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Inoculation with Single, Dual or Consortia of *Rhizobium leguminosarum* bv. *trifolli* , *Pseudomonas stutzeri* and *Anabaena* sp. and their effect on Yield Components of Rice Plant

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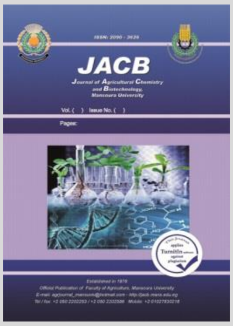

ABSTRACT

A set of six rice field inoculation trials was selected at two governorates in Egypt located at the Nile delta. These study was conducted to investigate the response yield components of three Egyptian rice varieties (Sakha 101, Giza 177 and Giza 178) to inoculation with three bacterial strains (*Rhizobium leguminosarum* bv. *trifolli* (RH) strain E11, *Anabaena* sp. strain ERC102 and *Pseudomonas stutzeri* strain ERP1) and their combination in the presence of three levels of nitrogen (48,96 and 144 kg N/ha.) . The results were obtained from means of yield parameters calculated all over the six experiments . The results show that increasing with inoculation in paddy yield , straw yield(15.26 ton/ha.), harvest index rang is within 33.13% and agronomic N-use efficiency compared with non- inoculated counterpart . The results of the agronomic N-use efficiency in the six experiments indicated that biofertilization can benefit grain production and then reduced agronomic N-use efficiency with increasing fertilizer-N doses. Finaly , the results concluded that the inoculation with bio-agents can reduce the need for additional fertilizer-N application to achieve and consequently higher grain yield.

Keywords : *Rhizobium leguminosarum ; Anabaena* sp. ; *Pseudomonas stutzeri ;* Rice plant ; Inorganic nitrogen fertilizer .

INTRODUCTION

Several species of rhizobacteria that flourish in plant rhizosphere may grow in, on, or around plant tissues, and investigation of their modes of action are stimulate plant growth . These rhizobacteria are known as PGPR (Plant Growth Promoting Rhizobacteria) Chi *et al* ,2005. The search for PGPR efforts are made as biofertilizers which must be tested for their efficiencies in establishment of effective plant microbe interrelationships that can benefit plant growth and crop performances while do not have pathogenic effects to plants, humans, animals or adverse impact on the environment. Inoculation with those rhizobacteria, when tested and found effective, can assist plant growth stimulation by providing fixed nitrogen or secretion of growth hormones that induce better plant growth, nutrient uptake and increased tolerance towards drought and/or salinity stress. Costs of fertilization with nitrogen and phosphorous chemical fertilizers is so commonly an economic limiting factor in crop production in most of the world, which can be mitigated by biofertilization (Anandaraj and Delapierre, 2010).

Several cyanobacterial strains belonging to *Nostoc* and *Anabaena* of the rhizosphere isolates of cyanobacteria, which were found efficient in nitrogen fixation (Prasanna *et al*., 2009 and Afify *et al*., 2018).

Endophytic bacteria, as defined by Hallmann *et al.*, (1997), who considered that the plant growth promotion and increased resistance against plant pathogens and parasites. Application of inoculation with some PGPR were enhancement the growth dynamics and seed yield of plants (Omara *et al.*, 2017) . In this connection, the strategy followed to achieve this goal consisted of consecutive step that can be summarized in study the possible rice growth and performance when used inocula preparations individually or in consortia in large-scale multilocation rice field experiments in the Nile delta.

MATERIALS AND METHODS

Source of Rhizobial strain

Rhizobium leguminosarum bv. *trifolii* (strain E11) was used in this study. It was found a microsymbiont N_2-fixer with berseem clover and an endocolonizer in rice roots. The results of those tests were published in Yanni *et al*. (1997and 2001) .

Source of *Anabaena* sp. and *Pseudomonas stutzeri*

The two isolates were obtained at “Blue-green Algae Research lab. Sakha Agricultural research Station, Kafr El-Sheikh” Egypt.

Media used

1- Semi solid malate N - free medium for isolation and growth of *Pseudomonas* (Dobereinar and Day 1976).
2- Modified Watanabe medium, with incubation under photosynthetic illumination of 14 hrs light and 10 hrs dark for 10 days under temperatures of 24 to 30 ^{o}C was used for isolation and growth of the cyanobacterium strain(El – Nawawy *et al.*, 1958).

Field experiments

Six rice field inoculation experiments was started at two governorates located at the central region of the Nile

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delta, two experiments at Kafr El-Sheikh and four at Algharbia , Egypt. The field soil textures heavy clayey to clay-loamy, originated over thousands of years from the annual Nile flood sediments. Soil mechanical parameters were measured (Jackson 1967) and tabulated (Table 1).

Preparation of field soil

The field soil was plowed three times in perpendicular directions. Nile tributary water was then introduced to a height of five to ten cm followed by further soil leveling under water.

Establishment of rice nursery

Rice nurseries (~1/12th of each test field area) were established adjacent to the experimental field approximately 30-35 days before manual transplantation of the young seedlings to the permanent field. Dried rice seeds were soaked in cloth bags for two days, then left for germination for one to two days depending on the rice variety. The emerged seedlings were uniformly broadcasted to a density of 115 g (dry seed basis) / m^2 during a wind calm period on the levee-enclosed nursery area covered with water to the depth of 3 cm. The herbicide Benzthiocarb "Saturn 50%" [S-(4-chlorobenzyl)-N, N diethylthiocarbamate] was applied at 4.8 L/ha four days later. Irrigation was then stopped for 3-4 days, followed by 3 to 4 cycles of irrigation and surface drainage until the seedlings well established. Irrigation water was increased concomitantly to head of standing water needed for the seedling growth stage.

Table 1 . Physico – chemical properties of soil used during this study .

Soil properties		Locations					
		Kafr El-Sheikh		Algharbia			
		Metobas	Fowa	Alsanta	Tanta		Koutor
		Kabel	Ashour	Algafaria	Alawkaf	Berma	Sagen
Chemical properties							
pH		8.04	7.78	7.99	8.08	8.21	8.15
EC (dS/m)		0. 48	0. 81	0. 33	0. 58	0. 28	0. 48
Soluble cations (meq/ 100 g)	Ca^{++}	1.60	2.70	1.20	1.20	0.90	1.00
	Mg^{++}	2.20	4.40	2.10	1.50	1.70	1.70
	Na^{+}	1.81	3.58	0.84	1.81	1.28	1.62
	K^{+}	0.12	0.16	0.30	0.10	0.14	0.30
Soluble anions (meq/ 100 g)	CO3--	0.00	0.00	0.00	0.00	0.00	0.00
	H CO3--	0.25	0.20	0.25	0.20	0.20	0.30
	Cl-	0.70	1.80	0.50	0.40	0.40	0.30
Organic matter%		2.30	1.90	1.65	1.86	2.30	2.00
Available N ppm		120	82	55	70	85	90
Available P ppm		12	11.5	9	11	11	12
Physical properties							
Sand %		39.58	34.28	42.39	11.50	27.00	25.00
Silt %		9.13	18.25	42.65	28.50	17.50	25.50
Clay %		51.29	47.47	14.96	60.00	55.50	49.50
Soil texture		clay	clay	clay loamy	clay	clay	clay

Transplantation of rice seedlings

Seedlings after 28-33 days of planting were gently uprooted from the nursery and manually transplanted to the experimental field sub-plots in groups of 2 or 3 seedlings at the corners of 20 x 20 cm squares (250,000 seedling groups/ha). Rhizobial, *Pseudomonas* or cyanobacterial inoculants, contained individual strains or mixtures inocula containing two or the three of them were prepared for testing in six farmer's field trials designed to contain sub-plots of 20 m^2 each. The used experimental design was the split-plot with three N-fertilization doses (48, 96 and 144 kg N/ha) as the main-plot treatments, seven inoculations and one non-inoculation control treatment as the sub-plot treatments. Four replicates were used for each treatments. Experiments conducted according to rice farming practices provided by the Agricultural Research Center (ARC), Egypt **.**

Treatments of post transplantation practices

The eight inoculation treatments contained:

1) Inoculation with the *Rhizobium leguminosarum* bv. *trifolii* (strain E11).
2) Inoculation with the *Pseudomonas stutzeri* (strain ERP-1).
3) Inoculation with *Anabaena* sp. (strain ERC102).
4) A balanced consortium (1/1 cfu) of E11 + ERP-1.
5) A balanced consortium (1/1 cfu) of E11 + ERC102.
6) A balanced consortium (1/1cfu) of ERP-1 + ERC102.
7) A balanced consortium (1/1/1 cfu) of E11 + ERP-1 + ERC102.
8) Non-inoculation control.

The inocula were prepared around two days before field use. For the treatments containing the rhizobial strain E11, the *Pseudomonas* strain ERP-1 or their consortia, inoculation was applied by manual broadcast of 720 g/ha peat-based inoculum (10^9 cfu / g). Application of the cyanobacterium strain ERC102 was as soil-based inoculum.

Irrigation

Water supply was continued regularly to maintain a standing head starting with 3 cm and subsequently increased to 10-15 cm as the plant height increased. Irrigation stopped 15 days before harvest.

Mineral fertilization

Potassium sulphate (48 % K_2O) was N/ha applied as urea (46 % N) . The amount of fertilizer-N recommended by the Field Crops Research Institute , ARC, Egypt .

Yield parameters

After harvest, weight of grain yield and straw were measured using the entire area of each of the 96-field plot of each experiment (no sub-sampling). The harvest index (percentage of grain yield / grain + straw yields) and the agronomic fertilizer N-use efficiency (kg grain yield / kg fertilizer-N) were calculated.

RESULTES AND DISCUSSION

Data of Table (2) presenting means of inoculation with the *Rhizobium leguminosarum* bv. *trifolii* along with 48 kg N/ha produced mean of grain yield ranged from 7.970 to 8.901ton/ha, increased to 8.591 to 9.333 and 9.013 to 9.450 ton/ha for the cases of application of fertilizer-N at 96 and 144 kg N/ha, respectively. Inoculation with the N_2-fixer *Pseudomonas stutzeri* strain ERP1 and the cyanobacterium strain ERC102 resulted in increases that followed the same pattern as those which obtained by inoculation with the *R .leguminosarum* bv. *trifolii* strain E11. Concomitant increases were obtained by inoculation with each of them. Highly l increases were obtained when the amount of fertilizer-N increased from 48 to 96 and 144 kg N/ha. The data refer to additional increases accompanied use of biofertilizer consortia of two or the three tested microorganisms, especially when the cyanobacterium strain ERC102 was included in the preparation. The mean values calculated for paddy yield over the 6 field experiments showed their least ranges of 7.970 ± 0.806, 8.591 ± 0.961 and 9.013 ± 0.818 when 48, 96 or 144 kg fertilizer-N/ha were used without inoculation. The corresponding values with inoculation increased to 9.967 ± 1.909, 10.047 ± 0.699 and 9.978 ± 0.972 ton/ha obtained using the *R. leguminosarum* bv. *trifolii* strain E11 plus the cyanobacterium strain ERC102, when the N-fertilizer was applied at 48, 96 or 144 kg N/ha, respectively.

Table 2. **Effect of inoculation with *Rhizobium leguminosarum* bv. *trifolii, Pseudomonas stutzeri* and/or *Anabaena* sp. on paddy yield in six locations in the Egypt Nile delta.**

Inoculants	Mean of grain yield over 6 field experiments (ton/ha)		
	48N	96N	144N
E 11	8.901 ± 1.179	9.333 ± 0.655	9.450 ± 1.029
ERP1	8.688 ± 0.945	8.832 ± 0.884	9.742 ± 0.824
ERC102	9.439 ± 0.831	9.865 ± 1.598	11.023 ± 1.300
E11 + ERP1	9.210 ± 2.038	9.164 ± 0.969	9.476 ± 0.845
E11 + ERC102	9.967 ± 1.909	10.047 ± 0.699	9.978 ± 0.972
ERP1+ERC102	9.138 ± 1.340	9.600 ± 0.968	10.014 ± 1.001
E11+ERP1+ERC102	8.767 ± 0.967	9.930 ± 0.644	10.259 ± 0.645
Control	7.970 ± 0.806	8.591 ± 0.961	9.013 ± 0.818

E11: *Rhizobium leguminosarum* bv. *trifolii* (strain E11). ERP1: *Pseudomonas stutzeri* (strain ERP-1). Inoculation with a soil-based inoculum of the cyanobacterium strain ERC102.

Previous field inoculation studies on rice have used N_2-fixing cyanobacteria, *Azospirillum*, *Azotobacter* and other candidates as biofertilizer inoculants (Omar *et al.* 1993, Shahaby *et al.* 1993 and Yanni and Abd El-Fattah 1999 & Afify *et al*., 2018).They concluded that inoculation with cyanobacteria positively contributed to rice plant growth, paddy yield and grain quality, with positive turnover effects on soil fertility and productivity of next crop(s). However, this is also confirmed in this study which clearly documenting similar positive effects of inoculation with N-fixing cyanobacteria (Abou – Elatta , 2018) .

Straw production

Means of the produced straw calculated from results of the six experiments (Table 3) show that over the six experiments, the lowest straw production was 11.530 + 1.904 ton/ha recorded for the treatment contained inoculation with the *R. leguminosarum* bv. *trifolii* strain E11 plus the *Pseudomonas stutzeri* strain ERP1 along with application of 48 kg N/ha. The highest mean value over the six experiments was recorded for biofertilization by the *Pseudomonas* plus the cyanobacterium along with N-fertilization by the higher dose of 144 kg N/ha, which yielded a mean of 15.261 + 4.702 ton straw/ha. The statistically significant increases in straw yield resulting are showing that inoculation with certain endophytic strains of rhizobia in lab growth champers (Yanni *et al.* 1997) and greenhouse experiments (Biswas *et al.* 2000a,b and Chi *et al.* 2005). Straw biomass production was found responsive to chemical N-fertilization and, in contrast, biofertilization boosted rice grain production. This differential result can be considered as the preferred positive benefit of inoculation (more grain production for human consumption rather than excessive vegetative biomass for animal consumption and wasteful burning) in lab growth champers (Yanni *et al.* 1997) and greenhouse experiments (Biswas *et al.* 2000a,b and Chi *et al.* 2005).

Table 3. **Effect inoculation with *Rhizobium leguminosarum* bv. *trifolii, Pseudomonas stutzeri* and/or *Anabaena* sp. on straw in six locations in the Egypt Nile delta.**

Inoculants	Mean of produced straw over 6 field experiments (ton/ha)		
	48N	96N	144N
E 11	13.090 ± 2.457	12.426 ± 2.083	13.753 ± 2.261
ERP1	13.559 ± 4.393	13.654 ± 3.183	13.842 ± 2.327
ERC102	12.192 ± 2.677	12.889 ± 2.812	14.059 ± 2.477
E11 + ERP1	11.530 ± 1.904	13.831 ± 3.330	14.072 ± 2.183
E11 + ERC102	12.683 ± 2.512	13.475 ± 3.456	13.394 ± 2.493
ERP1+ERC102	12.973 ± 2.964	14.313 ± 4.376	15.261 ± 4.702
E11+ERP1+ERC102	12.779 ± 2.825	13.638 ± 2.599	14.797 ± 3.466
Control	12.428 ± 2.926	12.855 ± 2.748	13.218 ± 2.088

E11: *Rhizobium leguminosarum* bv. *trifolii* (strain E11). ERP1: *Pseudomonas stutzeri* (strain ERP-1). Inoculation with a soil-based inoculum of the cyanobacterium strain ERC102.

The harvest index

Results of the harvest index (percent of paddy yield/above ground biomass at harvest) presented cumulatively summarized in Table (4), show increases due to application of the tested inoculation treatments comparing to their corresponding non- inoculation controls. For the non-inoculation controls, straw production figures fluctuated between a minimum of 34.11 % produced with application of 48 kg N/ha to 47.27% for the application of 96 kg N/ha. The range for the treatments received biofertilization is within 33.13% recorded in case of application of 96 kg N/ha up to 49.18% with application of 48 kg N/ha. Seems that increasing fertilizer-N application dose, when diploid with biofertilization, contributed to increased vegetative rather than reproductive growth. This result is consistent with previous work (Yanni *et al.* 1997, Dazzo *et al.* 2000 and Yanni *et al.* 2001) showed that rhizobial inoculation and N-fertilization contributed to rice vegetative growth and grain yield in parallel, with a single exception in a field research program resulted in data published by Yanni *et al.* (1997), where an excessive N-fertilization dose (above the recommended) was applied just for comparison reasons, and resulted in detrimental lodging decreased the grain yield. While , harvest index indicates the percentage ratio of grain yield to grain + straw yield the no significant was recorded in the interaction between algal inoculation and nitrogen fertilization (Abou - Elatta , 2018).

Table 4. **Effect inoculation with *Rhizobium leguminosarum* bv. *trifolii, Pseudomonas stutzeri* and/or *Anabaena* sp. on the harves index in six locations in the Egypt Nile delta.**

Inoculants	Mean of the harvest index over 6 field experiments		
	48N	96N	144N
E 11	40.85 ± 5.14	42.63 ± 5.45	41.03 ± 5.42
ERP1	41.12 ± 6.26	40.08 ± 7.35	41.70 ± 5.13
ERC102	44.25 ± 4.93	42.50 ± 4.41	44.25 ± 3.86
E11 + ERP1	44.23 ± 4.87	41.43 ± 6.24	40.57 ± 5.04
E11 + ERC102	43.17 ± 5.21	43.58 ± 4.84	43.10 ± 4.37
ERP1+ERC102	41.78 ± 4.80	42.23 ± 7.42	40.73 ± 6.97
E11+ERP1+ERC102	41.27 ± 5.48	42.83 ± 4.03	41.57 ± 4.65
Control	39.82 ± 5.72	40.67 ± 6.60	40.95 ± 5.87

E11: *Rhizobium leguminosarum* bv. *trifolii* (strain E11). ERP1: *Pseudomonas stutzeri* (strain ERP-1). Inoculation with a soil-based inoculum of the cyanobacterium strain ERC102.

The agronomic fertilizer N-use efficiency

The data in Table 5, indicate concomitant treatment induced pronounced decreases in these case with the increase in N-fertilizer application dose. Meanwhile, inoculation increased the figures with all the tested treatments of biofertilization and under the three tested chemical fertilizer doses, comparing with the corresponding counterparts. The data definitely assure that productivity of the fertilizer when applied in the smaller dose of 48 kg N/ha is much higher than what found with application of96 or 144 kg N/ha. The corresponding data in case of biofertilization and application of 48 kg N/ha was 190.4 kg paddy/kg fertilizer-N, which came comparable with 166.1 recorded in case of the non-inoculated control that treated with no N-fertilizer or biofertilization treatments.

Table 5. **Effect inoculation with *Rhizobium leguminosarum* bv. *trifolii, Pseudomonas stutzeri* and/or *Anabaena* sp. on the agronomic fertilizer N-use efficiency in six locations in the Egypt Nile delta.**

Inoculants	Mean of N-use efficiencies over 6 field experiments		
	48N	96N	144N
E 11	185.5 ± 24.6	95.2 ± 7.7	65.7 ± 7.1
ERP1	181.0 ± 19.7	92.0 ± 9.2	67.7 ± 5.7
ERC102	196.7 ± 17.3	102.8 ± 16.6	76.2 ± 9.1
E11 + ERP1	191.9 ± 42.5	95.5 ± 10.1	65.8 ± 5.9
E11 + ERC102	204.8 ± 37.7	104.7 ± 7.3	69.3 ± 6.7
ERP1+ERC102	190.4 ± 27.9	100.0 ±10.1	69.5 ± 7.0
E11+ERP1+ERC102	183.5 ± 19.7	103.4 ± 6.7	71.3 ± 4.5
Control	166.1 ± 16.8	89.5 ± 10.0	62.6 ± 5.7

E11: inoculation with the rice root endo-colonizer *Rhizobium leguminosarum* bv. *trifolii* (strain E11). ERP1: inoculation with the *Pseudomonas stutzeri* (strain ERP-1). Inoculation with a soil-based inoculum of the cyanobacterium strain ERC10.2

The corresponding data in case of application of 96 kg N/ha along with biofertilization was 104.7 , dropped to 89.5 that recorded for the corresponding non-inoculated control treatment. The tables recorded in case of application of 144 kg N/ha were 69.3 kg paddy yield in case of inoculation, down to 62.6 for the non-inoculation treatments. The lot of comparisons displayed here clearly indicates that contribution of biofertilization to paddy yield negatively affected by increasing the amount of applied N-fertilizer. Meanwhile, biofertilization increased productivity of the fertilizer-N unit by 14.7 % in case of application of 48 kg N/ha against 10.7 and 10.8 % in cases of application of 96 and 144 kg N/ha, respectively. The results here are informative and clearly refer to necessity of biofertilization to diminish the current excessive use of N-fertilizer in paddy fields that adversely affect rice production economy and environment soundness.

CONCLUSION

The values in the six experiments indicated that biofertilization with some microorganisms can benefit grain production with increasing fertilizer-N doses, providing the desired result that inoculation can reduce the need for additional chemical fertilizer-N application to achieve higher grain yield.

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التلقيح الفردي والمزدوج أو الخليط بكل من ***Rhizobium leguminosarum* bv. *trifolii* , *Pseudomonas stutzeri* or *Anabaena* sp** على المكونات المحصولية لنبات الأرز

عايـــدة حـــافظ عفيفـــى[1]،فتحـــى إســـماعيل علـــى حوقـــة[1]، أحمـــد محمـــود الســـواح[1] ، يوســـف جـــرس ينـــى[2] و عبد الجواد يوسف السعدنى[2]

[1]قسم الميكروبيولوجيا الزراعية ـ كلية الزراعة ـ جامعة المنصورة – المنصوره ـ مصر

[2]محطة البحوث الزراعية ـ سخا – كفر الشيخ – مصر

أجريت هذه الدراسة للحصول على عزلة سيانوبكتيريا من مزارع الأرز النامي في مناطق بشمال ووسط الدلتا ووجد أنها سلالة *Anabaena* sp. (strain ERC102) وكذلك عزلة سيدوموناس ووجد أنها *Pseudomonas stutzeri* واستخدم معهما سلالة *Rhizobium leguminosarum* bv. *trifoli*i (strain E11) وتم دراسة تأثير التلقيح بهذه الميكروبات في لقاحات منفردة أو في خليط على القياسات المحصولية (محصول الحبوب طن/هكتار , محصول القش طن/هكتار ، دليل المحصول والكفاءة المحصولية) لثلاثة أصناف من الأرز هي: جيزة 177 ، جيزة 178 ، سخا 101 في وجود ثلاثة معدلات مختلفة من التسميد الآزوتى (48 , 96 , 144 كجم نيتروجين للهكتار) . وقد أدى التلقيح عموما إلى زيادة في محصول الحبوب والقش ودليل الحصاد والكفاءة المحصولية مقارنة بالمعاملات غير الملقحة. وبناءا على ما سبق يمكن إستخدام هذه الأسمده البيولوجيه لتقليل إستخدام الأسمده الكيميائيه الملوثه للبيئه و الباهظة التكاليف .