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DECREASING NITROGEN FERTILIZER BY USING SOME BIOFERTILIZERES FOR RICE CROP UNDER SALINE SOIL CONDITIONS

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Abstract
A field trail was conducted at South Port Said, Egypt, during the two successive summer seasons of 2013 and 2014 to study the physiological response of rice Giza 178 cultivar grown under saline soil conditions to decreasing the mineral nitrogen fertilizer from 145 to 72 or 107 kg N/ha with adding bio-fertilizers i.e. yeast extract, azolla, blue green alga and Rizobium rizobacter. Results indicated that plant height, leaf area index (LAI), crop growth rate (CGR), net assimilation rate (NAR), photosynthetic pigments, dehydrogenase activity (DHA), soluble sugars, antioxidant enzymes [catalase (CAT), polyphenol oxidase (PPO) and peroxidase (POD)], potassium content, panicle length, panicle weight, 1000- grain weight, grain and straw yields were increased when plants received 107 kg N/ha plus spraying yeast extract, followed by adding azolla, then blue green alga and Rizobium rizobacter without significant difference with the treatment of 145 kg N/ha. While, significant increases were recorded in proline, sodium contents, and sodium/potassium ratio in leaves when plants received 72 Kg N/ha with Rizobium rizobacter followed by blue green alga then azolla and finally yeast extract as compared with other treatments.

Key words: Bio-fertilizer, nitrogen fertilizer, rice, salt stress.

1. INTRODUCTION

Rice (*Oryza sativa* L.,) is considered one of the most important summer crops in North Delta and new reclaimed lands in Egypt. Rice is one of the most important cereal crops of the world, grown in a wide range of climatic zones, to nourish the mankind. Because of the importance of rice for supplying food, there is a demand for higher grain yield per unit area. It has been suggested that by 2025, global rice production must increase by more than 50% over the mid-1990 levels to meet that demand (Peng and Yang, 2003; Walker et al., 2008). Rice crop considered moderately sensetive to salt stress, and rice cultivars have a wide tolerant variation to salt stress (Khan and Abdallah, 2003; Kandil et al., 2010; Zayed et al., 2012). However, minimizing the environmental pollution risks, reducing production costs and increase grain yield and farmer's income, are considered the most important goal in rice production all over the world (Koutroubas and Natos, 2003).
Bio-fertilizers are formulations of beneficial microorganisms, which upon application can increase the availability of nutrients by their biological activity which help to improve the soil properties. Bio-fertilizer has been identified as an alternative way for increasing soil fertility and crop production in sustainable farming (Itelima et al., 2018). The main idea of bio-fertilizers is that secretion of various plant growth and health promoting substances (Pandya and Saraf, 2010). Bio-fertilizers are low cost, effective and renewable source of plant nutrients to supplement chemical

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fertilizers (Boraste et al., 2009; Shrivastava and Kumar 2015). However, mineral nitrogen fertilizers as they have a good effect on plant productivity, also have a pollutant effect on the environment and affects human and animal health (Wopereis et al., 2006).

Previous studies pronounced that yeast is the richest source of protein, as well as amino acids like lysine, tryptophan etc., contains minerals and elements, namely calcium, cobalt, iron etc. and the best sources of the B-complex vitamins such as B1, B2, B6 and B12. However, yeast extract is a valuable source of phytohormones especially, cytokinins, so yeast extract enhances cell division and enlargement and works as a readily available growth supplement for plants and subsequently improve plant production and crop yield (Amer, 2004; Ghoname et al., 2009).

Azolla is a free-floating, eco-friendly, fast-growing water fern having symbiotic, heterocyst-forming and N_2-fixing cyanobiont, *Anabaena azollae* in their leaf cavities, which fulfils the N requirement of the symbiotic system through N_2-fixation. Due to N_2-fixation, organic carbon and available phosphorus, it is mostly used in agriculture to improve the fertility of soil and to replace the use of chemical fertilizer. The flooded rice fields are an ideal environment for the growth of azolla and that is why it is also extensively used as a most suitable biofertilizer for the rice fields to improve the N-balance within the few weeks (Prasanna et al., 2008; Bhuvaneshwari and Singh, 2015). Azolla, besides able to supply nitrogen to the rice crop, it is beneficial in rice fields for bringing some of changes like decreasing soil pH and water temperature, inhibit NH_3 volatilization, prevent weeds and mosquito proliferation (Pabby et al., 2004).

Cyanobacteria or blue green algae (BGA, *Anabaena* sp.) as nitrogen-fixing microorganisms are a diverse group of prokaryotes that form complex associations with bacteria and green algae. Application of BGA in the rice paddy fields promotes rice growth and yield (Begum et al., 2011; Shridhar, 2012; Roy and Srivastava, 2013).

Plant growth promoting rhizobacteria (PGPR) application may be proper in developing strategies to facilitate plant growth indirectly by reducing plant pathogens, or directly by facilitating the uptake of nutrients from the environment, by influencing production of phytohormone (e.g. auxin, cytokinin and gibberellins) and siderophores and adverse salt stress conditions (Vessey, 2003; Kohler et al., 2006). PGPR strains, that IAA-overproducing, enhancing plant protection against salt stress due to high antioxidant enzyme activities (Bianco et al., 2009). However, PGPR can increase quantity and quality of crop yield, efficiency of chemical fertilizers and tolerance to abiotic stresses as one of the suitable ways to conserve environment (Baset and Shamsuddin, 2010; Moussa and Youssef, 2012).

Therefore, this study was designed to evaluate the influences of different bio-fertilizers with decreasing the nitrogen fertilizer on growth and yield of rice plants and the possibility to ameliorate salt stress.

2. MATERIALS AND METHODS

The present work was carried out at South Port Said Gov., Egypt (31˚ 08’ 42˝ N 32˚ 17’ 36˝ E) during two successive summer seasons of 2013 and 2014 to study the physiological response of rice plants grown in saline soil to some biofertilizers and decreasing nitrogen fertilizer for growth, dehydrogenase activity, soluble sugars, antioxidant enzymes, yield, yield components, photosynthetic pigments and proline contents as well as Na^+/K^+ ratio.

The experiment was laid out in randomized complete block design (RCBD) with four replicates and each plot was 10.5 m^2.

The soil physical and chemical properties of the experimental site in seasons 2013 and 2014 are presented in the Table 1.

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Table 1. The soil physical and chemical properties of the experimental site in seasons 2013 and 2014.

Season	EC (dSm^{-1})	pH (1:2.5)	Texture	Cations (ml equevlant/L)				Anions (ml equevlant/L)			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{-}
2013	7.57	8.05	clay	9.84	11.64	62.24	2.34	-	1.01	65.9	19.15
2014	7.21	7.98	clay	10.05	11.56	60.07	2.45	-	1.03	64.21	18.89

Rice grains cv. Giza 178 were sown on 6th and 11th June in the first and second seasons, respectively. 36 kg P_2O_5/ha was added as calcium super phosphate (15.5% P_2O_5) to soil preparation. Nitrogen treatments in the form of urea (46% N) was added in three doses at 20, 35 and 50 days after sowing (DAS).

The treatments were as follows:145 kg N/ha (Control), 72 kg N/ha + Yeast extract, 72 kg N/ha + Azolla, 72 kg N/ha + Blue Green Alga, 72 kg N/ha + *Rizobium rizobacter*, 107 kg N/ha + Yeast extract, 107 kg N/ha + Azola, 107 kg N/ha + Blue Green Alga, 107 kg N/ha + *Rizobium rizobacter*.

Where: - Yeast extract was sprayed as 10 g/L at 25 and 40 DAS,
- Azola was added fresh as 1.2ton/ha at 25 DAS,
- Blue Green Alga (BGA) was sprayed as 4liter/ha at 25 and 40 DAS,
- *Rizobiumrizobacter* sprayed as 25liter/ha at 25 and 40 DAS.

The yeast, azola, cyanobacteria and Rizobium rizobacter were supplied by Agricultural Microbiology Dept., Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

Other, cultural practices were applied according to the methods being adopted for growing rice crop in the locality.

To calculate growth traits, five plants were randomly taken from each plot at 65, 80 and 95 days after sowing (DAS). Plants were dried at 70 °C in oven to a constant weight. According to Hunt (1990) formulas, the following traits were determined:

- Leaf area index (LAI) = leaf area per plant/ground area occupied by plant
- Net assimilation rate, in g/m^2/day (NAR) = $(W_2-W_1)(\log_e A_2-\log_e A_1)/(A_2-A_1)(t_2-t_1)$.
- Crop growth rate, in g/m^2/day (CGR) = $(W_2-W_1)/(t_2-t_1)$.

  Where:
  - A_2-A_1= differences in leaf area between two samples (cm^2).
  - W_2-W_1= differences in dry matter accumulation of whole plants between two samples in (g).
  - t_2-t_1= Number of days between two successive samples (day).
  - $\log_e$ = Natural logarithm.

- Soil microbial activity was evaluated in rice soil rhizosphere samples at 65, 80 and 95 DAS, in terms of dehydrogenase activity [µg triphenylformazan (TPF) g dry $soil^{-1}day^{-1}$], which determined according to Skujins (1976).

At 80 days after sowing (DAS), the following traits were determined:

- Photosynthetic pigments (chl a, chl b and carotenoides) in mg/g fresh weight, according to Metzener et al. (1965).
- Soluble sugars using modifications of the procedures by Yemm and Willis (1954).
- Catalase, polyphenol oxidase and peroxidase activities according to Kar and Mishra (1976).

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- Leaf proline concentration, in µg/g fresh weight, according to Bates et al. (1973).
- Potassium and sodium content in rice plant asmmole/kg dry weight, according to Allen et al. (1974).

Harvesting took place at 3/10/2013 and 9/10/2014 in the first and second seasons, respectively. At harvest time, plant height (cm), panicle length (cm), panicle weight (g), 1000-grain weight (g), grain and straw yields (t/ha) were determined.

Data of the two seasons were statistically analyzed according to Steel and Torrie (1980).

3. RESULTS AND DISCUSSIONS

Growth and growth analysis:

Plant height, crop growth rate and net assimilation rate

Data in Table 2 indicate that plant height, crop growth rate and net assimilation rate decreased with decreasing nitrogen dose to 72 kgN/haplus biofertilizers compared to 145 kg N/ha. When rice plants received 107 kg N/ha and biofertilizers insignificant differences were achieved with control treatment. In general terms, those behaviors of rice plant height, CGR and NAR were caused by the influence of nitrogen content in the medium, which could be supplied through different ways and means, as reported earlier (Bhuvaneshwari and Kumar 2013; Bhuvaneshwari and Singh, 2015). Therefore, application of bio-fertilizers with mineral nitrogen not only serve to provide plants with significant amount of nitrogen, but also enables a better use of nitrogen added by mineral fertilization and thereby, a higher dry mass production. Similar results were recorded with application of biofertilizers on rice byPimratch et al. (2015); on faba bean by El-Shafey et al. (2016) and on maize by Abido et al. (2017).

Table 2: Plant height, crop growth rate (CGR) and net assimilation rate (NAR) of rice plants as affected by bio-fertilization in saline soil for two summer seasons of 2013 and 2014.

Treatments	Plant height (cm)		CGR (g/m^2/day) (65-80day)		CGR (g/m^2/day) (80- 95 day)		NAR (g/m^2/day) (65-80day)		NAR (g/m^2/day) (80- 95 day)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
145kg N/ha (Control)	90.50	91.00	26.54	26.72	29.90	30.08	11.34	11.36	10.79	10.81
72 kg N/ha + yeast extract	82.75	83.00	21.76	21.89	24.33	24.43	8.31	8.32	7.68	7.70
72 kg N/ha + Azola	81.50	82.00	21.34	21.60	24.19	24.29	8.28	8.29	7.64	7.66
72 kg N/ha + BGA	82.25	82.50	21.27	21.46	23.98	24.02	8.20	8.21	7.60	7.61
72 kg N/ha + Rhizobium	82.00	82.25	20.62	20.79	23.23	23.58	8.17	8.18	7.58	7.59
107 kg N/ha + yeast extract	90.25	90.50	26.23	26.40	29.82	29.92	11.32	11.33	10.77	10.79
107 kg N/ha + Azola	90.00	90.00	26.07	26.21	29.62	29.77	11.30	11.31	10.74	10.75
107 kg N/ha + BGA	89.50	89.75	25.88	25.98	29.41	29.50	11.28	11.29	10.71	10.72
107 kg N/ha + Rhizobium	89.00	89.50	25.31	25.63	29.20	29.32	11.25	11.26	10.68	10.70
LSD 0.05	**1.46**	**1.44**	**0.3**	**0.2**	**0.19**	**0.17**	**0.16**	**0.17**	**0.17**	**0.18**

Where:BGA= blue green alga.

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Leaf area index (LAI)

The effect of decreasing nitrogen dose with application some bio-fertilizers on rice plant leaf area index is presented in Fig. (1). Rice plants received 72 kg N/ha plus biofertilizers were decreased in LAI at 65, 80 and 95 DAS as compared with plants received 145 kg N/ha (control) in the two seasons. While, plants received 107 kg N/ha plus bio-fertilizers showed insignificant increase in leaf area index with plants that received 145 kg N/ha. The stimulatory effect of yeast extract, azolla, BGA and *Rizobium rizobacter* can be attributed to the increased contents of different nutrients as well as the high concentration of protein, vitamin B and natural plant growth regulators such as cytokinins (Fathy and Farid, 1996). The physiological roles of vitamins and amino acids in bio-fertilizers can increase the metabolic processes and levels of endogenous hormones which in turn encourage vegetative growth of rice plant (Amprayn et al. 2012). Those results are in harmony with that obtained by Radwan et al., (2008); Abbas et al., (2015); Bhuvaneshwari and Singh (2015).

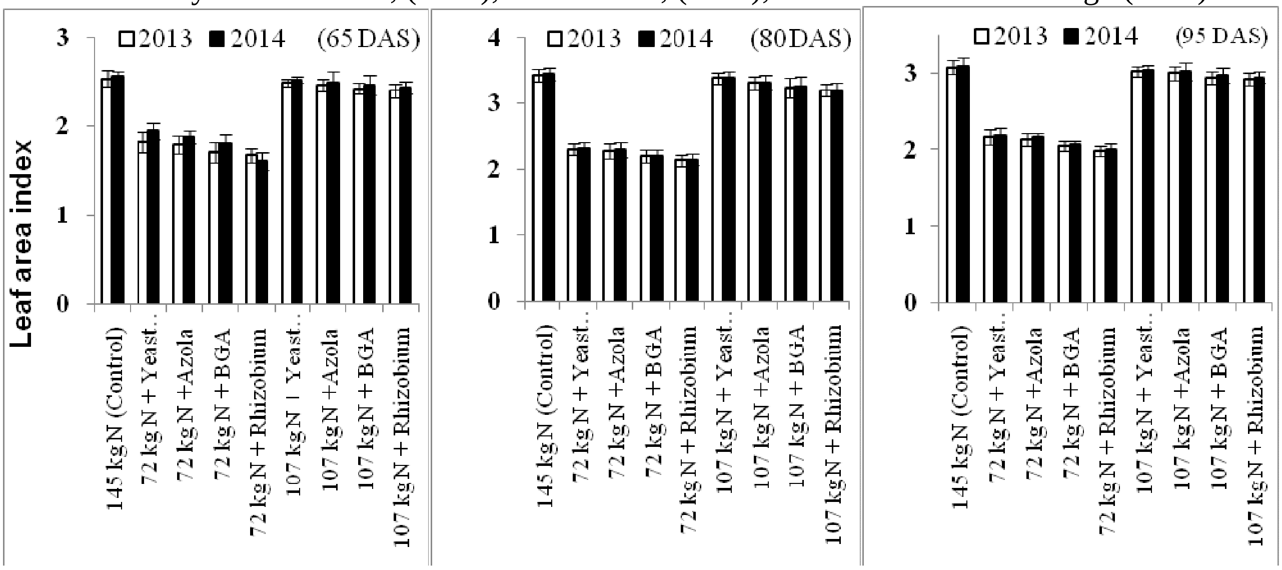

Fig. 1. Effect of different nitrogen doses and bio-fertilizers on rice leaf area index in two summer seasons

Dehydrogenase activity

Dehydrogenase activity (DHA) was determined as a criterion of respiration rate and total microbial activity in the rhizosphere of rice plant under different investigated treatments. Figure (2) illustrated that application of bio-fertilizers gave higher values of DHA, where the highest values of DHA were achieved when plants received 107 kg N/ha plus azola, BGA or *R. rizobacter* in the two seasons as compared to 145 kg N/ha treatment without biofertilizers. Data also revealed that nitrogen had a major impact in DHA reaching the highest activity at 145 kg N/ha. In addition, dehydrogenase activity in various treatments were higher after 95 DAS. This may due to the difference in multiplication rate of different soil microorganisms which usually be maximum during boating and grain filling stages. Such differences could be attributed to the qualitative and quantitative changes in the nature of root exudates during different growth stages. These results are in harmony with Abbas et al. (2015); El-Gamal and El Hawary (2016).

Photosynthetic pigments

Chlorophyll a, chl b and carotenoids in rice plants treated by some bio-fertilizers with reducing nitrogen dose under salt affected soil are presented in Fig. 3. Rice plants received 72 kg N/ha plus

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yeast extract, azolla, BGA or *R. rizobacter* showed significant decrease in photosynthetic pigments. While, application of 107 kg N/ha plus biofertilizers had insignificant increase in the photosynthetic pigments as compared to plants received 145 kg N/ha in the two seasons. In this regard, the negative effects of abiotic stress on photosynthetic pigments may be due to the inhibition of chlorophyll biosynthesis or increase of its degradation by chlorophyllase enzyme, which is more active under stresses.Regulation of metabolic and developmental processes by photosynthetic pigments often depends on nitrogen supply, therefore, the assay of rice photosynthetic pigment contents may serve to optimize rice fertilization technologies. However, the potential effect of bio-fertilizers is providing the nutrient elements needed by plants besides some other beneficial compounds that help plants to withstand abiotic stress conditions. Results of the present study are in agreement with that reported by Al-Erwy et al. (2016).

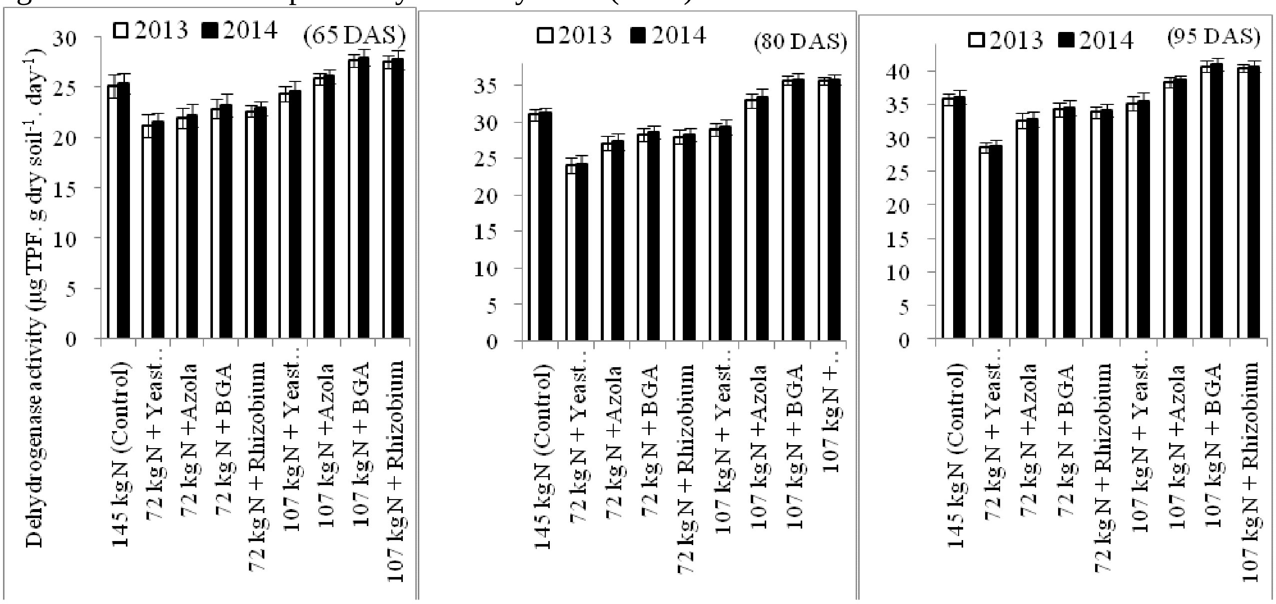

Fig. 2. Effects of different nitrogen doses and bio-fertilizers on microbial activities expressed as soil dehydrogenase activity at growth periods in two summer seasons

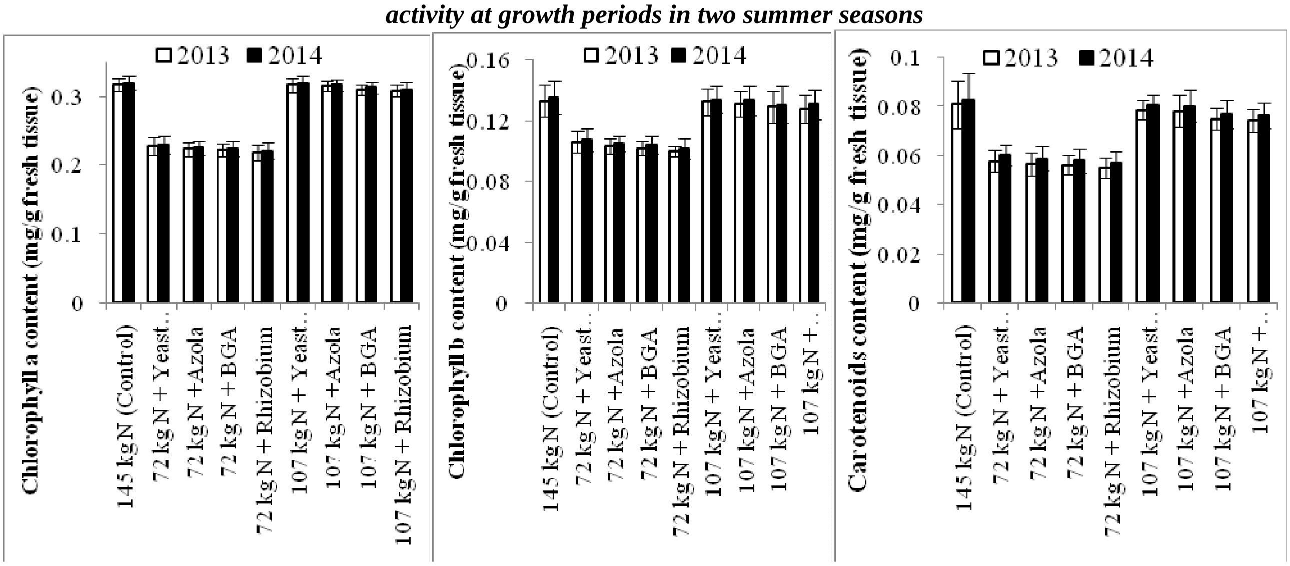

Fig. 3. Effects of different nitrogen doses and bio-fertilizers on photosynthetic pigments (chlorophyll a, b and carotenoids) in leaves of rice plants in two summer seasons

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Soluble sugars and antioxidant enzymes

The soluble sugars content and antioxidant enzymes; (catalase (CAT), polyphenol oxidase (PPO) and peroxidase (POD)) activities in leaves of rice plants were presented in Fig. 4. Rice plants treated with 72 kg N/ha plus bio-fertilizers had the lowest values of soluble sugars, CAT, PPO and POD in the two seasons as compared with plants treated with 145 kg N/haonly as control. While, rice plants received 107 kg N/ha and biofertilizers had insignificant difference in soluble sugars, CAT, PPO and POD compared to control. It seems that when plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and singlet oxygen (O_2) are produced. These ROS may initiate destructive oxidative processes such as lipid peroxidation, chlorophyll degredation, protein oxidation and damage to nucleic acids. Antioxidant enzymes' activity was increased when plants were exposed to stress. Improvement of plant growth under stressful environments could be due to the significant role of the enzymatic antioxidant system in alleviation of oxidative impact, and this mechanism has been generally pointed out in wheat (Mandhania et al., 2006). Ma et al. (2011) reported that bio fertilizers can improve plant tolerance to salinity, drought and flooding, and enable plants to survive under unfavorable environmental conditions. Results of the present study are in agreement with that reported by Babaei et al. (2017).

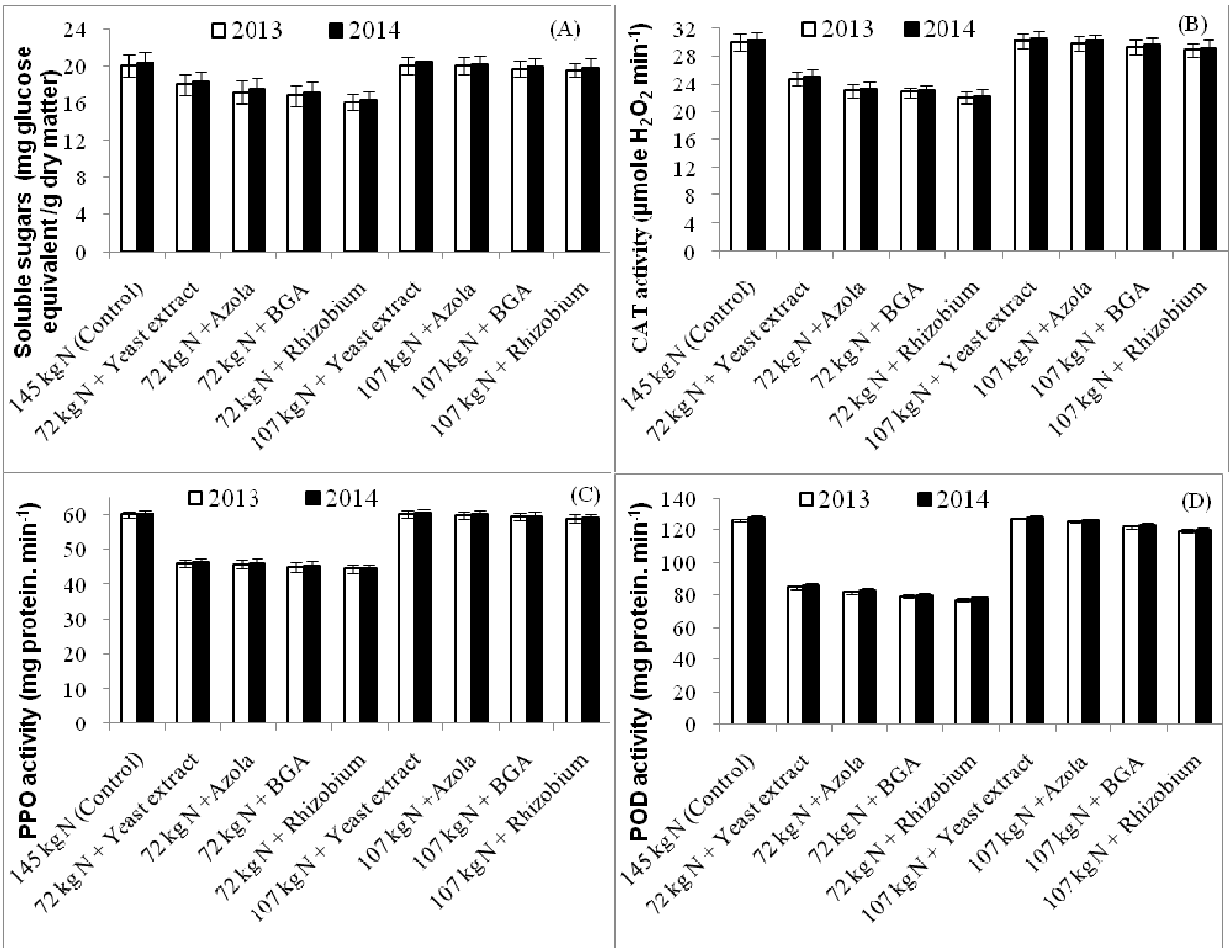

Fig. 4. Effects of different nitrogen doses and bio-fertilizers on soluble sugars content (A), catalase activity (B), polyphenol oxidase activity (C) and peroxidase activity (D) in leaves of rice plants in two summer seasons

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Proline sodium, potassium concentrations and Na^+/K^+ ratio

Data of proline, sodium and potassium contents as well as Na^+/K^+ ratio of rice leaves are shown in Table 3. The highest values of proline, sodium content and Na^+/K^+ ratio were found from application of 72 kg N/ha with biofertilizers especially with *R. rizobacter* in the two seasons as compared with control plants received 145 kg N/ha. While, plants received 107 kg N/ha plus biofertilizers had decreases in proline, sodium content and Na^+/K^+ ratio and insignificant with plants received 145 kg N/ha. On the other hand, the highest value of K^+ content was obtained in rice plants treated with 145 kg N/ha and plants received 107 kg N/ha plus bio-fertilizers application. While, the lowest values were gained from application of 72 kg N/ha plus bio-fertilizers especially *R. rizobacter* in the two seasons. The bio-fertilizers application could improved the biotic stress effect and subsequently proline content decreased.

Table 3. Proline, sodium (Na^+), potassium (K^+) concentrations and Na^+/K^+ ratio of rice leaves at 80 DAS as affected by bio-fertilization in saline soil for two summer seasons of 2013 and 2014

Treatments	Proline (µg /g FW)		Na^+ Leaf content (mmole/kg)		K^+ Leaf content (mmole/kg)		Na^+/K^+ ratio	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
145kg N/ha (Control)	93	91	349	347	587	589	0.59	0.59
72 kg N/ha + yeast extract	119	117	541	539	436	439	1.24	1.23
72 kg N/ha + Azola	122	120	543	541	431	434	1.26	1.25
72 kg N/ha + BGA	123	121	545	543	424	427	1.29	1.27
72 kg N/ha + Rhizobium	128	126	549	546	420	423	1.31	1.29
107 kg N/ha + yeast extract	92	90	353	351	581	584	0.61	0.60
107 kg N/ha + Azola	96	94	355	353	578	581	0.61	0.61
107 kg N/ha + BGA	98	96	358	356	577	580	0.62	0.61
107 kg N/ha + Rhizobium	100	98	360	358	575	578	0.63	0.62
LSD 0.05	**13**	**13.3**	**17.9**	**16.8**	**14.42**	**16.6**	**0.06**	**0.07**

Yield and yield components

Data in Table 4 shows the panicle length, panicle weight, 1000-grain weight, grain yield, straw yield as well as harvest index of rice plants. Plants treated with 72 kg N/ha plus bio-fertilizers (yeast extract, azolla, BGA and *R. rizobacter*) showed significant decrease in yield as compared with plants received 145 kg N/ha. However, application of 107 kg N/ha plus bio-fertilizers showed an increase in yield with insignificant difference as compared to control. This increment of grain yield may be due to the positive effect of yeast and other bio-fertilizers which play an important role in assimilation processes of rice plants (Abbas et al. 2015; Bhuvaneshwari and Singh, 2015). Moreover, yeast and other biofertilizers were suggested to participate in a beneficial role during vegetative and reproductive growths through improving flower formation and yield in some plants

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(Lonhienne et al., 2014). Biofertilizers are also capable of producing growth promoting substances, as auxins and hormones (IAA and GA3), amino acids and vitamins in their cultures which reflected on enhancing yield and reduce nitrogen application fertilizer (El-Shahat et al., 2014; Hassan and Bano 2015; Mohamed and Almaroai, 2016; Mohamed et al., 2018).

Table 4. Yield and yield components of rice plants as affected by bio- fertilization in saline soil for two summer seasons of 2013 and 2014

Treatments	Panicle length (cm)		Panicle weight (g)		1000-grain weight (g)		Grain yield (t/ha)		Straw yield (t/ha)		Harvest index	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
145kg N/ha (Control)	22.25	22.50	3.81	3.83	24.73	24.90	6.88	7.09	10.23	10.63	40.23	39.99
72 kg N/ha + yeast extract	18.75	18.88	2.81	2.84	19.51	19.67	5.02	5.08	8.04	8.41	38.41	37.66
72 kg N/ha + Azola	18.38	18.50	2.77	2.79	19.35	19.50	4.90	4.98	7.83	8.05	38.49	38.23
72 kg N/ha + BGA	18.00	18.13	2.74	2.77	19.11	19.23	4.74	4.81	7.77	7.77	37.91	38.23
72 kg N/ha + Rhizobium	17.75	17.88	2.72	2.74	18.97	19.05	4.57	4.65	7.78	7.78	37.00	37.41
107 kg N/ha + yeast extract	22.25	22.63	3.80	3.85	24.69	24.92	6.92	7.13	10.22	10.51	40.37	40.44
107 kg N/ha + Azola	22.00	22.25	3.77	3.79	24.57	24.67	6.59	6.71	10.19	10.19	39.26	39.69
107 kg N/ha + BGA	21.50	21.75	3.75	3.77	24.08	24.16	6.36	6.48	10.08	10.08	38.68	39.12
107 kg N/ha + Rhizobium	21.13	21.25	3.73	3.75	23.82	23.95	6.19	6.32	9.96	9.96	38.32	38.82
LSD 0.05	**0.76**	**0.74**	**0.18**	**0.18**	**0.23**	**0.25**	**0.39**	**0.41**	**0.43**	**0.39**	**1.50**	**1.30**

4. CONCLUSIONS

In view of the obtained results, it can be conclude that application of 107 kg N/ha plus biofertilizers in forms of yeast extract, azolla, cyanobacteria or *R. rizobacter* in rice fields can reduce 25% of nitrogen fertilizer requirements and could improve the salinity stress in salt affected soils at East Delta region of Egypt.

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