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Response of Phosphate Solubilising Inoculants (Jumpstart) on Biochemistry and Yield of Rice (*Oryza sativa L.*)

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A B S T R A C T

Phosphorus plays a significant role in several physiological and biochemical activities in plants. Phosphorus in soils is immobilized due to formation of insoluble complexes such as iron and aluminium hydrous oxides and calcium carbonate. Phosphate-solubilizing microorganisms (PSM) play an important role in insoluble phosphates into soluble forms involves processes of acidulation, ion chelation and exchange reactions. Present study, a field experiment was carried out to study the physiological and yield response of phosphate solubilising inoculants (*JumpStart*) on rice variety CO 47 under machine transplanting rice ecosystem. The present study revealed that the treatment P_3S_3 (100% P + *JumpStart* 10E5) was recorded increased total chlorophyll (3.03 mg g^{-1}) and higher leaf carbohydrate (82.06 mg g^{-1}) at 50% flowering stage. The lower enzyme activity of acid phosphatase (0.022 µmol of pNPP g^{-1} min^{-1}) and ATPase (29.37 µg of Pi g^{-1} h^{-1}) were recorded by treatment P_3S_3 (100% P + *JumpStart* 10E5) over control at 50% flowering stage. The uptake of phosphorus by plants was found higher in P_3S_3 treatment observed at all stages of crop growth. At 50% flowering stage, phosphorus (69.26 kg ha^{-1}), uptake were observed higher in P_3S_3 treatment than control. The maximum grain yield of 8426 kg ha^{-1} was registered by P_3S_3 (100% P + *JumpStart*) with an increase of 16.4 per cent over control. This might be due to the significant increment of major yield components such as number of productive tillers per plant, panicle dry weight and number of grains per panicle by the application of 100 % recommended phosphorus with seed treatment of *JumpStart* 10E5.

Introduction

Phosphorus is an essential nutrient required by rice (Kim *et al.*, 1998) and it has a defined role in plant metabolisms such as root development, photosynthesis, nutrient transport within the plant, Meiosis, phospholipid in cell walls and reproductive parts of plant (Rasipour and Asgharzadeh, 2007). The judicious and proper use of phosphorous in rice markedly increases the yield and quality of rice. Without adequate supply of plant with phosphorous, plant cannot attain its maximum yield.

Low level of available P in soils is one of the major constraints for rice production in the world. This is particularly apparent under

upland conditions commonly characterized by poorly fertile, erodible, badly leached, highly acidic, and P-fixing soils, normally with little or no fertilizer applied (IRRI, 1997). Even under lowland conditions, P deficiency is identified as a main factor limiting the performance of modern rice varieties to approach their optimum yields. Application of P fertilizers is a quick remedy for P deficiency in rice soils. However, nonorganic fertilizers are not always available to a large sector of poor rice farmers. Besides, some rice soils that are low in available P can also fix it into a highly less soluble mineral. Dobermann *et al.*, (1998) estimated that more than 90% of the added fertilizer P may rapidly be transformed to P forms that are not easily available to plants.

The microorganisms perform an important role in agriculture by supplying nutrients to plants and reduce the demand of chemical fertilizers (Cakmakci *et al.,* 2006). Particularly, phosphate solubilizing microorganisms are able to solubilize unavailable soil P and enhance the yield of crops (Adesemoye and Kloepper, 2009). Phosphate solubilizing microorganisms (PSMs) are ubiquitous, and their numbers vary from soil to soil.

The use of P solubilizing microorganisms improves the soil fertility and increase the crop production to fulfill the requirement. Further, the efficiency of these microorganisms to meet P requirement of crops will depend greatly on their impact under practical farming conditions. *Penicillium bilaii* (a phosphorus solubilising fungus) is a newly isolated soil fungus that has profound effect on solubilization of phosphorus. The efficacy of *Penicillium bilaii* has been test verified in various crops such as wheat, canola, chick pea, mustard and lentil elsewhere. However, the usefulness of *Penicillium bilaii* has not been tested in rice crop under machine transplanting system.

Keeping this in view, the present study was conducted at field level to evaluate the performance of different inoculation of *Penicillium bilaii* in combination with three levels of phosphatic fertilizer on biochemistry, P uptake and yield of rice.

Materials and Methods

The Present investigation was carried out in the field no. H7a of Department of Rice, Tamil Nadu Agricultural University, Coimbatore during *Kharif* season of 2015. Rice variety CO 47 seed material collected from Department of Rice. Before sowing, three bags (1 kg each) of rice seeds were soaked for 24 hours. Seeds were then seed treated with three different population of Phosphate solubilising inoculants – *Penicillium bilaii* (*Jump Start*) at the rate of 160 mg per Kg of seeds and 6 ml of water was added and agitated for one minute as per the treatments and compared with the recommended seed treatment of Azophos at the rate of 20g kg^{-1} of seeds and control (No seed treatment).The experimental field with provisions made for draining excess water was well irrigated, puddled and leveled by leveler to minimize undulations, field conditions were maintained for two days to make the land convenient for machine transplanting.

The field experiment was laid out in split plot design with three replications. The treatments are as follows three main plot of three different P levels P1- 0 % (0 kgP ha^{-1}), P2- 50 % P (25 kg P ha^{-1}) and P3-100%P (50 kgP ha^{-1}) (Recommended dose). Sub plot of five different seed treatments S1- No Seed Treatment, S2- *JumpStart* 10E4 (0.00967 mg kg^{-1} of seeds), S3-*JumpStart* 10E5 (0.0967 mg $kg^{-1}$of seeds), S4- *JumpStart* 10E6 (9.667 mg kg^{-1} of seeds) and S5- Azophos at the 20g

kg-1 of seeds. Five plants were selected at different growth stages for recording observation. Total Chlorophyll content in leaves was estimated by using the method described by Hiscox and Israelstam (1979) and expressed in mg g^{-1} fresh weight. The total carbohydrate content of the leaf and root samples were estimated by following the method suggested by Fales (1951) and all expressed in mg g^{-1} on dry weight basis.

Acid phosphatase activity of the seedling roots were determined by the method of Hooley (1984).Chlorophyll content in leaves was estimated by using the method described by Hiscox and Israelstam (1979) and expressed in mg g^{-1} fresh weight. Adenosine Triphosphatase activity of the rice root was determined by the method of Umbreit *et al.*, (1964) and the enzyme activity was expressed as µg of Pi/g fresh weight/hr

Uptake of phosphorus nutrients were calculated as the product of total dry matter production (kg ha^{-1}) with the corresponding nutrient content of the plant parts at active tillering (AT), panicle initiation (PI), flowering (F) and grain filling (GF) stage (Hartemink *et al.*, 2000).

The yield and its components were recorded at harvest (Yoshida *et al.*, 1972). The data collected were subjected to statistical analysis in split plot design by Gomez and Gomez (1984).

Results and Discussion

Total chlorophyll

Total chlorophyll content is an imperative indicator of crop growth. There was a general decrease in chlorophyll content observed from flowering to grain filling stage. Hence, measurement of chlorophyll indirectly explains the efficiency of the photosynthesis and photosynthate production. Total chlorophyll content was steadily increased up to 50% flowering stage (3.03 mg g^{-1}) and thereafter it decreased at grain filling stage (2.13 mg g^{-1}) due to initiation of senescence phase (Table 1). During senescence, the total chlorophyll was altered due to shift in the macro nutrient concentration. The content of total chlorophyll content of leaf decreased due to deficiency of phosphorus in 0% P with no seed treatment, which is 25% low when compared to 100% P + *JumpStart* 10E5 at 50% flowering stage. The increase of chlorophyll content is due to enhanced absorption of irons from rhizosphere and increased translocation to the shoot without any chelation inside the root (Wang *et al.*, 2009). Similar findings were reported by Mehrvarz and Chaichi (2008) that increase in chlorophyll content and photosynthesis rates with inoculation of PSB in aerobic rice.

Total carbohydrate

Total carbohydrate includes all those carbohydrates which can be used in the plant body as a source of energy or as building material. The determination of the total carbohydrate content is of greater significance than that of individual carbohydrates. In this study, increased amount of leaf carbohydrate was seen in P_3S_3 (100% P + *JumpStart* 10E4) during 50% flowering stage.

The control treatment, P_1S_1 had only 52.04 mg g^{-1} of leaf carbohydrate which is 57.69% lower than P_3S_3 treatment (Table 2). This increase due to higher solubilizing ability of *Penicillium bilaii* combined with 100% P application increased the availability of phosphorus in soil resulted in higher leaf carbohydrate content (Prasanna, 2013). Degroot *et al.*, (2003) found a reduction in starch and soluble sugars with decreasing phosphorus application. Low phosphorus condition largely affects the carbohydrate

content in tomato (Ramezan Ali Khavari, 2008).

Acid phosphatase enzyme activity

Acid phosphatase enzyme activity is responsible for P hydrolysis from organic compounds, favouring P mobilization and translocation. An increase in root phosphatase activity was often correlated with decrease in phosphorus level in root as well as leaf (Muthaiya, 2010). In this study, the treatment with 0% P + No seed treatment at 50% flowering stage recorded higher root acid phosphatase activity, which is 84.93% higher than 100 % P + *JumpStart* 10E5 (P_3S_3) (Table 3). Yun *et al.*, (2001) found that the acid phosphatase enzyme secretion from roots enhanced protein release to acidify the rhizophere and mobilize phosphorus which improves the acquisition and reutilization, thus helping the plants to grow under P deficit conditions. Jeong Hyun Lim *et al.*, (2003) reported that acid phosphatases (APases) play a role in the release of phosphate in organic complexes in soil.

Adenosine triphosphatase (ATPase) activity

Light energy absorbed by chlorophyll is converted into stable chemical energy and drive ATP formation via ATPase in the chloroplasts. ATPase is the metabolic enzyme integral to ATP hydrolysis and ATP serve to transport chemical energy in cell to drive metabolism (Pederson and Carafoli, 1987). Root ATPase activity was greatly influenced by different P levels and *Penicillium bilaii* (a phosphorus solubilising fungus) in our study. The values were found to increase from active tillering stage to 50% flowering stage. However, the value was higher in 0% P than at 50% and 100% P levels. At 50% flowering stage the ATPase activity at 0% P was 57.48 and 48.9% higher in comparison with 100% P levels (Table 4). Hong Shen *et al.*, (2006) found that P starvation enhanced the activity of plasma membrane H^+-ATPase in soybean roots.

Fig.1 Effect of P levels and *Penicillium bilaii* inoculants on grain yield (kg/ha) of rice

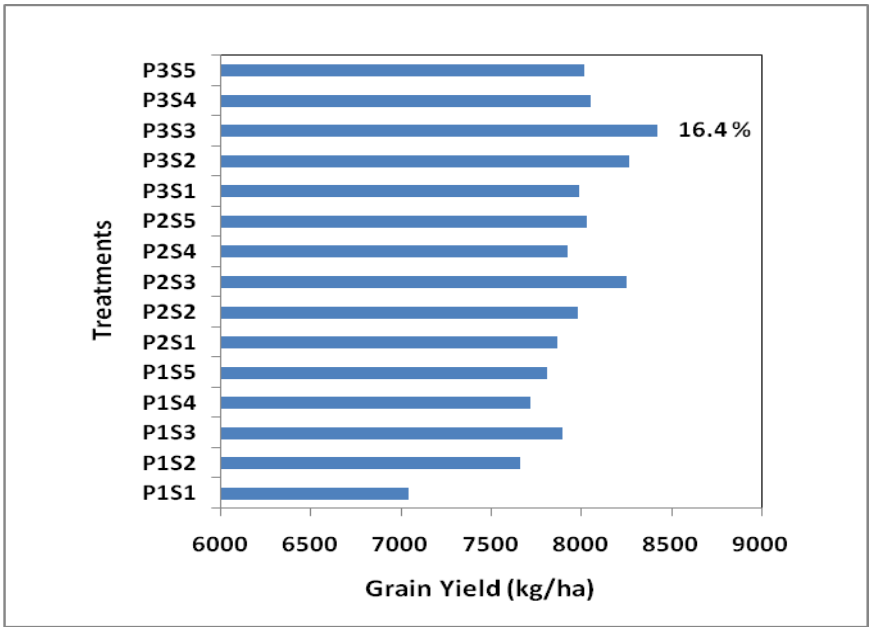

Table.1 Effect of P levels and *Penicillium bilaii* inoculants on total chlorophyll (mg g^{-1}) at different growth stages of rice

Treatments	AT (40 DAS)	PI (55 DAS)	F (85 DAS)	GF (105 DAS)	Mean
P_1S_1	1.23	1.75	2.42	1.78	**1.80**
P_1S_2	1.38	2.06	2.64	1.87	**1.99**
P_1S_3	1.42	2.25	2.76	1.90	**2.08**
P_1S_4	1.36	1.91	2.58	1.85	**1.93**
P_1S_5	1.32	1.84	2.46	1.84	**1.87**
P_2S_1	1.35	1.87	2.51	1.83	**1.89**
P_2S_2	1.50	2.18	2.76	1.89	**2.08**
P_2S_3	1.58	2.39	2.87	1.95	**2.20**
P_2S_4	1.44	2.05	2.65	1.88	**2.01**
P_2S_5	1.40	1.94	2.58	1.86	**1.95**
P_3S_1	1.43	1.95	2.63	1.87	**1.97**
P_3S_2	1.62	2.31	2.85	2.03	**2.20**
P_3S_3	1.66	2.57	3.03	2.13	**2.35**
P_3S_4	1.54	2.27	2.72	1.98	**2.13**
P_3S_5	1.47	2.16	2.66	1.93	**2.06**
Mean	**1.45**	**2.10**	**2.67**	**1.91**	
S at P SEd	**0.0466**	**0.0041**	**0.0033**	**0.0226**	
CD (P= 0.05)	**0.0961**	**0.0085**	**0.0069**	**0.0466**	

P_1 0% Phosphorus P_3 100% Phosphorus S_3 *JumpStart* 10E5 (0.966 g/kg of seed)
P_2 50% Phosphorus S_1 No seed treatment S_4 *JumpStart* 10E6 (9.662 g/kg of seed)
S_2 *JumpStart* 10E4 (0.097 g/kg of seed) S_5 Azophos (20 g/kg of seed)

Table.2 Effect of P levels and *Penicillium bilaii* inoculants on leaf carbohydrate content (mg g^{-1}) at different growth stages of rice

Treatments	AT (40 DAS)	PI (55 DAS)	F (85 DAS)	GF (105 DAS)	Mean
P_1S_1	25.30	42.01	52.04	40.21	**39.89**
P_1S_2	37.10	52.34	60.23	48.31	**49.50**
P_1S_3	42.50	57.19	67.32	53.64	**55.16**
P_1S_4	33.58	48.03	58.02	45.03	**46.17**
P_1S_5	30.20	42.25	56.12	43.12	**42.92**
P_2S_1	31.70	48.43	61.39	45.07	**46.65**
P_2S_2	44.24	60.12	71.33	57.43	**58.28**
P_2S_3	53.57	68.46	78.04	64.24	**66.08**
P_2S_4	36.21	56.26	65.21	53.67	**52.84**
P_2S_5	34.78	52.32	63.54	48.10	**49.69**
P_3S_1	38.23	54.05	68.56	52.09	**53.23**
P_3S_2	52.05	66.45	78.21	64.25	**65.24**
P_3S_3	62.36	74.65	82.06	73.69	**73.19**
P_3S_4	46.02	62.06	73.42	60.26	**60.44**
P_3S_5	41.25	58.40	70.29	57.44	**56.85**
Mean	**40.61**	**56.20**	**67.05**	**53.77**	
S at P SEd	**0.131**	**0.123**	**0.110**	**0.118**	
CD (P= 0.05)	**0.271**	**0.254**	**0.227**	**0.243**	

P_1 0% Phosphorus P_3 100% Phosphorus S_3 *JumpStart* 10E5 (0.966 g/kg of seed)
P_2 50% Phosphorus S_1 No seed treatment S_4 *JumpStart* 10E6 (9.662 g/kg of seed)
S_2 *JumpStart* 10E4 (0.097 g/kg of seed) S_5 Azophos (20 g/kg of seed)

Table.3 Effect of P levels and *Penicillium bilaii* inoculants on acid phosphatase activity (µmol of pNPP g^{-1} min^{-1}) at different growth stages of rice

Treatments	AT (40 DAS)	PI (55 DAS)	F (85 DAS)	GF (105 DAS)	Mean
P_1S_1	0.081	0.131	0.146	0.117	**0.119**
P_1S_2	0.055	0.064	0.084	0.061	**0.066**
P_1S_3	0.041	0.052	0.070	0.054	**0.054**
P_1S_4	0.060	0.097	0.096	0.083	**0.084**
P_1S_5	0.074	0.104	0.125	0.102	**0.101**
P_2S_1	0.068	0.114	0.132	0.106	**0.105**
P_2S_2	0.047	0.051	0.063	0.045	**0.052**
P_2S_3	0.036	0.042	0.048	0.039	**0.041**
P_2S_4	0.053	0.063	0.089	0.053	**0.065**
P_2S_5	0.061	0.082	0.103	0.087	**0.083**
P_3S_1	0.045	0.091	0.115	0.082	**0.083**
P_3S_2	0.031	0.046	0.058	0.038	**0.043**
P_3S_3	0.024	0.028	0.022	0.027	**0.025**
P_3S_4	0.033	0.051	0.074	0.041	**0.050**
P_3S_5	0.037	0.055	0.095	0.062	**0.062**
Mean	**0.050**	**0.071**	**0.088**	**0.066**	**0.069**
S at P SEd	**0.00975**	**0.00076**	**0.00066**	**0.00045**	
CD (P= 0.05)	**0.02013**	**0.00156**	**0.00136**	**0.00092**	

P_1 0% Phosphorus P_3 100% Phosphorus S_3 *JumpStart* 10E5 (0.966 g/kg of seed)
P_2 50% Phosphorus S_1 No seed treatment S_4 *JumpStart* 10E6 (9.662 g/kg of seed)
S_2 *JumpStart* 10E4 (0.097 g/kg of seed) S_5 Azophos (20 g/kg of seed)

Table.4 Effect of P levels and *Penicillium bilaii* inoculants on ATPase enzyme activity (µg of Pi g^{-1} h^{-1}) at different growth stages of rice

Treatments	AT (40 DAS)	PI (55 DAS)	F (85 DAS)	GF (105 DAS)	Mean
P_1S_1	21.41	33.22	57.48	26.85	**34.74**
P_1S_2	17.47	28.16	52.66	23.51	**30.45**
P_1S_3	13.39	25.62	49.86	18.55	**26.86**
P_1S_4	18.27	30.73	54.59	21.84	**31.36**
P_1S_5	20.17	31.27	56.15	25.07	**33.17**
P_2S_1	19.04	30.92	54.21	24.68	**32.21**
P_2S_2	15.54	24.80	43.34	19.37	**25.76**
P_2S_3	10.71	19.44	37.29	14.78	**20.56**
P_2S_4	16.48	26.46	48.47	20.47	**27.97**
P_2S_5	18.48	28.52	53.94	21.52	**30.62**
P_3S_1	17.53	27.49	49.74	20.24	**28.75**
P_3S_2	12.57	19.66	35.35	16.48	**21.02**
P_3S_3	8.38	15.63	29.37	12.67	**16.51**
P_3S_4	13.85	22.15	40.91	18.39	**23.83**
P_3S_5	15.54	26.44	46.09	19.69	**26.94**
Mean	**15.92**	**26.03**	**47.30**	**20.27**	**27.38**
S at P SEd	**0.946**	**1.116**	**1.594**	**1.005**	
CD (P= 0.05)	**1.953**	**2.302**	**3.289**	**2.075**	

P_1 0% Phosphorus P_3 100% Phosphorus S_3 *JumpStart* 10E5 (0.966 g/kg of seed)
P_2 50% Phosphorus S_1 No seed treatment S_4 *JumpStart* 10E6 (9.662 g/kg of seed)
S_2 *JumpStart* 10E4 (0.097 g/kg of seed) S_5 Azophos (20 g/kg of seed)

Table.5 Effect of P levels and *Penicillium bilaii* inoculants on phosphorus uptake (kg ha^{-1}) at different growth stages of rice

Treatments	AT (40 DAS)	PI (55 DAS)	F (85 DAS)	GF (105 DAS)	Mean
P_1S_1	5.91	21.67	38.99	25.58	**23.04**
P_1S_2	11.78	36.16	68.20	40.35	**39.12**
P_1S_3	16.27	42.50	75.33	48.20	**45.58**
P_1S_4	8.55	30.27	58.13	36.56	**33.38**
P_1S_5	6.20	25.46	46.59	32.35	**27.65**
P_2S_1	10.53	27.71	49.24	38.16	**31.41**
P_2S_2	17.30	46.68	78.67	47.67	**47.58**
P_2S_3	24.74	58.88	84.08	53.15	**55.21**
P_2S_4	11.74	38.75	65.18	41.78	**39.36**
P_2S_5	9.84	34.17	58.43	38.73	**35.29**
P_3S_1	12.01	39.61	65.86	45.92	**40.85**
P_3S_2	23.43	52.40	86.70	54.49	**54.26**
P_3S_3	34.75	65.85	92.91	67.96	**65.37**
P_3S_4	17.47	44.67	77.85	48.45	**47.11**
P_3S_5	14.64	40.45	68.57	43.41	**41.77**
Mean	**15.01**	**40.35**	**67.65**	**44.18**	
S at P SEd	**0.128**	**0.162**	**0.218**	**0.128**	
CD (P= 0.05)	**0.264**	**0.334**	**0.450**	**0.264**	

P_1 0% Phosphorus P_2 50% Phosphorus P_3 100% Phosphorus S_1 No seed treatment S_2 *JumpStart* 10E4 (0.097 g/kg of seed) S_3 *JumpStart* 10E5 (0.966 g/kg of seed) S_4 *JumpStart* 10E6 (9.662 g/kg of seed) S_5 Azophos (20 g/kg of seed)

The plasma membrane derived from active proteoid roots of P-deficient plants showed a more than two times higher ATPase activity than P-sufficient roots. Ullrich *et al.,* (1984) reported that a decrease in cytoplasmic pH and membrane depolarization stimulate a plasma membrane H+-ATPase that pumps protons out of the cell to maintain the intracellular pH and thus provides the proton driving force for Pi uptake. Organic acids are a common constituent and are effective in releasing soil P through a number of mechanisms. It includes reduction in rhizosphere pH that occurs in response to organic anion exudation, wherein H^+ ions are released as counter ions (Ryan and Angus, 2003).

Phosphorus uptake

Phosphorus is absorbed mainly during the vegetative growth; therefore most of its absorbed from is re-translocated in fruits and seed during the reproductive stages. Phosphorus uptake at active tillering stage is crucial to rice crop for higher grain yield. In the present study, at active tillering stage, phosphorus uptake was increased in 100% P level combined with *Penicillium bilaii* (*JumpStart* 10E5) seed treatment than control treatment by more than four times which is 71.5 % higher than control treatments (P_1S_1) as shown in (Table 5). This might be due to phosphorus solubilizing fungal inoculation which produced significant amount of organic acids and have enormous potential for increasing available P to the plant vicinity, simultaneously enhanced P uptake. The organic acid produced by *Penicillium bilaii* (Phosphorus Solubilising Fungus) complexes with the metal cations viz., calcium, aluminium, and iron, thereby helping in solubilization of native phosphorus and reduction in phosphorus sorption. Similar to the result of present study phosphorus solubilizing bacteria (*Bacillus subtills*), Phosphorus solubilizing fungus (*Aspergillus awamori*) and AM fungus (*Glomus fasciculatum*) increasing growth, N and P

uptake in green gram (Zaidi and Khan, 2006).

The data on the performance of phosphorus levels revealed a significant positive relation of grain yield with the increased phosphorus application. The highest grain yield (16.4 %) was recorded in P_3S_3 (100% P + *JumpStart* 10E4) compared to control treatment (P_1S_1) (Fig. 1). Similar to the present results, Xie*et al.*, (2007) reported that application of phosphorus fertilizer increased straw yield (9.4 t ha^{-1}) compared to control treatment (8.7 t ha^{-1}) in rice crop.

The higher levels of dry matter production and grain yield upon fungal inoculation was mainly due to higher enzyme activities in the rhizosphere and better nutrient availability besides the production of the plant growth regulators by PSM. Increase in the grain yield and straw yield of wheat was also observed when inoculated with phosphorus solubilising microorganism (PSM) (Kumar *et al.*, 1999).

In conclusion, application of recommended P fertilizer (50 kg ha^{-1}) along with the seed treatment of *JumpStart* 10E5 (0.966 g/ kg of seed) under machine transplanting system is suggested as a sustainable way for increasing rice yield. PSFs mainly *Penicillium bilaii*is very effective for increasing plant available Phosphorus in soil as well as the growth and yield of rice. Therefore, the use of the *Penicillium bilaii* through bio-inoculants has enormous potential for making use of ever increasing fixed Phosphorus in the soil and natural reserves of phosphate rocks. There is a need to explore *Penicillium bilaii* with greater efficiency and synergy with other microbes interacting with plants.

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