Impact of Cyanobacteria Inoculation on Rice (*Orize sativa*) Yield Cultivated in Saline Soil

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Abstract: A Field experiment was conducted in the clayey soil of the farm at Sahl El-Hossynia Agric. Res. Station in EL-Sharkia - Governorate, Egypt. The institute farm is located at 31° 8' 12.461" N latitude and 31° 52' 15.496" E longitude. Rice crop (*Oryzae sativa*) was planted during the summer season of 2009 to study the effect of cyanobacterial inoculation (Cyano.) under different levels of mineral nitrogen fertilization under saline soil condition on rice yield components and some soil biological activity. Results indicated that, in general, applying cyanobacteria inoculation to rice plant enhanced the growth of rice plant resulting in significant increases in rice straw and grains yields compared to uninoculated treatments. The treatment of 75% N + Cyano recorded significantly the highest rice straw and grains yields compared to the other tested treatments (50, 75 and 100% N and 50% N + Cyano). Also the treatment of 75% N + Cyno. increased significantly the total contents of N, P & K in rice plants. On the contrary, inoculation with cyanobacteria decreased significantly Na^+ content of rice plants. Inoculation with cyanobacteria increased total proline content of rice plants compared to the other examined treatments. Inoculation with cyanobacteria had positively affected the soil fertility through enhancing rice rhizosphere soil biological activity in terms of total count bacteria, carbon dioxide evolution, dehydrogenase activity and nitrogenase activity. Generally, the inoculation with cyanobacteria along with reduced mineral nitrogen amount can help in growing rice under saline soil condition.

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Keyword: Saline Soil – rice crop – cyanobacteria inoculation.

1. Introduction

Rice is important cereal crop in Egypt. It is also a major cash crop for the farmers and handsome amount of foreign exchange is earned through its export. Thus, its role in strengthening the economy of the country may not be neglected. Moreover**,** salinity is one of the major problems in arid and semiarid regions and has a great impact on reducing soil fertility and productivity of crop plants **(Sairam *et al*., 2002)**.

The main problem at Sahl El-Hossynia soil is related to high salinity conditions. Soil degradation caused by salinizations and sodication were of universal concern. Saline >4 dSm^{-1} at 25 °C, or salt affected soil is a major environmental issue, as it limits plant growth and development, causing productivity losses **(Qadir *et al*., 2008)**. Salt affected soils are characterized by excessively high levels of water- soluble salts, including sodium chloride (NaCl), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$), among others. In the salinity case, Na Cl is a major salt contaminant in the soil. It has a small molecule size and when oxidized by water, producing sodium ions (Na+) and chloride ions (Cl^-), which are easily absorbed by the root cells of higher plants and transferred to the whole plant using xylem uploading channels, also cause ionic and osmotic stresses at the cellular level of higher plants, especially in susceptible species **(Rodriguez and Rubio, 2006).** Improving the fertility of the saline soil is an utmost necessary from the agriculture point of view. There are reports that some cyanobacteria can grow successfully on saline soil where most plants with the exception of halophytes fail to grow. The fertility of saline soil can be improved by using cyanobacteria. Cyanobacteria a group of gram negative photoautotrophic bacteria are the one of the most primitive component of our mother earth and it was the cyanobacteria in the Archaean and Proterozoic Eras (2.7 billion years ago) that were responsible for creating our oxygenic atmosphere through their photosynthetic activities. Cyanobacteria also may be the most important nitrogen-fixing agents in many agricultural soils **(Rodrigo and Eberto, 2007).** 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 in soil biomass after their death and decomposition, preventing weeds growth **(Alam *et al*., 2014).** Soil based cyanobacterial inoculum that are prepared from saline soils and that are adapted to areas ecological problem may effectively be used in N economy and improved fertility of these soils **(Aziz**

and Hashem, 2003). Cyanobacteria inoculation is a low-cost technology in rice field **(Roy *et al*., 2013).** The climate resistant spores of cyanobacteria are formed under unfavorable environment conditions that posses a special structure (heterocyst), which contains nitrogenase enzyme that playing a vital role in the atmospheric N_2- fixation. Heterocyst forming species are special in N_2- fixation and are able to convert N_2 to ammonia, nitrites and nitrates, which can be absorbed by plants for the construction of potential to reclaim high saline soil and application of cyanobacteria in rice field reduces the usage of chemical urea **(Kumar and Rao, 2012)**. **Sharma *et al*. (2012)** stated that cyanobacteria are distributed worldwide and improve the growth and development of the plants, with which they share the habitat, because they contribute to soil fertility in many ecosystems, produce various biologically active substances and have higher efficiency in biosorption of heavy metals (bioremediation). Cyanobacterial nitrogen fixation is closely linked with their growth and fixed nitrogen is not made available to the plants until the organisms die and their organic nitrogen is mineralized **(Roy *et al*., 2013)**. Cyanobacteria benefits in 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).** Cyanobacteria can be helpful in agriculture as they have the capability to fix atmospheric nitrogen to soil.

Salinity is among the worst scourges of agriculture. One effective mechanism to reduce damage from salt stress is the accumulation of high intracellular levels of osmoprotectant compounds. These compounds include proline, ectoine, betaines, polyols, and trehalose and have evolved in many different organisms including cyanobacteria. Salt stress is one of the most serious factors limiting the productivity of rice, the staple diet in many countries. Gibberellic acid has been reported to reduce Na Cl-induced growth inhibition in some plants including rice (**El-Shimy and Ismail, 2007).** They added that cyanobacterium, *Anabaena oryzae* was found to be able to grow in defined medium containing Na Cl up to 300mM. Intracellular free proline was found to increase rapidly in response to osmotic stress by NaCl.

Since salinity is a major limitation to crop yield so it would be meaningful to study whether cyanobacteria inoculation is useful in supporting rice plants in survival in the presence of salts in the soil.

The present study aims to evaluate the effect of cyanobacteria inoculation on the growth promotion of rice plants under saline soil condition. As well as, the effect of cyanobacteria inoculation on rice yield components and soil biological activity in terms of total count bacteria**,** dehydrogenase activity (DHA), nitrogenase activity (N-ase) and carbon dioxide evolution were determined.

2. Materials and Methods

A Field experiments was conducted in a silty clay soil at Sahl El-Hossynia Agric. Res. Station Farm in EL-Sharkia Governorate, Egypt during the summer season of 2009 to study the effect of cyanobacterial inoculation (Cyano) under different levels of mineral nitrogen fertilization under saline soil condition on rice yield components and some soil biological activity. The farm is located at 31° 8' 12.461" N Latitude and 31° 52' 15.496" E Longitude. The experimental soil was then divided into 15 plots (3m X 3.5m) representing 5 treatments with three replicates in split plot design. Some physical and chemical characteristics of the studied soil are presented in Table (1).

Table (1): Some physical and chemical properties of the investigated soil

Soil property	Value
Particle size distribution (%)	
Coarse sand	5.40
Fine sand	4.20
Silt	40.40
Clay	50.00
Textural class	Silty clay
Chemical properties	
pH (Saturated paste extract)	8.09
EC dS m^{-1} (Saturated paste extract)	10.09
Organic matter (%)	0.62
Soluble cations and anions (meq L^{-1})	
Ca^{2+}	26.30
Mg^{2+}	24.70
Na^{+}	59.80
K^{+}	01.50
CO_3^{2-}	00.00
HCO_3^{-}	04.27
Cl^{-}	75.80
SO_4^{2-}	32.23
SAR	11.84
ESP	13.91
Available macronutrient contents (mg kg^{-1})	
N	62.21
P	7.00
K	133.00

The experiment comprises five treatments as follows:

1- 100 % N (Recommended nitrogen rates).
2- 75 % N from the recommended nitrogen rates.
3- 50 % N from the recommended nitrogen rates.
4- 75 % N + Cyanobacteria inoculation (Cyano.).
5- 50% N + Cyano.

Dried flakes from the soil based cyanobacteria inoculum (**Vennkataraman, 1972**) were inoculated to rice plants 30 days after sowing at the rate of 7 kg ha^{-1}. Cyanobacteria inoculum (Cyano.) is composed of a mixture of *Anabaena fertilissima*, *Anabaena anomala* and *Nostoc muscorum*, which were previously isolated from salt affected soils at the mentioned location.

The treatments received phosphorus in the form of superphosphate (15 % P_2O_5) at a rate of 250 Kg ha^{-1} added basically before sowing during soil preparation. Nitrogen added in the form of urea (46 % N) at rates 360 Kg ha^{-1} (100% the recommended dose), 270 Kg ha^{-1} (75 % N) and 180 Kg ha^{-1} (50 % N) kg ha^{-1} according to suggested treatment in three split equal doses after 15, 30 and 60 days from sowing. While, potassium was not added because the applied soil contained enough potassium amount, that is quite enough to meet the requirement for rice cultivation. Irrigation was done to keep the rice plants in water logged condition till 15 days before harvesting.

Rice rhizosphere soil was sampled after 90 days from sowing to determine the soil biological activity in terms of total count bacteria **(Allen, 1959)**, dehydrogenase activity (DHA) **(Casida *et al*., 1964)**, nitrogenase activity (N-ase) **(Hardy *et al*., 1973)** and carbon dioxide evolution **(Pramer and Schmidt, 1964).** Meanwhile, full sun expanded rice leaves were also sampled from each plot to determine their proline content **(Gilmour *et al*., 2000)** and pigments contents in terms of Total chlorophyll, Chlorophyll a & b and carotenoids as described by **Burnison (1980).**

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, and then weighed up to a constant dry weight, ground and prepared for digestion according to **Page *et al*. (1982).** The digests were then exposed to the estimation of N, P, K and Na **(Cottenie *et al*., 1982).**

3. Results

The present study was undertaken to evaluate the inoculation with cyanobacteria inoculum which was previously isolated from the rhizosphere of rice plants cultivated in salt affected soil in terms of its ability to promote plant growth and enhance soil fertility in a rice crop grown under saline soil condition.

Plant analyses:

Effect of cyanobacteria inoculation combined with different nitrogen levels on rice yield components and total N, P, K and Na contents of rice crop under different nitrogen rates and saline soil condition:

Data in Tables (2 & 3) show the effect of cyanobacteria inoculation combined with different nitrogen rates under saline soil condition on rice yield components and total N, P, K and Na contents of rice crop under saline soil condition. Results in Table (2) indicated that inoculation of rice plants with cyanobacteria along with both reduced nitrogen rates of 75 and 50 % increased significantly both straw and grain yields compared to same nitrogen treatments alone without cyanobacteria inoculation. The corresponding values were 9.72 and 6.23 ton h^{-1} against 7.37 and 5.45 ton h^{-1} for straw yield in response for 75 and 50 % N with and without cyanobacteria inoculation, respectively. The relative values for grain yield were 7.84 and 6.29 ton h^{-1} and 6.51 and 4.62 ton h^{-1} for grain yield in response for 75 and 50 % N with and without cyanobacteria inoculation, respectively. However, the highest values of both straw and grain yields of rice were recorded due to the rice plants that received 75 % N + Cyano. treatment. These high values were 9.72 ton h^{-1} (Straw yield) and 7.84 ton h^{-1} (grain yield). Also these high values were significantly higher than the control treatment (100 % N), 75 and 50 % N and 50 % N + Cyano. treatments. On the other hand, the inoculation with cyanobacteria had not significantly affected 1000 - grain weight.

Due to total N, P and K contents of rice crop under saline soil condition shown in Table (3), results exhibited the same trend achieved for straw and grain yield of rice in response of cyanobacteria inoculation combined with different nitrogen levels. The treatment of 75 % N + Cyano. recorded the significantly the highest total N, P and K contents compared to the other tested treatments. The corresponding highest values were 31.20 (N), 3.95 (P) and 16.98 (K) kg ha^{-1} for rice straw and 47.28 (N), 4.18 (P) and 4.45 (K) kg ha^{-1} for rice grains.

While as for total sodium contents of both rice straw and grains, the behavior was in contrast for total N, P and K contents in both rice straw and grains, since inoculation with cyanobacteria led to significantly decrease the total Na content in both rice straw and grains compared to the other examined treatments. Also, the treatment of 75 % N + Cyano. scored the least significant values of total sodium contents of both rice straw and grains compared to the other tested treatments. These relative least Na values were 2.60 and 0.93 kg ha^{-1} for both straw and grains, respectively.

Effect of cyanobacteria inoculation combined with different nitrogen rates on pigments and proline contents of rice plants under different nitrogen rates and saline soil condition after 90 days from sowing:

Data in Table (4) show the effect of cyanobacteria inoculation combined with different nitrogen rates under saline soil condition on pigments and proline contents of rice plants under different nitrogen rates and saline soil condition. Results in Table (4) revealed that inoculation of rice plants with cyanobacteria in combination with of 75 and 50 % N rates increased total chlorophyll, carotene and proline contents of rice plants. Again the treatment of 75 % N + Cyano. recorded the highest amounts of total chlorophyll, carotene and proline contents of rice plants compared to the other tested treatments including the treatment of 50% N + Cyano.. The corresponding values for these high amounts were 14.22, 4.65 and 117.45 mg g^{-1} fresh weight due to total chlorophyll, carotene and proline contents of rice plants respectively. Also the treatment of treatment of 50 % N + Cyano. gave almost higher amounts of these parameters than those recorded by the nitrogen treatments without cyanobacteria inoculation including the recommended nitrogen treatment of 100 % N.

Table: (2): Rice yield component as affected by cyanobacteria inoculation under different nitrogen rates and saline soil condition

Treatments	Nitrogen Rates (%)	1000-grain yield (g)	Straw yield (ton ha^{-1})	Grain Yield (ton ha^{-1})
Without *cyano.	100 (Control)	25.20	9.09	7.47
	75	25.90	7.37	6.51
	50	24.96	5.45	4.62
With cyano.	75	26.96	9.72	7.84
	50	25.58	6.23	6.29
L. S. D.@ 5 %		**ns	0.28	0.31

***Cyano. = Cyanobacteria; **ns. = Not Significant**

Table: (3): Total nutrients content of rice plants as affected by cyanobacteria inoculation under different nitrogen rates and saline soil condition

Treatments	Nitrogen Rates (%)	N		P		K		Na	
		Kg ha^{-1}							
		Straw	Grains	Straw	Grains	Straw	Grains	Straw	Grains
Without *cyano.	100 (Control)	25.08	32.16	2.37	1.88	14.64	3.51	5.95	1.25
	75	17.64	26.88	2.00	1.50	12.02	2.25	4.55	1.43
	50	14.76	22.44	1.63	1.05	10.32	1.85	4.73	1.15
With cyano.	75	31.20	47.28	3.95	4.18	16.98	4.45	2.60	0.93
	50	29.28	41.40	3.35	3.13	14.58	3.50	2.88	0.82
L. S. D.@ 5 %		6.36	8.44	0.70	0.98	2.23	0.34	1.20	0.53

***Cyano. = Cyanobacteria**

Table: (4): Pigments and proline contents of rice plants as affected by cyanobacteria inoculation under different nitrogen rates and saline soil condition after 90 days

Treatments	Nitrogen Rates (%)	Total chlorophyll	Carotene	Proline
		mg g^{-1} fresh wt.		
Without cyano.	100 (Control)	10.12	2.78	83.12
	75	8.10	2.01	85.03
	50	6.13	1.91	70.80
With cyano.	75	14.22	4.65	117.45
	50	12.15	3.14	110.62

***Cyano. = Cyanobacteria**

Rice rhizosphere soil biological activity analyses:

Effect of cyanobacteria inoculation combined with different nitrogen rates on the soil rhizosphere biological activity after 90 days from sowing:

Data in Table (5) show the effect of cyanobacteria inoculation combined with different nitrogen rates on the soil rhizosphere biological activity in terms of total count bacteria, carbon dioxide evolution, dehydrogenase activity and nitrogenase activity after 80 days from sowing. Results revealed that cyanobacteria inoculation increased the soil biological activity compared to un-inoculated treatments. Results revealed that any of total count bacteria, carbon dioxide evolution, dehydrogenase activity (DHA) and nitrogenase activity (N-ase) recorded higher values in response to cyanobacteria inoculation combined with 50 or 75 % N than the treatments of 50, 75 and 100% N without cyanobacteria inoculation. These high values were 18 x 10^5 cfu g^{-1} dry soil day^{-1}(Total count bacteria), 185.36 mg CO_2 g^{-1} dry^{-1} (CO_2 evolution), 540.52 mg TPF g^{-1} dry soil day^{-1} (DHA) and 2145.18 µmole C_2H_2 g^{-1} dry soil day^{-1} (N-ase) for the treatment of 75% N + Cyano. followed by 15 x 10^5 cfu g^{-1} dry soil day^{-1}(Total count bacteria), 165.78 mg CO_2 g^{-1} dry^{-1} (CO_2 evolution), 475.85 mg TPF g^{-1} dry soil day^{-1} (DHA) and 1345.36 µmole C_2H_2 g^{-1} dry soil day^{-1} (N-ase) for the treatment of 50% N + Cyano.. However, the treatment of 100% N (control) gave the highest values of these parameters amongst the un-inoculated treatments. On the other respect the lowest values were due to the treatments of 50% N. The corresponding values were 9 x 10^5 cfu g^{-1} dry soil day^{-1} (Total count bacteria), 132.25 mg CO_2 g^{-1} dry^{-1} (CO_2 evolution), 280.28 mg TPF g^{-1} dry soil day^{-1} (DHA) and 1007.12 µmole C_2H_2 g^{-1} dry soil day^{-1} (N-ase).

Table: (5): Effect cyanobacteria inoculation on Soil biological activity under different nitrogen rates and saline soil condition

Treatments	Nitrogen Rates (%)	Total count bacteria x 10^5 **cfu g^{-1} dry soil day^{-1}	CO_2 Evolution mg CO_2 g^{-1} dry soil	***DHA mg ****TPF g^{-1} dry soil day^{-1}	*****N-ase µmole C_2H_2 g^{-1} dry soil day^{-1}
Without *cyano.	100 (Control)	13	150.12	420.16	1518.25
	75	12	144.45	360.36	1198.75
	50	9	132.25	280.28	1007.12
With cyano.	75	18	185.36	540.52	2145.18
	50	15	165.78	475.85	1345.36

***Cyano. = Cyanobacteria; **cfu = Colony formed unit^{-1}; ***DHA = Dehydrogenase activity; ****TPF = Tri-phenyl formazan; *****N-ase = Nitrogenase activity**

4. Discussion

Salinity is one of the most important abiotic stresses affecting plant growth and development, as well as productivity, especially in rice crops. Under saline conditions, the plant cell is confronted by osmotic stress, ionic toxicity and nutritional disorder **(Patade *et al*., 2008).** Several strategies have been proposed to alleviate the degree of cellular damage caused by abiotic stress and to improve crop salt tolerance. Among them, inoculation with the salt tolerance cyanobacteria strains **(Eletr *et al*., 2013).** In the present study, rice yield had significantly improved in the saline soil (EC 10.09 dSm^{-1}) and were able to withstand the salt stress in presence of cyanobacteria inoculation combined with 75% N. This improvement in rice crop due this treatment had surpassed that achieved by the use of 100% N the recommended nitrogen rate. In this context, **Eletr *et al*. (2013)** revealed that wheat and rice yields (straw and grain) along with total content of macronutrients (N, P and K) had increased significantly in response to cyanobacteria inoculation under saline soil condition as compared to control treatment 100% N under same condition. **Sharma *et al*. (2012)** indicated that cyanobacteria have the ability to fix the atmospheric nitrogen and possess some soil phosphate solubilizing species that solubilize the insoluble phosphate through excreting for organic acids that solve the common problem of P Chemical fixation in all types of soils **(Gaur, 1990)**, thus both N and P become available to plants sharing in the increase of both straw and grains yield. In contrast inoculation with cyanobacteria led to decrease the sodium total content of rice plants. **Thomas and Apte (1984)** explained the effects of sodium on the metabolism of cyanobacteria due to the N_2-fixing filamentous species. Na^+ is required for nitrogenase activity in *Anabaena torulosa, Anabaena* L-31 and *Plectonema boryanum.* The features of this requirement have been mainly studied in *Anabaena torulosa.* The need for Na^+ is specific and cannot be replaced by K^+, Li^+, Ca^{2+} or Mg^{2+}. This trend explains that cyanobacteria consume Na^+ ion from the saline soil habitat leading to reduce the total sodium content in rice plants. Due to the accumulation of both total chlorophyll and proline in rice plants, it was noticed that cyanobacteria inoculation led to increase their accumulation in rice plants. This was due to that cyanobacteria excrete exopolysacharide, growth promoting substances (indole acetic acid and Gibberellic acid), amino acid (proline) and consume sodium in their metabolic process to fix nitrogen, all these features led rice plant to withstand the adverse condition of salt stress **(Rodrigues *et al*., 2006)**.

In the current study, cyanobacteria inoculation enhanced the soil biological activity in terms of total count bacteria, carbon dioxide evolution, dehydrogenase activity (DHA) and nitrogenase activity (N-ase). In this concern**, Zulpa *et al*. (2008)** studied the effect of cyanobacteria products of *Tolypothrix tenuis* and *Nostoc muscorum* on the microbiological activity and the nutrient content of the soil. The biomass and extracellular products of both strains increased the soil microbial activity. *N. muscorum* and *T. tenuis* biomasses increased the soil oxidizable C (15%; 14%), total N (10%; 12%) and available P (22%; 32%), respectively. *T. tenuis* extracellular products increased by 28% oxidizable carbon and *N. muscorum* extracellular products increased by 15% the available phosphorus. These are caused the soil biological activity to be increased also because they are a continuously renewable carbon source. **Caire *et al.* (2000)** established that cyanobacteria can increase the soil enzymatic activity. **Aref and EL- Kassas (2006)** found that cyanobacteria inoculation to maize field enhanced significantly any of total count bacteria, cyanobacteria count, CO_2 evolution, dehydrogenase and nitrogenase activities compared to the control treatment received no inoculation. They explained that biofertilization with cyanobacteria led to increase microorganisms' community and in turn soil biological activity in soil through increasing the organic matter and microbial activity. **Prasanna *et al.* (2009)** revealed that under greenhouse conditions, rice pots inoculated with cyanobacterial isolated from the rhizosphere of diverse rice varieties had significantly enhanced soil microbial biomass carbon, total bacterial count, dehydrogenase activity and nitrogenase activity up to two-fold increase compared to the control treatment without cyanobacteria inoculation. They explained that cyanobacteria as diazotrophic microorganisms are the logical choice for supplying most of the soil's enzyme activity because of their large biomass, high metabolic activity and relatively larger amounts of extra cellular enzymes than plants or animals. The activity of enzymes can be used as an index of the fertility status of soil as they essentially integrate the effects of climate, crops and soil amendments and edaphic properties.

Conclusion

This study provides evidence regarding the significance of cyanobacterial inoculation to rice plants cultivated in salt affected soil, not only as agents help the rice plants to withstand the adverse effect of salt stress, but also as significant contributors to enhanced microbial activity, plant-growth promotion, reduction of approximately 25% of the mineral nitrogen required for rice production, thereby emphasizing their significance in protecting environment from pollution and the sustainable management of the rice in saline ecosystem. Further studies at different locations are being undertaken to reach the level of recommendation.

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