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Assessment of *Penicillium bilaii* inoculation in wheat (*Triticum aestivum* L.) for improving growth, yield and phosphorus availability in Mollisols of India

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Abstract: A field experiment was carried out with an aim to study the influence of two strains of *Penicillium bilaii* (PB -201 and PB-208) inoculation along with superphosphate application on growth, yield and P uptake of wheat (cv. PBW-343) and, also to examine the inoculation effect on P availability, forms of P and soil properties in Mollisols of Uttarakhand, India. The results showed that both strains of *P. bilaii* effectively solubilized tri-calcium phosphate in Pikovskaya agar medium, which was much higher over native fungal isolates. Wheat seed inoculation with *P. bilaii* strains along with superphosphate levels significantly influenced shoot height, shoot dry weight, number of total and effective tillers, yield attributes, yield components, tissue content and uptake of P. The treatment T_7 (*P. bilaii*, strain PB-208 + 50% P) has resulted into the highest amount of shoot height (87.9 cm at 90 DAS), shoot dry weight (1.5 and 3.8 g at 60 and 90 DAS, respectively), grain (66.8 q ha^{-1}) and straw yield (42.7 q ha^{-1}) and P uptake (26.5 kg ha^{-1}). The Olsen-P, organic carbon, dehydrogenase activity and fungal populations also increased in soil inoculated with *P. bilaii* strains combined with superphosphate application compared to the control soil. The conjoint use of the fungal strains with or, without P fertilization has developed an antagonistic interaction that has caused decline in yield, tissue content and uptake of P and its availability in soil. In conclusion, it is possible to reduce the rate of soluble P-fertilizer added by 50% without reducing yield, if wheat is inoculated with P-solubilizing fungi like *P. bilaii*.

Keywords: Mollisols, *Penicillium bilaii*, Phosphorus, P-solubilizing fungi, Wheat

INTRODUCTION

Phosphorus (P) deficiency is a constraint to plant growth worldwide (Khan and Joergensen, 2009). Deficiency of P leads to severe growth retardation, reduced tillering in cereal, poor quality and low yields (Havlin *et al*., 2009). To increase agricultural production, large amounts of P fertilizers are widely applied. Unfortunately, about 85-90% of the added P as chemical fertilizer becomes unavailable to plants in the year of application due to adsorption and precipitation with Fe, Al and Ca in the soil (Brady and Weil, 2008; Khan and Joergensen, 2009). Additionally, the conventional methods of P fertilization cause unsatisfactory and unsustainable environmental and economic problems and, lead to an overall reduction in soil fertility (Xiao *et al*., 2008). Hence, researchers are desperate to find alternate strategies that can ensure sustainable crop productions while protecting the soil health and environment. In this context, use of microbial inoculants capable of phosphate solubilization in agriculture represent an environmentally-friendly and economically sound alternative to the more expensive superphosphates and possess a greater agronomic utility (Khan *et al*., 2007).

Several soil microorganisms have the ability to solubilize insoluble mineral and organic phosphates, converting them into soluble forms that are available to plants through different mechanisms, such as acidification, chelation, exchange reactions and production of organic acids (Rodriguez *et al*., 2004; Relwani *et al*., 2008). *Penicillium bilaii* (PB), a rhizospheric filamentous fungus, is considered to be a key group of soil microflora involved in P cycling. The fungus is reported to increase dry matter production, growth rate, yield, and P uptake in many field grown crops in growth chamber and field experiments (Kucey *et al*., 1989, Saber *et al*., 2009). PB inoculation increased the $NaHCO_3$-extractable P and the incidence of P-solubilizing fungi in the rhizosphere (Kucey, 1988). The major low molecular weight organic acids produced by PB are oxalic and citric acid (Cunningham and Kuiack, 1992). This indirect evidence suggests that PB may increase the availability of P to the plant by releasing organic acids, which may act to acidify localized areas of the rhizosphere, or act as a chelator of cationic partners of the phosphate anion (Kucey, 1988). Most inoculants based studies have focused on the ability of specific fungi or bacteria to directly solu-

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bilize P from inorganic pools in the soil leading to increased soluble P available for plant uptake (Whitelaw, 2000). Inoculants may however, potentially increase soil soluble P by other mechanisms such as releasing P from organic pools.

Interaction between *P. bilaii* and wheat (*Triticum aestivum* L.) as a host plant is of great significance because, solubilization of insoluble phosphates and its subsequent availability can increase growth and grain yield production in wheat. Despite much of the work has gone into the study on P solubilization through inoculation of P solubilizing microorganisms with phosphate-rock (RP), tri-calcium phosphate (TCP) or, even without phosphate fertilization (Tarafdar and Rao, 1996; Wahid and Mehana, 2000; Singh and Reddy, 2011), there have been very few developments that have led to commercially available P solubilizing inoculants in combination with superphosphate that have a consistent plant growth response in soils. The main objective of our study was to investigate the effect of *P. bilaii* inoculation on growth, yield, yield attributing parameters and uptake of phosphorus in wheat. We also intended to measure the influence of *P. bilaii* inoculation on various forms of phosphorus and its availability and some selected soil properties.

MATERIALS AND METHODS

Fungal strains and media: Two effective strains of *P. bilaii viz.* PB-201 and PB-208 were procured from M/s. Novozymes Pvt. Ltd. Bengaluru, India for conducting the experiment. The procured *Penicillium* strains were maintained as slant culture on Pikovaskaya (PKV) agar medium modified by Sundara Rao and Sinha (1963). Morphologically all the colonies formed on PKV agar medium were uniform without any contaminants.

P solubilization: Qualitative estimation of phosphate solubilization efficiency of the fungi was carried out by performing the experiment of halo zone formation around the fungal growth after inoculation on PKV agar medium, amended with tri-calcium phosphate (TCP) and, incubated for 3 and 7 days at 28±2°C. In addition, appropriate serial dilutions of soil were also made and dilutions were plated on PKV media to isolate soil fungi present in the study site capable of dissolving phosphates. The fungal isolate from soil and procured *P. bilaii* strains were incubated and observed for development of clear halo zone around the colonies. Transparent zone of clearing around microbial colonies indicate the extent of phosphate solubilization (Sundara Rao and Sinha, 1963). The basic objective of this experiment was to observe the phosphate solubilization efficiency of the procured *P. bilaii* strains compared to soil fungal isolates. The decoding of isolates isolated from soil and procured culture of *P. bilaii* strains are given below:

Code	Detail
Pb_1	*P. bilaii* PB-201
Pb_2	*P. bilaii* PB-208
Pb_1+Pb_2	*P. bilaii* PB-201 and PB-208
F_1W_1	Fungi 1 white 1
F_2W_3	Fungi 2 white 3
F_3B_1	Fungi 3 black 1

Here, F_1W_1, F_2W_3 and F_3B_1 were the unidentified phosphate solubilizing fungi isolated from soil. The terms like 'white' and 'black' referred to here, was designated on the basis of colour of the fungal spores.

Study site and soil: The study was carried out in the *rabi* season (cooler winter-spring period, December 2009 to April 2010) at GBPUA&T, Pantnagar, Uttarakhand, India (29°N, 79°3′E; 244 m msl). The site is characterized as humid sub-tropical having hot dry summers and cold winters with annual maximum and minimum air temperature usually ranges between 40 to 3 °C, respectively. The mean annual rainfall ranges from 1300 to 1500 mm, mostly confined from June to September. The total precipitation during the course of this study was 35.6 mm and average temperature ranged from 11.7 to 28.9 °C. The soil of study area is derived from calcareous alluvium and, has been classified as Mollisol (Typic Hapludolls, USDA Classification). Table 1 shows initial properties of the soil (0-15 cm).

Experimental design and treatments: Seeding of wheat (*cv.* PBW-343) was done in December, 2009. Prior to seeding, seeds were treated with the respective *P. bilaii* strains by using 6 ml of fungi inoculum kg^{-1} wheat seed. The treated seeds were dried for half an hour under shed and, then sown in individual plots (2.4 × 3.0 m) at 5 cm soil depth. Soil was irrigated once before sowing to ensure adequate soil water for seedling establishment and, then regularly during crop growth to maintain sufficient soil moisture. A uniform dose of 120 kg N ha^{-1} in three equal splits (pre-sowing, 45 and 70 days after sowing) and 40 kg K_2O ha^{-1} as basal was applied through urea and muriate of potash, respectively. Other recommended agronomic practices were also followed throughout the experiment for raising the crop. Phosphorus, through superphosphate (16% P_2O_5 approx.), was applied in accordance to the treatments and, a recommended dose of 65 kg P_2O_5 ha^{-1} was considered as 100% P. The following treatments consisting combinations of different levels of superphosphate with

Treatments	
Uninoculated + 0% P	T_1 (Control)
Pb_1 + 0% P	T_2
Pb_2 + 0% P	T_3
Pb_1 + Pb_2 + 0% P	T_4
Uninoculated + 50% P	T_5 (Control)
Pb_1 + 50% P	T_6
Pb_2 + 50% P	T_7
Pb_1 + Pb_2 +50% P	T_8
Uninoculated + 100% P	T_9 (Control)
Pb_1 + 100% P	T_{10}
Pb_2 + 100% P	T_{11}
Pb_1 + Pb_2 + 100% P	T_{12}

and without *P. bilaii* strains were laid out in randomized block design in four replications:

The crop was harvested at 131 days after sowing (DAS), dried in shed and the grains were separated and, yield was recorded plot wise for grain and straw.

Growth and yield attributes: Four growth parameters *viz.* shoot height, shoot dry weight and number of total and effective tillers, were selected for the study of growth in wheat. Five plants from each plot were selected randomly and, shoot height was measured at 60 and 90 DAS and averaged. For dry weight, three plants were uprooted from each plot at 60 and 90 DAS, washed and then dried at 70°C to constant weight, and their dry weights were averaged. The number of total and effective tillers was measured at harvesting from a square meter area of net plot area. Yield attributes were estimated in terms of number of spikelets per ear, number of grains per ear and weigh of 1000 grains (test weight). Ten ears were collected randomly from each plot at harvesting and, number of spikelets and grains were counted and averaged.

P uptake: The harvested samples were analyzed for P contents in grain and straw by digestion with HNO_3:$HClO_4$ (4:1) mixture and, subsequent determination by vanadophosphomolybdic acid (Jackson, 1973). Dry matter yield and nutrient content of grain and straw were used to determine uptake of P.

Soil analysis: Composite soil samples of 0-15 cm depth were collected after harvesting. Soil samples were air dried, processed and sieved through 2 mm sieve and stored for further analytical purpose. A portion of soil sample was separated prior to air drying and stored at 4°C for determination of dehydrogenase activity (DHA) and fungal population. Available P in soil was determined following Olsen method (Olsen *et al*., 1954). Inorganic P (P_i) was measured by non-ignition and, organic P (P_o) was determined following by ignition method (Walker and Adams, 1958). Total P (P_t) was estimated by digestion with $HClO_4$ and, the digested residue was further fused with Na_2CO_3 to extract all remaining P from each sample (Jackson, 1973). The soil was also analyzed for pH, organic C (Walkley and Black, 1934), mineralizable-N (Subbiah and Asija, 1956) and acetate-extractable K (Jackson, 1973). DHA in samples was measured by reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) using colorimetric procedure of Tabatabai (1982). Population of fungi was determined by plate count method using Martin's Rose Bengal streptomycin agar medium (Subba Rao, 1986). Colonies were counted in replicates of 10^{-3}, 10^{-4} and 10^{-5} dilutions and their averages were multiplied with dilution factor to obtain the fungal population (colony forming unit, CFU). The laboratory and field data were analyzed statistically for analysis of variance (ANOVA) by procedure outlined by Gomez and Gomez (1984). The level of significance was tested at $p \leq 0.05$.

RESULTS

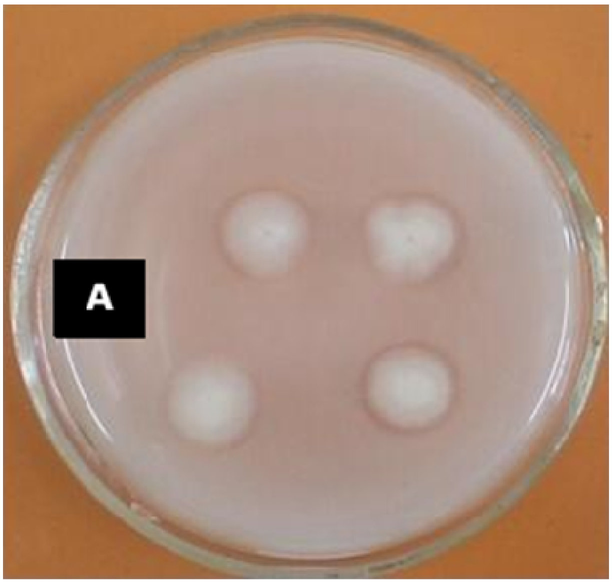
In vitro **phosphate solubilization efficiency of *Penicillium* strains and soil isolates:** The tested fungi showed different abilities to dissolve tri-calcium phosphate in PKV agar medium. Phosphate solubilizing efficiency of soil isolates and *P. bilaii* strains was graded based on diameter of halo zones by assigning the positive (+) marks and given in Table 2 and Fig. 1. Higher number of (+) marks indicates better solubilization efficiency. It is clearly seen from the table that phosphate solubilization efficiency of the procured *P. bilaii* strains was much higher over the fungal isolates from soil. It is also evident that phosphate solubilization by the combined inoculation of *P. bilaii* strains (Pb_1+Pb_2) was less, as compared to the strains individually. They can be arranged in a decreasing order, according to their efficiency, as follows: $Pb_2 > Pb_1 > Pb_1 + Pb_2 > F_2W_3 > F_1W_1 > F_3B_1$.

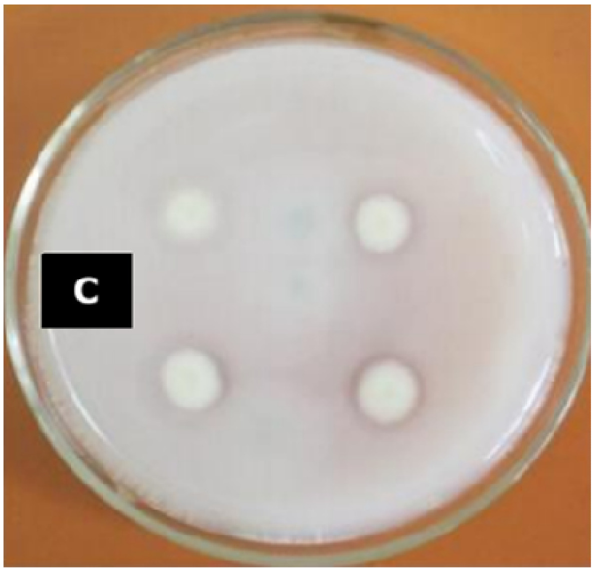
Growth and yield attributes: The shoot height ranged from 41.4 to 53.9 and 76.3 to 87.9 cm among different treatments at 60 and 90 DAS, respectively (Fig. 2a). It can be seen from the figure that highest shoot height was recorded in T_{10} and T_7 at 60 and 90 DAS, respectively. And, the lowest was recorded in T_1 at 60 and 90 days after sowing. However, inoculation effect of both the strains of PB along with phosphate fertilization on shoot height was statistically non-significant. The treatments significantly increased shoot dry weight at 60 DAS to their corresponding control while, they failed to influence the same at 90 DAS. Like shoot height, T_7 also registered the maximum shoot dry weight at both 60 and 90 DAS (Fig. 2b). The total and effective tillers m^{-2} ranged from 219.0 to 257.3 and 205.0 to 249.0 in T_1 and T_7, respectively (Fig. 3a). They were also statistically significant compared to their respective control. It was interesting to observe that individual effect of the fungal strains was more than their conjoint use, without P fertilization, in all of the growth parameters. Even, the effect of conjoint use of the fungal strains with P fertilization on plant growth was at par, with their lone use combined with P fertilization. Number of spikelets and grains ear^{-1} were also affected significantly by the treatments. Highest increase in numbers was observed in T_7 while, the lowest was recorded in T_1 (Fig. 3b). Like other yield attributes, maximum test weight was also recorded in T_7 (44.3 g).

Yield components and P uptake: Table 3 indicates that the yield components of the tested crop significantly increased as a result of soil inoculation with *P. bilaii* strains. However, this effect was not same for both the inoculants. Moreover, the influence of *P. bilaii* (strain PB-208) on grain and straw yield was higher over the combined inoculation of the strains, with P fertilization. The influence of the treatments T_2, T_3 and T_4 on grain yield was at par as compared to their respective control (T_1). Inoculation of Pb_2 with 50% P (T_7) increased the highest grain and straw yield

Table 1. Initial soil and microbial properties of the experimental site.

S.N.	Soil property	Values	Method employed	Reference
1.	Mechanical-analysis		Hydrometer	Piper (1967)
	Sand (%)	50		
	Silt (%)	30		
	Clay (%)	20		
	Textural class	Sandy loam		
2.	pH	7.47	1:2 (w/v) soil to water suspension	Jackson (1973)
3.	Electrical conductivity (dS m^{-1})	0.42	Conductivity bridge	Jackson (1973)
4.	Organic C (g kg^{-1})	7.1	Chromic acid oxidation	Walkley and Black (1934)
5.	Cation exchange capacity (meq 100 g^{-1})	17.6	Sodium acetate	Jackson (1973)
6.	Mineralizable N (mg kg^{-1})	84.4	Alkaline permanganate	Subbiah and Asija (1956)
7.	Acetate-extractable K (mg kg^{-1})	82.1	1 N CH_3COONH_4, pH 7.0	Jackson (1973)
8.	Olsen P (mg kg^{-1})	12.0	0.5 M $NaHCO_3$, pH 8.5	Olsen *et al*., (1954)
9.	Inorganic P (mg kg^{-1})	66.9	Non-ignition	Walker and Adams (1958)
10.	Organic P (mg kg^{-1})	135.6	Ignition	Walker and Adams (1958)
11.	Total P (mg kg^{-1})	206.1	Na_2CO_3-fusion	Jackson (1973)
12.	Dehydrogenase activity (µg TPF g $soil^{-1}$ 24 h^{-1})	215.1	Colorimetric	Tabatabai (1982)
13.	Fungal population, CFU (propagules g $soil^{-1}$)	29.5×10^3	Serial dilution	Subba Rao (1986)





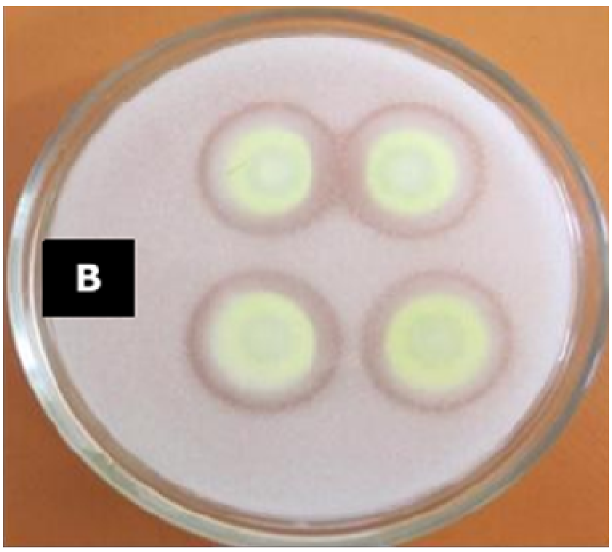


Fig. 1. *Phosphate solubilization efficiency of Penicillium strains. (A) P. bilaii strain PB-201; (B) P. bilaii strain PB-208 and (C) combination of both strains.*

Table 2. Comparative phosphate solubilization efficiency of soil isolates and *P. bilaii* strains.

Code	Comparative solubilization efficiency*
Pb_1	+ + + + +
Pb_2	+ + + + + +
Pb_1+Pb_2	+ + + +
F_1W_1	+ +
F_2W_3	+ + +
F_3B_1	+

*Average of 3 and 7 days of incubation, Pb_1: *P. bilaii* strain PB-201, Pb_2: *P. bilaii* strain PB-208 of wheat by 21.7 and 26.3 per cent, respectively, over 50% P alone (T_5). However, the influence of T_6 and T_8 on grain and straw yield was at par compared to T_5. The treatments T_{10}, T_{11} and T_{12} increased the grain yield by 9.0, 16.3 and 6.0 per cent, respectively, over 100% P (T_9) alone. However, the effect of all the treatments, except T_{11}, was also at par.

Table 3 reveals that phosphorus content in grain and straw and its uptake significantly increased in soil treated with either phosphate compound and inoculated with PB strains, corresponding to the controls. The table also indicates that the individual PB strain resulted in higher increase in P content and uptake over their conjoint use with or, without P fertilization. The inoculation of Pb_2 with 50% P (T_7) resulted in highest increase in P content in grain and straw and its uptake. The increase was by 38.0 and 38.1 per cent for P content in grain and straw, respectively and 53.4 per cent for P uptake, over T_9.

Forms of P: The levels of inorganic (P_i), organic (P_o) and total P (P_t) were increased with increasing level of P fertilization, either inoculated with individual *P. bilaii* strains, or in combination (Table 4). They were also statistically significant compared to their respective control. However, a non-significant decline was observed in Olsen-P when P fertilization increased from 50% to 100% inoculated with individual strains or, conjoint use (Table 4). It is clearly seen from the table that Olsen-P ranged from 11.7 to 15.4 mg kg^{-1} soil and T_7 resulted in maximum level amongst the treatments. P_i, P_o and P_t ranged from 63.74 to 81.60, 131.62 to 165.75 and 195.40 to 247.40 mg kg^{-1} soil, respectively. The share of organic P to total P on an average ranged from 16 to 46 per cent among different treatments.

Soil properties: Data pertaining to various soil properties are presented in Table 5. Two strains of *P. bilaii* with different levels of P showed significant differences in pH and organic C over respective controls. An overall decrease was observed in soil pH in all of the treatments corresponding to the controls. However, an opposite phenomena was recorded in organic C. The treatments failed to influence mineralizable-N and acetate-extractable K in soil however, a non-significant decline in their levels was observed compared to the controls. Table 5 reveals a significant increase in fungal population (CFU) and DHA activity in soil after harvest. The fungal population and DHA activity of the experiment varied between 67.35×10^3 to 156.25×10^3 CFU and 829.9 to 1385.3 mg TPF g^{-1} 24 h^{-1}, respectively.

DISCUSSION

In the present study, two strains of *P. bilaii* were tested for their efficiency to solubilize tri-calcium phosphate compared to native fungal isolates present in soil. Higher phosphate solubilization efficiency of the procured *P. bilaii* strains over the soil fungal isolates indicates that whatever phosphate solubilization and, its subsequent availability to wheat occurred was mainly contributed by *P. bilaii* strains. *Penicillium* strains have been studied for their phosphate solubilizing activity and used as biofertilizers (Whitelaw, 2000). As phosphorus is known to initiate cell division and enlargement processes, the increase in growth parameters among different treatments over their respective controls could probably be due to increased mobilization of phosphorus made soluble by P-solubilizing fungi from soil reserves (Reyes *et al*., 2002). Improvement in crop growth with inoculation with P-solubilizing microorganisms is reported by many authors (Wakelin *et al*., 2007; Singh and Reddy, 2011; Patil *et al*., 2012). Seshadri *et al*. (2000) reported that the native soil P is utilized for growth and development of the organism when applied to soil environment. In this experiment, P was applied in readily soluble form, which perhaps fulfilled the demand for P by the *P. bilaii* strains. This was probably the reason that the growth parameters were much higher in the treatments with P fertilization as compared to without P fertilization, inoculated with *P. bilaii* strains. It may also be because that application of P fertilizer facilitated proper and timely availability of phosphorus to the crop, resulting in an increase in plant growth. This also indicates that P-solubilizing microorganisms when applied without P fertilization are not much effective in improving plant growth. Similar kind of observation in maize is also reported by Patil *et al*. (2012). In addition to solubilization of insoluble phosphates, the P-solubilizing microorganisms are also known to produce phytoharmones, growth promoting substances, vitamins, amino acids and other substances responsible for enhanced plant growth (Singh and Reddy, 2011; Patil *et al*. 2012). Improvement in wheat growth parameters, as a result of inoculation with *P. bilaii* strains, could also be supported by this. Ramezanpour *et al*. (2010) reported that inoculation of phosphorus solubilizing bacterial strain of *Pseudomonas* significantly increased spike length, number of panicle, number of spikelet per panicle and weight of 1000 grains in rice. A similar kind of observation with *P. bilaii* strains is also recorded in this experiment. Improvement in growth parameters perhaps also lead to an increase in yield attributes. This could again be ascribed to adequate P supply to wheat by the *P. bilaii* strains through phosphate solubilization. Increase in yield attributes also reflected in the yield of wheat.

The highest amount of yield components under the treatment T_7 could be explained on the basis that values of the highest available P was recorded in the same treatment. Under field condition, crop yield depends on the availability of P. A similar kind of result in soil inoculated with *P. pinophilum* in wheat and faba bean is also reported by Wahid and Mehana (2000). They concluded that soil inoculation with *P. pinophilum* resulted in an increase in available P, which in turn gave the highest yield in both crops. It was astonishing

Table 3. Effect of inoculation of two *P. bilaii* strains with different levels of phosphorus application on yield, content and uptake of P in wheat.

Treatments	Yield (q ha^{-1})		P content (%)		P uptake (kg ha^{-1})
	Grain	Straw	Grain	Straw	
T1: Uninoculated + 0% P	50.6	31.0	0.22	0.16	11.8
T2: Pb_1 + 0% P	53.4	35.0	0.28	0.18	16.1
T3: Pb_2 + 0% P	55.7	36.6	0.32	0.19	18.7
T4: Pb_1 + Pb_2 + 0% P	57.8	34.5	0.27	0.17	15.1
$S.E_m.\pm$	2.3	1.6	0.01	0.01	1.07
LSD ($p\leq0.05$)	7.6	4.8	0.04	0.02	3.07
T5: Uninoculated + 50% P	54.8	33.8	0.26	0.19	15.2
T6: Pb_1 + 50% P	61.4	38.3	0.33	0.24	21.7
T7: Pb_2 + 50% P	66.8	42.7	0.40	0.29	26.5
T8: Pb_1 + Pb_2 + 50% P	60.5	34.7	0.32	0.23	19.4
$S.E_m.\pm$	2.8	2.0	0.01	0.01	1.05
LSD ($p\leq0.05$)	7.2	5.2	0.05	0.02	3.00
T9: Uninoculated +100% P	56.3	34.6	0.29	0.21	17.3
T10: Pb_1 + 100% P	56.4	36.0	0.30	0.22	18.7
T11: Pb_2 + 100% P	65.4	38.6	0.36	0.23	25.2
T12: Pb_1 + Pb_2 + 100% P	59.2	35.2	0.29	0.21	17.3
$S.E_m.\pm$	2.5	1.8	0.01	0.01	1.09
LSD ($p\leq0.05$)	7.2	5.0	0.04	0.02	3.02

Pb_1: *P. bilaii* strain PB-201, Pb_2: *P. bilaii* strain PB-208, 100% P: 65 kg P_2O_5 ha^{-1}

Table 4. Effect of inoculation of two *P. bilaii* strains with different levels of phosphorus application on different forms of P in soil after harvest.

Treatments	Olsen P (mg kg^{-1})	Inorganic P (mg kg^{-1})	Organic P (mg kg^{-1})	Total P (mg kg^{-1})
T1: Uninoculated + 0% P	11.7	63.7	131.6	195.4
T2: Pb_1 + 0% P	13.0	70.5	142.9	213.5
T3: Pb_2 + 0% P	13.5	69.9	139.7	209.7
T4: Pb_1 + Pb_2 + 0% P	13.0	71.1	145.3	216.4
$S.E_m.\pm$	0.55	3.00	6.84	8.71
LSD ($p\leq0.05$)	1.60	8.65	10.5	17.5
T5: Uninoculated + 50% P	12.3	66.3	150.3	216.7
T6: Pb_1 + 50% P	13.7	73.9	151.2	225.3
T7: Pb_2 + 50% P	15.4	71.9	145.0	217.1
T8: Pb_1 + Pb_2 +50% P	13.6	76.2	151.1	227.5
$S.E_m.\pm$	0.51	3.00	6.00	8.00
LSD ($p\leq0.05$)	1.60	8.10	4.62	8.90
T9: Uninoculated + 100% P	12.6	69.4	166.2	235.6
T10: Pb_1 + 100% P	13.5	80.7	164.2	245.0
T11: Pb_2 + 100% P	13.9	78.7	162.7	241.5
T12: Pb_1 + Pb_2 + 100% P	13.4	81.6	165.7	247.4
$S.E_m.\pm$	0.50	3.00	3.85	9.00
LSD ($p\leq0.05$)	1.50	8.53	5.00	9.88

Pb_1: *P. bilaii* strain PB-201, Pb_2: *P. bilaii* strain PB-208, 100% P: 65 kg P_2O_5 ha^{-1}

to observe that the yield components were not increased further when P fertilization increased from 50 to 100%, inoculated with *Penicillium* strains. Therefore, it can be concluded that the initial soil P was already present in adequate quantity. It means that the adequate amount of P in the soil provided with enough resources for P-solubilizing microorganisms to provide high levels of available P, while application of high level of P fertilizer led to an antagonistic interaction with the fungal strains. Thus, it can be concluded that the high rate of P fertilizer application (65 kg P_2O_5 ha^{-1}) might lead to an antagonistic interaction with *P. bilaii*, especially in soil high in available P. Mehrvarz *et al*. (2008) obtained the maximum dry matter yield of 17.2 t ha^{-1} with sole application of 60 kg ha^{-1} P fertilizer which was not significantly different from using *Pseudomonas petida* combined with 60 kg ha^{-1} P fertilizer in barley. Thus, our observation confirms the finding of Mehrvarz *et al*. (2008). A non-significant decline in yield components with conjoint use of *Penicillium* strains over their individual use with 50 and 100% P indicates that, an antagonistic interaction between the *P. bilaii* strains also existed in this experiment. This antagonistic interaction was also evident in P content and its uptake by wheat. Patil *et al*. (2012) also recorded the maximum plant height, cob length and 1000

Table 5. Effect of inoculation of two *P. bilaii* strains with different levels of phosphorus application on various soil properties after harvest.

Treatments	pH	Org. C ($g\ kg^{-1}$)	Min-N ($mg\ kg^{-1}$)	Ac-K ($mg\ kg^{-1}$)	DHA (μg TPF g $soil^{-1}$ 24 h^{-1})	Fungal population ,CFU (propagules g $soil^{-1}$, $\times 10^3$)
T1: Uninoculated + 0% P	7.41	5.4	83.7	79.8	829.9	71.3
T2: Pb_1 + 0% P	7.36	5.8	83.5	79.6	954.6	79.1
T3: Pb_2 + 0% P	7.39	5.9	79.8	78.9	1205.8	108.3
T4: Pb_1 + Pb_2 + 0% P	7.36	5.4	82.5	79.3	1310.3	121.7
$S.E_m.\pm$	0.06	0.3	2.1	2.8	79.1	4.3
LSD ($p\leq0.05$)	0.19	0.9	ns	ns	227.7	12.6
T5: Uninoculated + 50% P	7.32	6.1	83.8	80.6	971.6	92.0
T6: Pb_1 + 50% P	7.28	6.3	80.3	78.3	1098.6	99.1
T7: Pb_2 + 50% P	7.21	6.7	75.2	78.8	1504.5	156.2
T8: Pb_1 + Pb_2 + 50% P	7.31	6.3	76.7	80.3	1385.3	139.1
$S.E_m.\pm$	0.05	0.3	3.1	2.8	87.6	4.3
LSD ($p\leq0.05$)	0.19	0.9	ns	ns	227.7	12.6
T9: Uninoculated + 100% P	7.49	6.2	81.2	79.4	865.3	76.7
T10: Pb_1 + 100% P	7.28	6.5	78.4	78.2	970.6	86.3
T11: Pb_2 + 100% P	7.34	6.6	76.9	77.2	1340.9	132.4
T12: Pb_1 + Pb_2 + 100% P	7.41	6.4	79.7	78.6	1333.5	131.6
$S.E_m.\pm$	0.06	0.2	2.5	2.8	84.3	4.3
LSD ($p\leq0.05$)	0.19	0.9	ns	ns	227.7	12.6

Org. C: Organic C; Min-N: Mineralizable N; Ac-K: Acetate-extractable K; DHA: Dehydrogenase activity; ns- Non-significant; Pb_1: *P. bilaii* strain PB-201; Pb_2: *P. bilaii* strain PB-208; 100% P: 65 kg P_2O_5 ha^{-1}

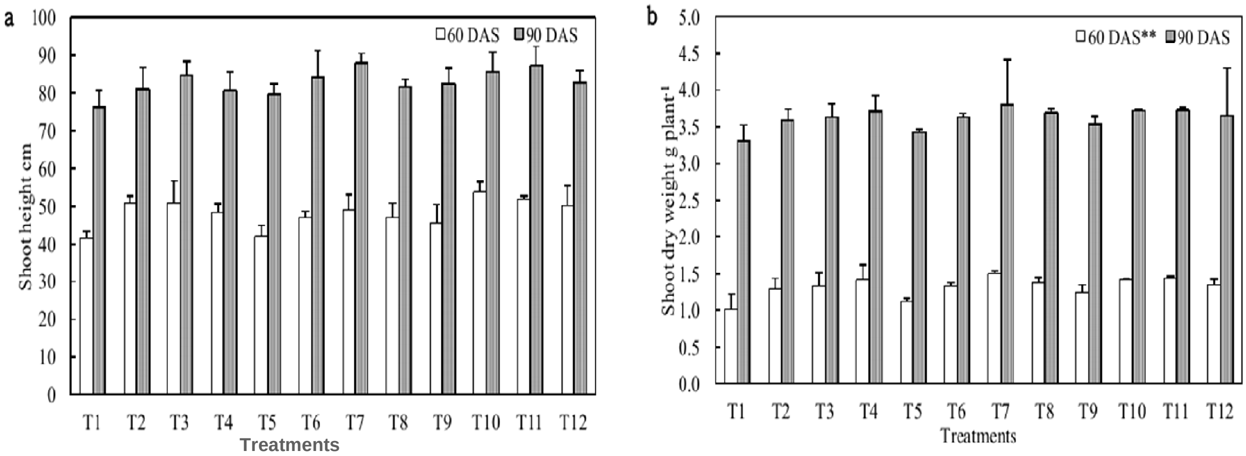


Fig. 2. *Effect of inoculation of two P. bilaii strains with different levels of phosphorus application on (a) shoot height and (b) shoot dry weight of wheat at 60 and 90 DAS. Bars indicate standard error (n=4). **Significant at p≤ 0.05.*

grain weight in maize in the treatment that received *P. bilaji* (Jump start 2) + 75 kg P_2O_5 ha^{-1}, which were at par with the treatments *P. bilaji* (Jump start 1) + *P. bilaji* (Jump start 2) + 75 kg P_2O_5 ha^{-1} and *P. bilaji* (Jump start 1) + *P. bilaji* (Jump start 2) + 38 kg P_2O_5 ha^{-1}. Therefore, our findings could be supported the observations made by Patil *et al*. (2012). Dual inoculation has often been shown to have no effect, or even a negative effect on plant growth and yield (Owen *et al*., 2014). For instance, positive growth responses have been reversed by dual inoculation, even though colonization rates of the multi-inoculants remained the same (Dodd and Ruiz-Lozano, 2012). The particular mechanism mediating the antagonism could not be identified from this single study and requires further investigation to confirm such observation. Gulden and Vessey (2000) observed that *P. bilaii* inoculation increased root hair production in pea and, they concluded that *P. bilaii* has the potential to greatly influence the nutrient absorptive capacity of roots. This increase in nutrient absorptive capacity could result in an increase in P uptake in crops. Highest increase in shoot P uptake also occurred in treatment that had the highest level of Olsen-P. This suggests that uptake of P by wheat was not necessarily associated with P solubilization by the fungus. Increased capacity of roots to absorb P could have also accounted for improved P uptake.

The major portion of the P that is applied to soil rapidly becomes converted into inorganic and organic fractions which are poorly available to plants for uptake (Brady and Weil, 2008). Filamentous fungi,

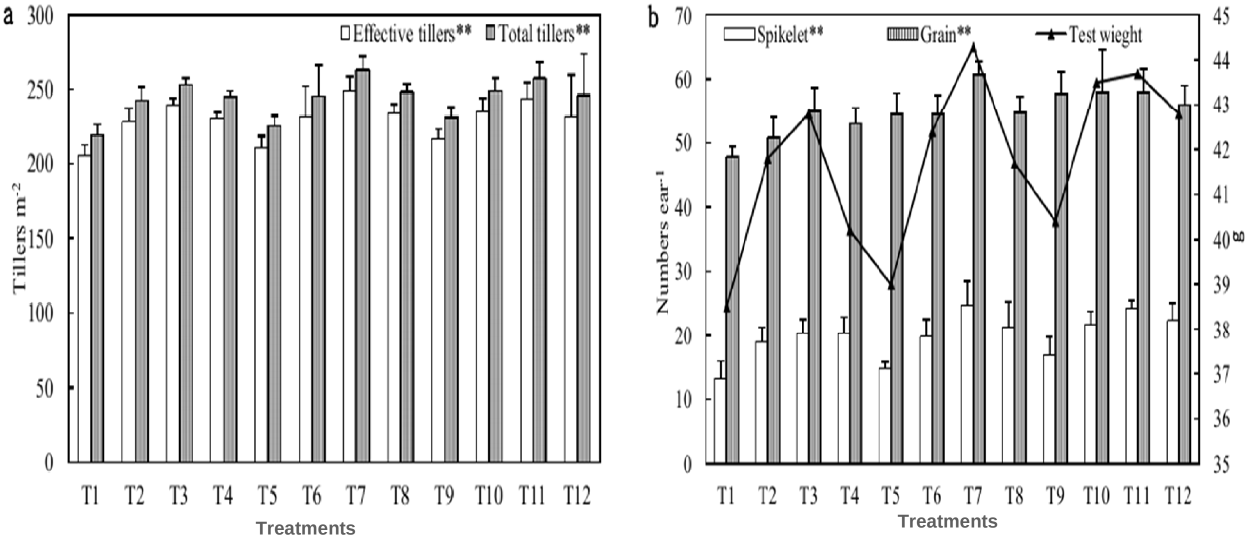


Fig. 3. *Effect of inoculation of two P. bilaii strains with different levels of phosphorus application on (a) effective and total tillers and (b) yield attributing characters of wheat at harvest. Bars indicate standard error (n=4). **Significant at p≤ 0.05.*

mainly *Aspergillus* and *Penicillium* are widely used to solubilize insoluble phosphates (Reyes *et al*., 2006). This ability is generally associated to the release of organic acids, such as citric acid, oxalic acid, malic acid and gluconic acid with ion chelation solubilize inorganic P sources (Reyes *et al*., 2006; Gadagi *et al*., 2007). Addition of P through readily soluble form as well as solubilization of inorganic P-fraction by the *P. bilaii* strains was mainly responsible for increased level of Olsen-P in soil after wheat harvest. Moreover, in absence of P fertilization, the *P. bilaii* strains were also able to solubilize insoluble phosphates that led to an increase in Olsen-P over the control. However, determination of the type of organic acids released and their quantification would reveal much more information regarding the difference between the two strains in solubilizing inorganic P. The decrease in Olsen-P level with concomitant increase in P fertilization has resulted in an increase in inorganic and organic P level in soil. This suggests that with the increase in P fertilization rate, P became converted into inorganic and organic P in soil. This is obvious but however, it seems that inoculation of *P. bilaii* strains did not have any influence in increasing Olsen-P level with the increasing rate of P fertilization. This result perhaps indicates that *P. bilaii* strains are not much effective in increasing available P in soil when combined with high rate of P fertilization.

Many microorganisms can mobilize P from inorganic compounds simply by lowering pH occurring as a result of general metabolic activity (Zaidi and Khan, 2007). The lowering of soil pH in this experiment suggests the release of organic acids by the fungal strains. The synthesis and discharge of organic acid by the fungal strains could acidify their surrounding environment that ultimately lead to the release of P ions from the insoluble P mineral by H^+ substitution. Nonetheless, a dramatic shift in pH would be required to increase its solubility, and, therefore mechanisms such as secretion of strongly chelating organic acids (or the conjugate anions) must be expressed to affect P solubilization (Wakelin *et al*., 2004). P fertilization along with P solubilizing microorganism has led better crop growth, which in turn released good amount of root exudates and assimilable organic C to the soil. This was probably the reason that organic carbon content was greater in inoculated treatments as compared to un-inoculated ones. This result is in agreement with the findings of Singh and Reddy (2011) who observed an increase in soil organic carbon when inoculated with *P. oxalicum* over control. Improved crop growth due to increased availability of P by *P. bilaii* strains has definitely increased the demand for other nutrients by wheat crop. Therefore, levels of mineralizable-N and acetate-extractable K were declined in inoculated treatments over the controls. The bulk increase in fungal population and dehydrogenase activity was probably due to good growth of the treated fungus and other fungal genera in soil and, thereby enhancing their activity in soil. Plant's active root system releases about 17% of photosynthate captured in the form of organic compounds into the rhizosphere, most of which is available to the soil microorganisms for their growth and optimum activity (Nguyen, 2003). Dehydrogenase activity in soil depends on the content of soluble organic carbon and, the increased organic C in soil could enhance the soil enzyme activities (Debnath *et al*., 2015). Release of root exudates and assimilable organic C to the soil by wheat perhaps maintained a favorable condition for microbial growth and activity which, in turn could have also resulted in an increase in fungal population and dehydrogenase activity in inoculated treatments.

Conclusion

From these results it was found that the influence of the two *P. bilaii* strains was not similar in terms of measured parameters in this experiment. Even, their conjoint use did not have the most effective influence over their individual use with or, without P fertilization. One of the major reasons for such difference is attributed to the nature and amount of organic acids released by them. Another reason is extended to the genotype and physiological state of the inoculated strains. The size and composition of the populations sustained by the rhizosphere is determined by several environmental factors like pH, mineral nutrients, water content, crop species and the presence of other microbial species. Therefore, selection of specific strain for a particular crop is of paramount importance and can support the shift towards sustainable production system. The phosphatic fertilizer in current use scenario requires a greater input that cannot be afforded by the small to marginal farmers of the developing nations like India. With the results of this experiment, it is possible to reduce the recommended dose of P-fertilizer added by 50% without reducing yield, if wheat is inoculated with P-solubilizing fungi such as *P. bilaii*.

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