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THE RESPONSE OF RICE PLANT TO PHOSPHATE - DISSOLVING BACTERIA AND AZOLLA UNDER TWO PHOSPHORUS FERTILIZATION RATES

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ABSTRACT

Two field experiments were carried out at Sakha Agric. Res. Station during two successive seasons 2002/2003 to study the effect of two sources of bio-fertilizers (phosphate dissolving bacteria ***Bacillus megatherium*** **and Azolla**) under two sources of chemical P fertilizer (as superphosphate or rock phosphate). It was found that both bio-fertilizers compared with control caused an increase in grain and straw yield of rice and macronutrients content (N,P and K) in grain at harvest stage as well as protein and total carbohydrate in grain of rice. Biofertilization under chemical P fertilizer was more effective than chemical P-fertilizer only. P- fertilizer superphosphate (SP) superiored rock phosphate fertilizer.

The present study recommended using of PDB plus Azolla with application of rock phosphate at rate of 150 kg/fed to obtain the highest rice grain yield with good quality.

INTRODUCTION

Rice is one of the most important cereal crops in Egypt, both for local consumption and export. Recently, its productivity in Egypt has scored the highest level all over the world being about 10 ton/ha. The growth of rice was found to be affected by phosphorus and nitrogen under Egyptian soil conditions. Phosphorus availability in soil is governed by many factors (pH, $CaCO_3$, organic matter and clay contents). In spite of the considerable addition of P to these soils, the level of available phosphorus decreases sharply after a short period from application (**Miller** ***et al.*****, 1990**). They revealed that under alkaline soil conditions, the available phosphorus in the added fertilizer is rapidly transformed to tricalcium phosphate, thus becomes unavailable to the plants. Various hypotheses were put forward to account the beneficial effects of microbial fertilizers in general and phosphobacterin in particular on plant growth. One can envisage many possible mechanisms for the mode of action of phosphate –dissolving bacteria (PDB). It has been repeatedly reported that the stimulating effect of PDB on plant growth is solely the result of releasing originally bound phosphorus compounds in soil **(Saber *et al.*, 1981).** On the other hand, **Curl and Truelove (1985) and El-Sayed (1999)** revealed that PDB plays an important role in releasing P from rock, tricalcium or other difficult P forms through producing organic and inorganic acid, as well as CO_2. These substances convert the insoluble forms of P into soluble ones. PDB also affects other nutrients rather than phosphorus, in this concern, **Alagawadi and Gaur (1988) and El-Sayed (1999)** reported that seed inoculation with PDB increased number of total bacteria generally and PDB particularly in the rhizospher zone and released ammonia from bound complex nitrogen compounds. Yet, increasing the uptake of N and K due to the application of PDB could be attributed to the depletion of such nutrients in building new tissues **(Nijjar, 1985).** In addition, phosphorus has an enhancing impact on plant growth and biological yield through its importance as an energy storage and transfer necessary for metabolic processes **(Nassar and Ismail, 1999).** It also raises the efficiency of plants to photosynthesis.

Because of increasing the costs of chemical nitrogen fertilizer much attention is now being focused on biologically fixed nitrogen for rice. Azolla biofertilizers had proved its success as an alternative source of chemical fertilizer in rice fields.

Many researchers in several countries stated that inoculation of rice under field conditions with azolla had benefited rice yield. There were increases in yields ranging from 20 to 30% over the control **(Abo-Soliman *et al.*, 1990; Singh and Singh,1990; Hegazy *et al.*,1995 and Abd El-Fattah *et al.*,1998).**

Inoculation of rice with azolla is a promising technique to reduce the amount of N-fertilizers for the crop. Also, biological N-fertilization, in general, could lead to avoidance of environmental pollution arising from N-mineral fertilization.

The present study was undertaken to examine the effect of seed inoculation with phosphate-dissolving bacteria (***Bacillus megathrium var. phosphaticum***) or Azolla or a combination of both with rock phosphate on growth, yield of rice and its chemical composition.

MATERIALS AND METHODS

Two field experiments were carried out on rice (***Oryza sativa***) Varity Giza 178 at Sakha Station during 2002/2003 seasons.

Representative surface soil sample (0-30cm) were taken before performance of the experiment where its physical and chemical analysis characteristics were determined as indicated in Table (1).

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

pH (1:2.5)	EC dS/m	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3	HCO_3^-	Cl^-	$SO_4^{=}$
8.20	4.80	19.00	10.00	33.0	0.52	--	2.60	26.00	29.94

Sand %	Silt %	Clay %	Texture grade
15.30	39.10	45.60	Clayey

Available N (ppm)	Available P (ppm)	Available K (ppm)
28.00	8.60	886.00

The techniques adopted for mechanical and chemical analysis were as the following:

1. Particle size distribution **(Kilmer and Alexander,1949).**
2. Soil pH, EC and soluble cations and anions **(Richards,1954).**
3. $CaCO_3$ and available N,P and K**(Jackson,1973)**

A strain of phosphate dissolving bacteria (PDB), and azolla were supplied from Soil Microbiology Department; Soils, Water and Environment Research Institute, Giza, Egypt.

The layout of experiments under consideration was randomized design with four replicates as follow:-

1. Superphosphate (SP) (100kg/fed.).
2. Rock phosphate (RP) (100kg/fed) without PDB or,azolla.
3. Rock phosphate (RP) (100kg/fed) with PDB.
4. Rock phosphate (RP) (100kg/fed.) with azolla.
5. Rock phosphate (RP) (100kg/fed.) with PDB and azolla.
6. Rock phosphate (RP) (150kg/fed) without PDB or azzola.
7. Rock phosphate (RP) (150kg/fed) with PDB .
8. Rock phosphate (RP) (150kg/fed) with azzola.
9. Rock phosphate (RP) (150kg/fed) with PDB and azzola.

All blocks were fertilized with nitrogen as injected gas of ammonium, and potassium as potassium sulphate (48%KO_2) as well as zinc sulphate with recommended rates.

Plant samples were taken at harvesting stage. The following parameters were determined and recorded:

- plant height(cm), Panicle length(cm),Panicle weight (g), 1000-grain weight(g)
- grain yield, straw yield and nitrogen percentage in grains which was determined according to **A.O.A.C.(1980)** using micro-kjeldahle procedure. Protein content is calculated as nitrogen percentage x 5.95. Total potassium of the plant material was determined by flame photometric methods as described by **Jackson (1973).** Phosphorus was determined calorimetrically according to **Troug and Mayer (1949).** Soluble and total carbohydrates were determined according to **Smith *et al.* (1956)**.

All data were tabulated and subjected to statistical analysis according to **Sendecor and Cochran (1967)**.

RESULTS AND DISCUSSION

1- The effect of various bio and phosphorous fertilizers on growth characters:

Data in Table (2) indicate that biofertilizer inoculated treatments significantly increased all studied characteristic compared with non-inoculated ones. In addition, there were insignificant differences between individual application of Azolla or phosphate dissolving bacteria (PDB). The combined treatment of Azolla plus PDB in addition to the application of 150kg rock phosphate/fed produced maximum values of plant height, panicle length, panicle weight and 1000- grains weight compared to treatments without biofertilizers. The improvement and increase in rice plant height and panicle characters may be due to fixed nitrogen obtained by the biofertilizers utilization which allows plants to grow and then increased leaf photosynthetic rates resulting in more accumulation of crop biomass that increase panicle length and panicle weight. These results are in harmony with those obtained by **Nayak *et al.*(1986); Yanni(1992) and Abou-Zeid *et al.*(1996).**

PDB enhances the rice characters by increasing the available P in soil which in turn promotes cell division and develops meristematic tissues **(Kundu and Gaur,1980) and Abo-El-Nour *et al.*(1996).** In the same respect, **(Ghazal 1987, Herzalla 1991 and Hammad,1994)** revealed that both nitrogen and Azolla significantly increased the rice plant height, panicle length and 1000 grains weight.

Table (2): Response of growth parameters to PDB, azolla and P-fertilization rates.

Treatments: Rate of P	Treatments: Bio fertilizer	Plant height (cm)	Panicle length (cm)	Panicle weight (g)	1000-grain weight (g)
SP 100kg/fed	-	89.3	22.50	2.2	23.20
R P 100kg/fed	Without PDB	87.2	21.3	2.0	22.4
	With PDB	88.9	21.6	2.1	22.9
	Azolla	89.9	21.4	2.1	22.8
	Azoll +PDB	90.6	21.9	2.2	23.1
RP 150kg/fed	Without PDB	90.6	22.3	2.1	22.6
	With PDB	91.6	22.6	2.3	23.4
	Azolla	91.2	22.6	2.2	23.3
	Azoll +PDB	92.7	23.7	2.5	25.3
L.S.D.at 0.05		0.9	n.s	n.s	1.1
0.01		1.3	n.s	n.s	1.6

SP = superphosphate
RP = Rock phosphate
PDB= phosphate dissolving bacteria.

2-The effect of various bio and phosphorus fertilizers on nutrients uptake:

Concerning the nutrients uptake, results in Table (3) show, generally, that the application of any phosphate source under study increased N,P and K uptake by grain. It is deserved to mention that the results of the named nutrients related to the treatment inoculated with P-dissolving bacteria combined with azolla recorded the highest values than the others.

With respect to phosphate rates, results showed that the increase of N,P and K uptake were in a harmony with increasing rates of phosphorus fertilizer under study. The increase in N,P and K uptake as affected by PDB, my be due to increasing growth as results of improvement the media of the root zone. These results are in agreement with those obtained by **Sattar and Gaur (1989), Chhabra and Jalali (1997), Farres (1997), El-Sayed (1999) and Abd El-Rasoul *et al.*(2002).** In this respect, **Marschner (1986)** described the enhancing effect of K uptake to the energy rich phosphates (in the from ATP) and the close relationship between K-uptake and ATP-ase activity. The positive effect of such treatments on NPK contents can be explained on the basis of stimulating the population of Azotobacter chrooccum in the root zone of the growing plants as the addition of phosphobacteria. This leads to increase the rate of N_2-fixation. **(Kundu and Gaur; 1980 and Abo El-Nour *et al.*, 1996)**

Table (3): Response of N.P,K and protein content of rice grain to PDB, azolla and P-fertilization.

Treatments		N Uptake kg/fed	P Uptake kg/fed	K Uptake kg/fed	Crude protein
Rate of P	Bio fertilizer				
Sp 100kg/fed	-	52.56	13.40	24.65	8.60
R P 100kg/fed	Without PDB	49.22	10.20	21.66	8.33
	With PDB	59.80	13.40	25.86	8.60
	Azolla	63.95	13.43	26.73	8.90
	Azoll +PDB	67.44	16.42	27.72	9.20
RP 150kg/fed	Without PDB	58.88	12.56	24.61	8.90
	With PDB	67.20	16.80	28.56	9.00
	Azolla	68.99	16.60	28.35	9.20
	Azoll +PDB	73.84	20.54	32.07	9.52
L.S.D.at 0.05		1.33	0.15	0.042	
0.01		1.92	0.20	0.058	

Regarding protein content in rice grain, it was clear that all treatments of azolla and PDB or combination between them increased protein content over the non-inoculated treatments. These results are in agreement with those obtained by **Ghazal *et al.* (1997), El-Shahat *et al.* (2002) and Atia (2002).**

3. The effect of various bio and phosphorus fertilization on rice yield

It is clear from Table(4) that the highest values of rice grain and straw yields as well as most of the yield components were obtained under the use of the highest rate of rock phosphate (150kg/fed) with azolla and phosphorus dissolving bacteria. On the other hand, the lowest values of rice grain and straw yields as well as its components were obtained under the use of rock phosphate only. The increased grain yield of rice in the PDB or azolla treatments resulted from an increase in both panicles weight and 1000 grains weight due to increasing the availability of nitrogen in these treatments. **Singh and Singh(1990), Jayarman(1991), Mandal *et al.*,(1993), Marazi *et al.*,(1993) and Sikander *et al.*,(1996a and b)** showed that the use of azolla increased plant growth, number of tillers, grain and straw yield significantly. The positive effect of combination between seed inoculation and rock phosphate fertilizer may be due to stimulating and increasing the activity of soil microorganisms in general and phosphate dissolving bacteria in particular.

Table (4): Effect of PDB, Azolla and P-fertilizers rates on rice yield.

Treatments: Rate of P	Treatments: Bio fertilizer	Grain yield (ton/fed)	Straw yield (ton/fed)	Total yield (ton/fed)	Harvest index
SP 100kg/fed	-	3.625	3.680	7.305	49.624
R P 100kg/fed	Without PDB	3.516	3.500	7.016	50.114
	With PDB	4.124	4.115	8.239	50.055
	Azolla	4.263	4.290	8.553	49.842
	Azoll +PDB	4.351	4.415	8.766	49.633
RP 150kg/fed	Without PDB	3.925	3.850	7.775	50.482
	With PDB	4.421	4.521	8.942	49.441
	Azolla	4.451	4.512	8.963	49.660
	Azoll +PDB	4.615	4.680	9.295	49.650
L.S.D.at 0.05		0.3318	0.2937		
0.01		0.4572	0.4047		

Harvest index = grain yield/(grain yield+straw yield) X 100

Concerning the interactive effect between phosphorus application rate and biofertilizers PDB and azolla, it was found that increasing phosphorus level not only increase rice growth but also increased nitrogen use activity and N cotent in the plant,**(Kondo *et al.*,1989).** Also, **Mian and Azmal(1989)** found that biomass production and N-fixation by azolla was better with application of phosphorus.

More recently, **Mukherijee and Rai (2000)** found that an interaction effect of biofertilizer and phosphorus nutrition increased the grain yield significantly over the application of P alone.

4. The effect of various bio and phosphate fertilizers on carbohydrate constituent in rice grain:

Interaction effect of biofertilizer and phosphorus nutrition increased the total carbohydrate production of rice (Table 5). High increase in total carbohydrate production was noticed in case of PDB or azolla and the highest rate of P-fertilizer (150kg RP /fed). On the other hand, grain contents of starch and TC were increased markedly by the higher P rates. In this connection, **Faleiros *et al.* (1996)** showed that P deficiency decreased starch production and endosperm dry weight, but with a minimal effect on the activities of ADP-glucose pryrophosphrylase and alanine transaminase. Also, **Mengel and Kirkby(1987)** illustrated that photosynthesis and translocation of photosynthatase from leaves to seeds was promoted in plants well supplied with P.

Results in Table(5) show also that all inoculated treatments have the same effect on increasing soluble, insoluble and total carbohydrates in grains of rice yield as compared with the control. This may be due to the increase in the concentration of photosynthetic pigments which was reflected on carbohydrates biosynthesis. The highest values of carbohydrates insoluble and total in the most samples were attained by the highest rate of P-fertilizer along with azolla and PDB. This means that biofertilizer enhanced the carbohydrate metabolism in the plants.

Finally, it can be observed that the soil available P content was further augmented as a result of seed inoculation with PDB and azolla at different rates of P-fertilization. The increase was more pronounced in the treatment of PDB and azolla in combination with the application of rock phosphate at 150 kg /fed.

Table (5): Effect of PDB, Azolla and P-fertilizers rates on the carbohydrate fraction of grain rice (g/100g dry weight)

Treatments: Rate of P	Treatments: Bio fertilizer	S.C	I.C	T.C	Starch %
SP 100kg/fed	-	11.77	66.65	78.42	59.96
R P 100kg/fed	Without PDB	12.07	65.77	77.84	59.19
	With PDB	12.25	67.80	80.05	61.02
	Azolla	12.52	67.34	79.86	60.61
	Azoll +PDB	11.99	68.19	80.18	61.37
RP 150kg/fed	Without PDB	12.53	65.78	78.31	59.20
	With PDB	12.50	67.95	80.45	61.16
	Azolla	12.14	68.24	80.38	61.42
	Azoll +PDB	12.06	68.86	80.92	61.97
L.S.D.at 0.05		n.s	n.s	n.s	
0.01		n.s	n.s	n.s	

S.C. = Soluble carbohydrate
I.C. = Insoluble carbohydrate
T.C.= Total carbohydrate

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استجابة نبات الارز للبكتيريا المذيبة للفوسفات والازولا تحت معدلين من الاسمدة الفوسفورية

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معهد بحوث الاراضى والمياه والبيئة– مركز البحوث الزراعية– الجيزة

تم تنفيذ تجربتين حقليتين فى محطة بحوث سخا خلال موسمى ٢٠٠٢/٢٠٠٣ لدراسة تأثير مصدرين من الاسمدة الحيوية(البكتيريا المذيبة للفوسفور كمصدر للفوسفور والازولا كمصدر للنتروجين) وذلك تحت معدلين من سماد الصخر الفوسفاتى(١٠٠كجم ، ١٥٠ كجم/ف) بالاضافة الى معاملة الكنترول(١٠٠كجم سوبرفوسفات/فدان بدون اضافة الاسمدة الحيوية)

وجد من الدراسة أن كلا مصدرى الاسمدة الحيوية مقارنة بالكنترول أدت الى زيادة كلا من محصولى الحبوب والقش للارز ومحتوى عناصر النتروجين والفوسفور والبوتاسيوم فى الحبوب عند مرحلة الحصاد.

- زاد محتوى الحبوب من البروتين والمكونات الكربوهيدراتية المختلفة
- تفوق التسميد الحيوى مع الكيماوى عن الكيماوى فقط
- تفوق سماد السوبر فوسفات الاحادى على صخر الفوسفات

من دراسة التفاعلات بين المعاملات أوصت النتائج باستخدام البكتيريا المذيبة للفوسفور +الازولا مع اضافة المعدل الثانى من الفوسفور للفدان فى صورة صخر فوسفات وذلك للحصول على اعلى محصول من الارز

كذلك توصى الدراسة بالمزيد من البحث لزيادة كفاءة سماد صخر الفوسفات مع التسميد الحيوى الفوسفاتى.