71.67c	7.00d-g	3.67fg	30.80c-e	7.10d	5.32f	42.82cd
	75	72.00bc	7.67c-e	7.00ab	31.05cd	8.18bc	6.93c-e	45.87a
	100	67.67de	7.33c-f	5.67cd	32.06ab	9.25a	7.32f	44.16a-c
Mean		68.75A	7.00B	5.33A	27.27B	7.21B	5.71C	43.98B
Bacteria + Cyanobacteria	0	64.33h-j	5.67hi	4.00e-g	15.40i	4.47ef	3.70gh	45.31ab
	50	67.67de	6.33f-h	4.33ef	31.10b-d	9.13ab	7.57a-c	45.31ab
	75	77.67a	9.00a	8.00a	32.07ab	9.87a	8.18a	45.34ab
	100	66.00fg	7.67c-e	6.67bc	32.32a	9.55a	7.87ab	45.16ab
Mean		68.92A	7.17AB	5.75A	27.72A	8.26A	6.73A	45.28A
Cyanobacterin	0	54.00m	5.67hi	0.33k	15.63i	4.73e	3.02h	38.92gh
	50	62.50k	8.33a-c	2.00hi	30.50c-e	9.27a	6.79de	42.28c-e
	75	57.00l	8.00b-d	6.67ab	30.36de	9.58a	7.28b-d	43.18c
	100	73.33b	8.67ab	6.00b-d	31.42a-c	9.67a	7.53b-d	43.80bc
Mean		61.71C	7.67A	3.75B	26.98B	8.31A	6.16B	42.05C
Mean of rate	0	60.13D	5.60C	2.33C	13.44D	4.26D	2.80C	39.11C
	50	66.77C	6.87B	3.33B	29.10C	7.97C	2.77B	41.66B
	75	68.43B	7.80A	5.80A	30.13B	8.75B	6.70A	43.15A
	100	69.40A	7.67A	5.01A	31.19A	9.25A	6.96A	42.90A

Regardless of NPK levels, the superior mean value of branches number/plant (7.67) was found with the commercial product (Cyanobacterin). While, NPK at 75 and 100% gave similar effect on branches number/plant (70.80 and 70.67). Same trend was found with panicles number/plant. On the other hand, cyanobacteria either alone or combined with bacteria achieved the highest mean values of panicles number/plant (5.33 and 5.75, respectively) apart from NPK levels. Weight of 1000 - grain was affected significantly by NPK levels which could be ranked in the following order: 100>75>50>0. The highest mean value of 1000 – grain weight (27.72 g) was achieved by N_2-fixing bacteria + cyanobacteria treatment. The highest mean values of both grain yield and harvest index were recorded due to N_2-fixing bacteria and cyanobacteria (6.73 ton. ha^{-1} and 45.28%, respectively). Inoculation with N_2-fixing bacteria+cyanobacteria and cyanobacterin gave the same statistically straw yields of 8.26 and 8.31 ton. ha^{-1}, respectively. The superior straw yield was found with 100%NPK. While, applying 75 and 100% NPK had the same significant highest effect on grain yield and harvest index. Cyanobacteria inoculation recorded the highly significant grain yield values of 7.57, 8.18 and 7.87 ton. ha^{-1} in response for 50, 75 and 100% NPK, respectively, these values were significantly higher than the mineral treatment of 100 % NPK (5.42 ton. ha-1) with relative increase of 39.67, 50.92 and 45.20%, respectively.

N, P, K and Na contents of rice

Total N, P, K and Na contents of rice (grain and straw) as affected by treatments and NPK levels under alkaline-saline soil condition are shown in Table (6). Inoculation enhanced the concentrations of N, P and K while, reducing Na in rice grain and straw comparing with the mineral treatments.

The total N and K% of grains reached their highest values (1.64 and 0.74%, respectively) by N_2-fixing bacteria+cyanobacteria treatment while, the treatments of N_2-fixing bacteria+cyanobacteria, cyanobacteria and

Cyanobacterin achieved the highest total P content of rice grain (0.17, 0.17 and 0.19%, respectively). As for straw, the highest total N (1.58%) and P (0.23%) were achieved by the co-inoculation of N_2-fixing bacteria+cyanobacteria and N_2-fixing bacteria treatments, respectively. However, the treatments had no significant effect on total K% of straw. 75% NPK resulted in the highest records of total N and P of grains in response to N_2-fixing bacteria+cyanobacteria and bacteria treatments (1.90 and 0.8%, respectively). While, NPK levels had the same significant effect on total K% of grains, same trend was found with Cyanobacterin in response to 75 and 100% NPK (Table 6).

The behavior of Na content was in contrast for total N, P and K contents in both rice straw and grains, since inoculation with biological treatments led to significant decrease in total Na content compared to mineral treatments which recorded the highest significant values of total sodium contents.

Table 6: Total nitrogen, phosphorus, potassium and sodium concentrations of rice plants as affected by inoculation under different NPK rates.

Treatments	NPK%	TN%		TP%		TK%		Na%	
		Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Mineral	0	0.76h	0.93f	0.14d	0.11c-f	0.27e	1.01e-g	0.71a	0.80a
	50	1.42ef	1.09c-f	0.14d	0.02h	0.43c-e	1.22b-d	0.12d	0.50bc
	75	1.50de	1.96a	0.05e	0.07e-h	0.54b-d	0.95f-i	0.71a	0.70ab
	100	1.55cd	1.01ef	0.13d	0.20b	0.56b-d	1.05d-f	0.12d	0.11d
Mean		1.31C	1.25B	0.12C	0.10C	0.45D	1.06AB	0.42A	0.53A
Bacteria	0	1.15g	1.04d-f	0.12d	0.05f-h	0.40de	0.83g-i	0.20d	0.80a
	50	1.69b	1.22b-e	0.13d	0.04gh	0.45c-e	0.88f-i	0.53ab	0.20d
	75	1.60b-d	1.26bc	0.21ab	0.80a	0.63a-c	0.81hi	0.12d	0.30cd
	100	1.58b-d	1.26bc	12d	0.03gh	0.52b-d	1.26bc	0.20d	0.20d
Mean		1.50B	1.20B	0.14B	0.23A	0.50CD	0.95B	0.26B	0.38B
Bacteria + Cyanobacteria	0	1.32f	1.37b	0.22a	0.15b-d	0.79a	0.97f-i	0.12d	0.11d
	50	1.63bc	1.91a	0.12d	0.17bc	0.68ab	1.64a	0.12d	0.14d
	75	1.90a	1.88a	0.11d	0.06e-h	0.79a	1.19b-e	0.20d	0.21d
	100	1.69b	1.15c-e	0.21ab	0.09d-g	0.68ab	0.79i	0.20d	0.18d
Mean		1.64A	1.58A	0.17AB	0.12BC	0.74A	1.15A	0.16C	0.16C
Cyanobacteria	0	1.20g	1.37b	0.16b-d	0.01h	0.81a	1.06d-f	0.30cd	0.16d
	50	1.42ef	1.19b-e	0.16b-d	0.02h	0.54b-d	1.08c-f	0.30cd	0.50bc
	75	1.60b-d	1.09c-f	0.25a	0.03gh	0.54b-d	0.92f-i	0.12d	0.11d
	100	1.67b	1.04d-f	0.11d	0.04gh	0.43c-e	1.22b-d	0.30cd	0.13d
Mean		1.47B	1.17B	0.17AB	0.03D	0.58BC	1.07A	0.26D	0.23C
Cyanobacterin	0	1.20g	1.28bc	0.24a	0.20b	0.44c-e	1.00e-h	0.20d	0.16d
	50	1.69b	1.20b-e	0.15cd	0.12c-e	0.55b-d	1.30b	0.20d	0.15d
	75	1.55cd	1.24b-d	0.20a-c	0.12c-e	0.70ab	1.22b-d	0.20d	0.22d
	100	1.66bc	1.20b-e	0.15cd	0.13c-e	0.72ab	0.79i	0.40bc	0.15d
Mean		1.52B	1.23B	0.19A	0.14B	0.60B	1.08A	0.25B	0.17C
Mean of rate	0	1.13C	1.20C	0.18A	0.10B	0.54A	0.97B	0.31B	0.41A
	50	1.57B	1.32B	0.14B	0.08B	0.55A	1.18A	0.14C	0.24B
	75	1.63A	1.49A	0.16AB	0.22A	0.52A	1.06B	0.40A	0.37A
	100	1.63A	1.13C	0.14B	0.10B	0.58A	1.02B	0.24B	0.15B

K/Na ratio in grains, straw and whole plant are illustrated in Figure (4). Obviously, all the biological treatments applied in this trial enhanced the absorption of nitrogen, phosphorus and potassium at the expense of sodium. Na^+ contents of rice revealed a reverse pattern to the other ion constituents. K^+/Na^+ ratio lower than one were achieved by mineral treatments at 0% NPK (0.85). The maximum (8.92) K^+/Na^+ ratio was found with N_2-fixing bacteria+cyanobacteria in response to 50% NPK followed by mineral treatments with100% NPK (7.0) and cyanobacteria with 75% NPK (6.35). As for K^+/Na^+ ratio for grain and straw, inoculation of N_2-fixing bacteria and cyanobacteria maximized K^+/Na^+ of grain and straw up to 6.58 and 11.71 corresponding to 0 and 75% NPK, respectively (Figure 5).

Chemical properties of soil

Data in Table (7) show the changes in some soil chemical properties in response to inoculation with different levels of NPK after 90 days from sowing under alkaline-saline soil condition.

Statistical analysis showed that all applied biological treatments increased significantly the soil total N, P and K% compared to the mineral treatments.

The highest significant values of total N, P and K% were 0.16, 0.40 and 0.37% due to the Cyanobacterin, N_2-fixing bacteria+cyanobacteria and bacteria, respectively. Also, their interactions with 75, 100 and 100% NPK resulted in highest significant values of N (0.19%), P (0.89%) and K (0.44%), respectively. Regarding the effect of NPK levels on soil content of total N, P and K, it was noticed that the highest records of N (0.14%) and P (0.33%) were due to 75% and 100%NPK, respectively. While, the highest contents of soil total K% (0.34 and 0.33%) were recorded by 50 and 100%NPK, respectively.

All treatments had insignificant effect on pH values while; EC records of all biological treatments were significantly lower than those of mineral treatments alone.

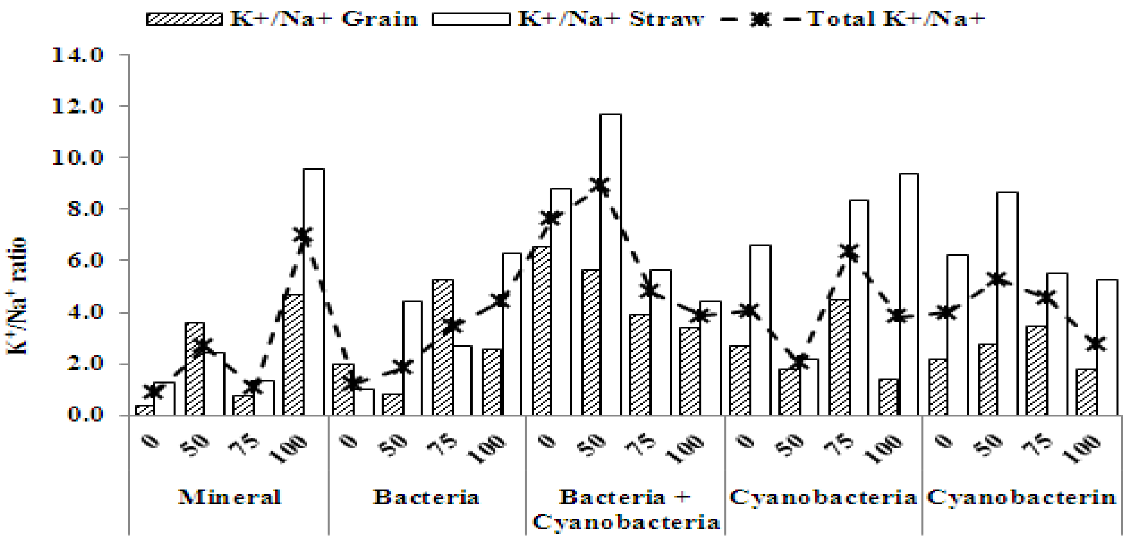

Fig. 5: Effect of inoculation and different NPK rates on K^+/Na^+ ratio of rice under alkaline-saline soil condition

Table 7: Effect of inoculation and different NPK rates on total NPK%, pH and electric conductivity (EC) of rice rhizosphere after 90 days from sowing under alkaline-saline soil conditions

Treatments	NPK%	TN %	TP %	TK %	pH (1:2.5)	EC (1:5) (dS.m^{-1})
Mineral	0	0.12g	0.13cd	0.31c-e	7.61a	3.70de
	50	0.14ef	0.15cd	0.28de	7.57a	7.30a
	75	0.13fg	0.21cd	0.36bc	7.73a	3.25fg
	100	0.16cd	0.20cd	0.31c-e	7.72a	3.40d-g
Mean		0.14C	0.17D	0.32B	7.66A	4.41A
Bacteria	0	0.15de	0.20cd	0.35bc	7.57a	3.10f-j
	50	0.12g	0.18cd	0.40ab	7.71a	5.20b
	75	0.18ab	0.17cd	0.27de	7.6a	3.20f-j
	100	0.15de	0.19cd	0.44a	7.66a	2.80ij
Mean		0.15AB	0.19C	0.37A	7.64A	3.58B
Bacteria + Cyanobacteria	0	0.16cd	0.27b	0.26e	7.61a	3.80d
	50	0.14ef	0.19cd	0.32c-e	7.63a	3.68de
	75	0.17bc	0.23cd	0.27de	7.73a	2.70jk
	100	0.10h	0.89a	0.31c-e	7.79a	3.00g-j
Mean		0.14BC	0.40A	0.29B	7.69A	3.30C
Cyanobacteria	0	0.12g	0.08d	0.20f	7.57a	3.25fg
	50	0.16cd	0.27b	0.36bc	7.38a	3.32e-g
	75	0.14ef	0.12cd	0.27de	7.70a	3.70de
	100	0.16cd	0.22cd	0.31c-e	7.65a	3.21f-h
Mean		0.15BC	0.35B	0.29B	7.58A	3.37C
Cyanobacterin	0	0.14ef	0.15cd	0.26e	7.67a	2.83h-j
	50	0.16cd	0.23cd	0.33cd	7.67a	4.50c
	75	0.19a	0.25c	0.30c-e	7.77a	3.50d-f
	100	0.14ef	0.16cd	0.27de	7.55a	2.40k
Mean		0.16A	0.20C	0.29B	7.67A	3.31C
Mean of rate	0	0.14B	0.17C	0.28B	7.61A	3.34B
	50	0.14B	0.20B	0.34A	7.59A	4.80A
	75	0.16A	0.20B	0.29B	7.71A	3.27B
	100	0.14B	0.33A	0.33A	7.67A	2.96C

Discussion

Soil–plant–microbe interactions are complex and known to influence the plant health and productivity. With increasing concern for food and environmental quality and dependence of modern agriculture on the application of chemical inputs, there exists a need to search for viable alternatives for sustainable agriculture (Piromyou *et al*., 2011).

In the present study, it was observed that seed-soaking in PGPR-bacteria suspension followed by two doses soil-drench and one dose foliar spray applications of all biological treatments all biological treatments N_2-

fixing bacteria, cyanobacteria and their combination at 30, 60, and 90 days after planting, respectively significantly promoted plant growth and yield productivity of rice under different levels of mineral NPK fertilizers in saline-alkaline soil. IRRI (1968), it was found that the rice gains tolerance during vegetative growth is usually correlated with the seedling age. Also, Del Valle and Babe (1947) studied the effect of salinity initiated 30, 60, and 90 days after planting and they found that salinity was most harmful at the youngest age. As the plants became older, their tolerance for salinity increased at 90 days and the plants were hardly affected by salt concentrations in the soil as high as 1.0%. Pearson and Bernstein (1959) reported that the salt tolerance of rice seedlings progressively increases from 1 to 3 to 6 weeks of age.

The enzymatic activity of the soil in this experiment was determined after 90 days of planting by assessing the activity of nitrogenase and dehydrogenase enzymes. Both exhibited significant increases due to the selected N_2-fixing N_2-fixing bacteria and cyanobacteria strains under different NPK levels and their highest records were achieved by the inoculation of cyanobacteria and bacteria with reduction of 25% of NPK.
These findings are supported by our previous investigations (Mostafa et al., 2011; 2013 and 2015) and the results obtained by Li *et al*. (2009); Rana *et al*. (2012). Higher activities of microbial enzymes were recorded in all the treatments that involved inoculation with cyanobacteria or bacterial strains or their combinations (Rana *et al*., 2015).

The plant rhizosphere is known to be the preferred ecological niche for different types of soil microorganisms due to availability of nutrients, which in turn, is intimately related to the successful production of crops and sustenance of soil fertility (Vessey 2003). Therefore, their inclusion in biofertilizer/biocontrol consortia can improve the nutrient mobilization and improve the nutrient status of soils and crops. Plant growth promoting rhizobacteria (PGPR) have become important partners in integrated nutrient management strategies in various crops, including cereals, spices, legumes and fodder plants (Zarrin *et al*. 2009; Gholami *et al*. 2009).
In the present study, it was observed that inoculation with selected isolates of PGPR/N_2-fixing bacteria and cyanobacteria significantly promoted plant growth parameters i.e., plant height, branches number/plant, panicles number/plant, weight of 1000-grain, straw yield, grain yield and harvest index (Table 5). Our earlier studies on a set of bacterial/cyanobacterial strains provided interesting results in terms of enhancing the plant growth and yields of sugar beet, wheat and rice crops in saline and calcareous soils besides enhancing soil fertility (Mostafa *et al.*, 2011; 2013; 2015). We indicated that all the treatments receiving cyanobacteria/bacteria co-inoculation have shown the best results in improving soil fertility, enhancing crop quality and productivity besides providing up to 50% of the recommended dose of mineral nitrogen. Therefore, the dose of N fertilizer applied was reduced in many crops. This prompted us to evaluate different combinations of the promising cyanobacteria with rhizobacteria in rice crop comparing with the well-established commercial biofertilizer Cyanobacteria under NPK limitation. The compatibility among the cyanobacterial and bacterial strains revealed interesting results. PGPR/N_2 rhizo- and cyano-bacteria had high potential to improve plant growth, biomass (grain and straw yields) and nutrients concentration especially when applied in combination. These results agreed with the results reported by Rana *et al*. (2015) who mentioned that the combined inoculation of *Anabaena oscillarioides*, *Brevundimonas diminuta* and *Ochrobactrum anthropi* significantly increased N, P, K, Fe, Zn, Cu, and Mn content in the grain and improved rice yield by 21.2% compared to the application of recommended dose of NPK fertilizers. Similar findings were reported by Prasanna *et al*. (2012).

Cyanobacteria benefit rice plants by producing growth-promoting substances followed by increasing the availability of P by excretion of organic acids was also exploited in the prevention of soil erosion process (Kumar and Rao, 2012).

Salinity entails ionic stress (mainly due to Na^+, Cl^- and SO^-_4), osmotic stress, and secondary stresses such as nutritional imbalances and oxidative stress for glycophytes (Zhu, 2002). Although Na^+ represents the major ion causing toxicity related to high salinity, some plant species are also sensitive to chloride, the major anion found in saline soils. High concentrations of Na^+ disturb osmotic balance and results in “physiological drought”, preventing plant water uptake. The importance of K+ transport systems and its cytosolic homeostasis under saline conditions have had an increasing interest and now it is becoming widely accepted that the ability of a plant to maintain a high cytosolic K+/Na+ ratio is vital in plant salt-tolerance mechanisms (Shabala and Cuin, 2007). Most crop plants are susceptible to salinity even when EC is <3.0 dS m^{-1} (Chinnusamy, 2005).
However, the total Na^+ content in rice crop was significantly decreased due to the application of biological treatments and therefore the K^+/Na^+ ratio increased from 7 (100% NPK, control) to 7.65 (N-fixing bacteria+cyanobacteria, 0%NPK) and 8.92 (N-fixing bacteria+cyanobacteria, 50%NPK). The significance of maintaining high cytosolic K^+/Na^+ ratio in salinity tolerance is becoming widely accepted as intracellular K+ and Na+ homeostasis bears importance for the activities of many cytosolic enzymes, for maintaining membrane potential and a suitable osmoticum for cell volume regulation. Cytosolic K^+ homeostasis could be maintained by preventing NaCl-induced K^+ leakage from the cell through the enhanced activity of H^+/ATPase. This could control voltage-dependent outward-rectifying K^+ channels and create the electron gradient necessary for secondary ion transport processes (Shabala and Cuin, 2007; Munns and Tester, 2008).

Regarding the role of PGPR in ameliorating salinity stress on rice crop, the production of plant growth promotors (indole acetic acid, gibberellic acid and abscissic acid) by rhizo- and cyano-bacteria has been taken as a main criterion in the selection of the microbial strains used in this study. This trial was carried out in soil EC, in water extract 1:5, ranging from 2.4 to 7.3 dS m^{-1} (Table 7) which is moderately saline according to Department of Natural Resources (DNR, 1997) and on the other hand, chosen the rice cv. Giza 178 rice as a tolerant cultivar of salinity up to 14% NaCl (Kandil *et al*., 2012). So, the effects of pre-sowing hormone treatment may be reflected well into the vegetative stage, when the basic infrastructure of plant physiology is laid down. Also, seed-soaking in the studied phytohormones sources, the bacterial culture solution, could stimulate seed germination faster than that in the control treatments via the exogenously applied PGBRs which strongly influenced the endogenous hormone levels of the seeds particularly GA3 and IAA which have been reported to increase germination percentage and seedling growth. The beneficial effects of microorganisms are also seen when seed-soaking was followed by soil-drench and foliar spray applications (Aly *et al*, 2008).

Our study illustrates that the cyanobacteria-bacteria combinations used in the present field experiment for enhancing the yield of rice may represent members of the beneficial microbiome for rice under salinity stress and NPK reduction. The increased mobilization of nutrients in the soil facilitates uptake by plants and enhances root growth and plant growth and yield.

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