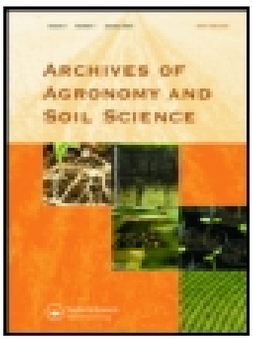

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Improvement of crop yield by phosphate-solubilizing *Aspergillus* species in organic farming

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ABSTRACT

Phosphate-solubilizing fungal strains were isolated from organically managed soil and tested for their ability to solubilize rock phosphate (RP), ferric phosphate and aluminium phosphate. These strains were identified as *Aspergillus tubingensis* and *Aspergillus niger* based on internal transcribed spacer sequence analysis. A field study was conducted in two different seasons in organically managed soil to test the efficacy of two strains, *A. tubingensis* (PSF-4) and *A. niger* (PSF-7) on the yield and soil fertility. RP was amended at the rate of 59 kg P_2O_5 ha^{-1} to study the effect of RP on soil fertility. The maize was grown in rainy season (July–October 2011) and wheat in winter season (November 2011–April 2012). Plant heights, shoot and root dry biomass and phosphorous (P) uptake in roots, shoots and grains were significantly increased due to inoculation in both crops. The yield of maize and wheat were significantly increased when inoculated along with RP fertilization. Organic carbon, P levels and soil enzyme activities were significantly increased due to inoculation. Results of present study suggested that *A. tubingensis* and *A. niger* improved the crop yield and soil fertility of organic farm when inoculated with RP fertilization.

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KEYWORDS
Phosphate-solubilizing fungi; *Aspergillus tubingensis*; *Aspergillus niger*; organic farming; maize; wheat

Introduction

Organic agriculture is a method of production that not only avoids the use of synthetic chemicals but also 'relies on ecological processes and biodiversity' (International Federation of the Agriculture Movement (IFOAM 2008)). Phosphorus is an essential mineral nutrient most commonly limiting the growth of crops. The phosphate fertilization of soils has always been important because vast areas of agricultural land are poor in soil fertility. Phosphorus deficiencies are limiting crop production in many agricultural soils worldwide. The nutrient reservoirs in the soil shrink when crops are removed from the field at harvest which creates a phosphorous (P) deficit, necessitating regular P addition to replace the harvested P (Nelson & Mikkelsen 2008). This leads to the need of frequent application of phosphate fertilizers to the soil. Use of P fertilizers on a regular basis has become a costly affair as its prices are continuously increasing and are also environmentally undesirable (Reddy et al. 2002). In addition, synthetic P fertilizers are prohibited for use by organic farming standards. As a result, locally available sources of raw rock phosphate (RP) have been recognized as alternative source of P fertilizers (Van Straaten 2007).

RP as such is not readily available to the plants in soils with a pH > 5.5–6.0. Because of this, extension services are reluctant to recommend it and farmers are hesitant to utilize RP directly. One approach for solubilization of RP in field conditions is the application of phosphate-solubilizing

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microorganisms (PSMs). PSMs solubilize insoluble form of phosphates by acidification, chelation and exchange reactions and also by production of organic acids (Chung et al. 2005). This process not only compensates for higher cost of manufacturing fertilizers in industry; it also mobilizes the fertilizers applied to the soil. In addition to P-solubilization, organic farming avoids the inputs of synthetic chemicals and their consequences. The build-up of a large and active soil microbial biomass is therefore critically important for sustaining the productivity of soils in organic farming systems (Tu et al. 2006). Phosphate-solubilizing fungi *A. tubingensis* and *A. niger* are known to increase the soluble P of the soil, resulting in better growth and higher yield of crop plants (Richa et al. 2008). Singh and Reddy (2012) suggested that inoculation of *A. tubingensis* and *A. niger* significantly enhance the fertilizer value of RP and yield of wheat and maize in alkaline soils. In the present investigation, efficacy of two P-solubilizing fungi isolated from organic farm was tested in improving the yield of maize and wheat in organic farming along with RP fertilization.

Materials and methods

Isolation of phosphate-solubilizing fungi

Nine fungal strains showing varied phosphate-solubilizing zones on Pikovskaya's (PKV) agar medium were isolated from rhizosphere soil samples of *Stevia rebaudiana* grown in organically managed farm at Pojewal (31.65° N, 76.26° E), Punjab, India. No synthetic fertilizers, pesticides and fungicides were used during the last 10 years in this farm. Animal manure, vermicompost and green manure are used to maintain the soil fertility. Soil samples were collected at 5–10 cm depth, brought to the laboratory and stored at 4°C. Samples were analysed for their physico-chemical properties. The soil texture in this farm was loamy sand, with pH 8.4 (1:2 soil:water ratio, using Deluxe water and soil analysis kit (Model 191 E)) and electric conductivity 0.14 m S cm^{-1}. The chemical constituents of the soil were organic carbon 0.4% (Walkley 1947), organic matter 0.7 mg g^{-1} of soil (Walkley 1947), available P 4.0 mg kg^{-1} (Olsen et al. 1954), total P 219 mg kg^{-1} (Kitson & Mellon 1944) and total nitrogen 0.03% (Piper 1966). For isolation of P-solubilizing fungi, soil samples were serially diluted in sterile physiological saline (0.85% NaCl in distilled water) plated on PKV agar plates (Pikovskaya 1948) supplemented with 0.5% tricalcium phosphate as the sole P source and incubated at 30°C. Fungal colonies showing zone of solubilization were selected and screened for RP (RP equivalent to 100 mg P_2O_5 100 ml^{-1}), ferric phosphate ($FePO_4$) and aluminium phosphate ($AlPO_4$) (equivalent to 5 mM P_2O_5) solubilization in PKV broth. RP used in this study was obtained from Rajasthan State Mines and Minerals, Ltd., Udaipur, India. The chemical constituents of the RP were 31.5% P_2O_5, 45.4% CaO, 3.4% MgO, 8.4% Al_2O_3, 3.1% fluoride, 0.044% organic carbon and 0.003% available P (Olsen P). Fungal isolates were inoculated (5.0 mm mycelial disc was cut from the periphery of the actively growing colony) in 100 ml PKV broth in 250 ml conical flask and incubated at 30°C on a rotary shaker (130 rpm) for 1 week. Reduction of pH, soluble P and the acid phosphatase activity were determined in the culture filtrates. Soluble P in the culture filtrate was estimated by chlorostannous reduced molybdo-phosphoric acid blue method (Jackson 1973). Acid phosphatase activity of these fungal isolates was determined according to the method described in Tabatabai and Bremner (1969). Organic acids exudated by fungal isolates during P-solubilization were determined using high performance liquid chromatography (HPLC) as described by Relwani et al. (2008). Fungal isolates were identified at the species level based on their morphological and sequence analysis of internal transcribed spacer (ITS) regions of nuclear ribosomal ribonucleic acid of fungi.

Field experiment

Two field trials in two different seasons (rainy season and winter season) were conducted in an organic field at Pojewal, Punjab. The maize was grown in rainy season (July–October, 2011) and wheat in winter (November 2011–April 2012). The organic farm used in this study was a field where no synthetic fertilizers had been used during the last 10 years. Field trials were conducted in a completely

randomized block design. Field trial consisted of six treatments, control; *Aspergillus tubingensis* (At); *Aspergillus niger* (An); Rock Phosphate (RP); Rock Phosphate + *A. tubingensis* (RP + At) and Rock Phosphate + *A. niger (*RP + An), each with three replicates. Each treatment plot was of 4 m × 4 m in size. RP was amended in respective plots at the rate of 59 kg P_2O_5 ha^{-1} before sowing. Seeds were treated with fungal spores before planting. Fungal inoculum was prepared by growing the fungi in PKV plates. The spores of *A. tubingensis* and *A. niger* were scraped and mixed with slurry containing 40% gum arabic and 10% sugar solution. The inoculum was applied to the seeds in the form of seed coat. For the seed coating of fungal strains, seeds were surface sterilized by dipping them in 95% ethanol for 3 min and 3% sodium hypochlorite for 5 min, and subsequently washing them with sterile distilled water followed by treatment with slurry containing 40% gum arabic and 10% sugar solution and fungal spores. The density of the inoculum on seed was assessed by serial diluting the seed suspended water and plating them on potato dextrose agar plates. Seeds treated with 40% gum arabic and 10% sugar solution that did not contain fungal spores served as a control.

During first year, maize variety DKC-9106 (20 kg ha^{-1}) was cultivated in the rainy season (July 2011). At the time of sowing, size of inoculum per maize seed was 2.3–2.9 × 10^5 spores/seed. All the plots were irrigated once before sowing to ensure proper germination of seeds and then regularly during crop growth. No chemical fertilizer was applied to the crop. The maize was harvested in October 2011. After maize harvesting, wheat variety PBW-621 (99 kg ha^{-1}) was sown in the winter season of November 2011. The field was tilled and irrigated without disturbing the experimental design. The size of the inoculum per wheat seed was 1.5–2.0 × 10^5 spores per seed. Wheat was harvested in April 2012.

Plant and soil analysis

In both seasons, 10 plants from each plot were randomly uprooted and the plant height was recorded. Roots, shoot biomass and seeds of these 10 randomly selected plants were then oven dried at 65°C for 72 hours and growth parameters such as root and shoot dry biomass were recorded. The plant samples were then ground to pass through a 0.5 mm sieve and analysed for total P content. Phosphorus concentration in plant roots, shoot and seed was determined by the method of Kitson and Mellon (1944). The yield of maize and wheat were recorded for whole remaining plot.

For analysis of different soil properties, soil samples to the depth of 5–10 cm were drawn carefully from rhizosphere from each plot randomly from five different places and homogeneous composite samples was prepared. Soil samples were stored at 4°C and analysed within a week for acid and alkaline phosphatase activity (Tabatabai & Bremner 1969), phytase activity (Heinonen & Lahti 1981) and dehydrogenase enzyme activity (Cassida 1977). For physiochemical analysis, soil samples were air dried under shade and then passed through 2.0 mm sieve. Soil samples of each plot were analysed for pH (1:2 soil:water ratio), organic carbon (Walkley 1947), available P (Olsen et al. 1954), total P (Kitson & Mellon 1944) and total nitrogen (Piper 1966). Maize and wheat grains from plots inoculated with P-solubilizing fungi were analysed for B1, B2, G1 and G2 aflatoxins by HPLC as described in Singh and Reddy (2012).

Statistical analysis

The data were analysed by analysis of variance (ANOVA) and the means were compared with Tukey's test at $P \leq 0.05$. All the analyses were performed by using Graph Pad Prism 5.1 software.

Results

Isolation and characterization of phosphate-solubilizing fungi

A total of nine fungal strains (PSF-1, PSF-2, PSF-3, PSF-5, PSF-6, PSF-7, PSF-8 and PSF-9) showing zone of solubilization on PKV agar plates were isolated. These isolates were further tested for their ability to solubilize RP, $FePO_4$ and $AlPO_4$. All the isolates were able to solubilize these phosphate sources and reduced the pH of the culture filtrate. Maximum reduction of pH was observed in RP amended medium compared to $FePO_4$ and $AlPO_4$. Soluble P levels and acid phosphatase activities were significantly higher in RP supplemented medium than $FePO_4$ and $AlPO_4$ (Table 1). HPLC analysis revealed that in presence of RP, the main organic acids exudated by these isolates were gluconic acid and succinic acid. Oxalic acid was the main organic acid produced by these isolates when grown in presence of $FePO_4$ and $AlPO_4$ followed by citric acid and formic acid (data not shown). Basic local alignment search tool analysis of ITS sequences revealed that isolate PSF-4 showed maximum similarity (99%) with *A. tubingensis* and other isolates (PSF-1, PSF-2, PSF-3, PSF-5, PSF-6, PSF-7, PSF-8 and PSF-9) showed (99 %) with *A. niger*. ITS gene sequences of fungi determined in this study were deposited in GenBank of NCBI under the accession numbers KJ410674, KJ410675, KJ410676 and KJ410677 for PSF-4, PSF-5, PSF-6 and PSF-7, respectively. *A. tubingensis* (PSF-4) and *A. niger* (PSF-7) were selected for further studies on the basis of their efficient P solubilization *in vitro*.

Field experiments

Significant improvement in plant growth parameters such as plant height, shoot biomass and root dry biomass and grain yield was observed in inoculated and RP fertilization treatments in both seasons. Results were more pronounced when there was a combined treatment of fungal inoculation and RP fertilization than individual treatments. Plant height and shoot biomass of maize were significantly higher in fungal inoculated treatments combined with RP fertilization. Root biomass also increased due to inoculation. A significant increase in maize yield (25 %) was attained with inoculation along with RP fertilization than individual treatments (Table 2). A significant increase in total P was observed in seeds, shoots and roots in maize due to inoculation of fungi together with RP fertilization as compared to control (Table 2). Wheat grown in winter season significantly increased its growth and yield due to inoculation of fungi compared to control treatments. Plant height, biomass were significantly increased when inoculated along with RP fertilization (Table 3). Wheat yield increased significantly (38%) when inoculated with *A. niger* along with RP fertilization compared to other treatments. P uptake in root, shoot and seed was increased due to inoculation and RP amendments. P uptake levels were significantly higher when fungi inoculated along with RP fertilization (Table 3). HPLC analysis revealed that aflatoxins such as B1, B2, G1 and G2 were absent in the grains.

Soil samples were analysed after harvesting each crop. pH of the soil was reduced from 8.33 to 7.67 due to inoculation of *Aspergillus* species. Compared to initial pH, all treatments were significantly reduced the pH of the soil after maize harvest. Organic carbon levels were significantly increased due to inoculation of *Aspergillus*. Available P was significantly increased in inoculation treatments along with RP fertilization compared to other treatments. Total P levels were also significantly increased in combined treatment of fungal inoculation and RP fertilization. Soil enzyme activities such as alkaline phosphatase, acid phosphatase, phytase and dehydrogenase were significantly increased in inoculated treatments. However, the effect was more pronounced when inoculation was done along with RP fertilization (Table 4).

Soil analysis after wheat harvest also showed similar results. pH of the soil significantly reduced in all the treatments where fungi were inoculated. Organic carbon levels were significantly increased due to inoculation. Available P levels were significantly higher in combined treatments where inoculation of fungi and RP fertilization was done. Total P levels increased in RP amended

Table 1. pH reduction, phosphate solubilization and acid phosphates activity of fungal isolates in different phosphate sources supplemented in PKV broth.

	pH			Soluble P (μg ml^{-1})			Acid phosphatase (μM *p*-NPP ml^{-1} h^{-1})		
Isolates	RP	$FePO_4$	$AlPO_4$	RP	$FePO_4$	$AlPO_4$	RP	$FePO_4$	$AlPO_4$
PSF-1	2.83 ± 0.12ab	4.60 ± 0.13a	4.37 ± 0.18a	337 ± 6 cd	189 ± 5ef	219 ± 12 cd	83 ± 3a	36 ± 4bc	49 ± 1b
PSF-2	2.80 ± 0.01ab	4.29 ± 0.29ab	3.87 ± 0.10bc	323 ± 5d	196 ± 3de	213 ± 7d	74 ± 3b	33 ± 4c	38 ± 3c
PSF-3	2.84 ± 0.08ab	4.14 ± 0.23bc	3.89 ± 0.11b	289 ± 7e	181 ± 6f	230 ± 8bcd	74 ± 1b	30 ± 3c	38 ± 1c
PSF-4	2.77 ± 0.02ab	4.0 ± 0.12bc	3.44 ± 0.30 cd	358 ± 10bc	225 ± 8ab	243 ± 4abc	91 ± 3a	47 ± 2a	55 ± 3ab
PSF-5	2.66 ± 0.10bc	4.12 ± 0.09bc	3.39 ± 0.13d	364 ± 3b	216 ± 6bc	232 ± 4bcd	87 ± 5a	45 ± 1a	51 ± 5ab
PSF-6	2.68 ± 0.05bc	3.87 ± 0.09bc	3.33 ± 0.03d	366 ± 16b	213 ± 3bc	249 ± 6ab	86 ± 2a	44 ± 2ab	51 ± 2ab
PSF-7	2.53 ± 0.06c	3.72 ± 0.15c	3.35 ± 0.20d	392 ± 7a	233 ± 3a	265 ± 6a	88 ± 1a	48 ± 3a	58 ± 1a
PSF-8	2.95 ± 0.15a	3.75 ± 0.09c	3.84 ± 0.07bc	230 ± 8f	218 ± 5bc	229 ± 10bcd	68 ± 2b	29 ± 5c	32 ± 2c
PSF-9	2.61 ± 0.04bc	3.85 ± 0.13bc	3.73 ± 0.04bcd	323 ± 6d	207 ± 5 cd	244 ± 13ab	72 ± 5b	34 ± 2c	36 ± 5c

Means sharing a common letter within the column are not significantly different at $P \leq 0.05$. Values are Mean ± SD ($n = 3$).

Table 2. Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) on growth parameters of maize (July–October 2011).

Treatments	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Grain yield (ton ha^{-1})	P uptake (mg kg^{-1})		
					Root	Shoot	Seed
Control	225 ± 3.3d	36 ± 1.9d	9.70 ± 1.0b	5.66 ± 0.14c	127 ± 10e	136 ± 8c	142 ± 10d
At	258 ± 4.7ab	58 ± 1.3bc	14.2 ± 0.9a	6.72 ± 0.06b	176 ± 8 cd	172 ± 15b	187 ± 11c
An	250 ± 3.0bc	64 ± 6.0b	15.0 ± 0.9a	6.74 ± 0.08b	194 ± 24bc	177 ± 10ab	196 ± 17bc
RP	238 ± 3.0 cd	51 ± 5.1c	10.0 ± 0.5b	6.55 ± 0.04b	148 ± 6de	170 ± 11bc	176 ± 12 cd
RP + At	271 ± 3.0a	74 ± 0.7a	15.3 ± 0.4a	7.03 ± 0.07a	228 ± 18ab	212 ± 17a	230 ± 14ab
RP + An	270 ± 1.8a	75 ± 1.2a	15.8 ± 0.9a	7.06 ± 0.07a	235 ± 10a	197 ± 14ab	238 ± 14a

Means sharing a common letter within the column are not significantly different at $P \leq 0.05$. Values are Mean ± SD ($n = 10$).

Table 3. Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) on growth parameters of wheat (November 2011–April 2012).

Treatments	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Grain yield (t ha^{-1})	P uptake (mg kg^{-1})		
					Root	Shoot	Seed
Control	99 ± 8d	1.44 ± 0.10c	0.66 ± 0.07c	3.85 ± 0.18c	222 ± 6c	38 ± 10c	227 ± 10d
At	115 ± 1bc	1.99 ± 0.12b	0.94 ± 0.05bc	4.50 ± 0.17b	335 ± 17b	63 ± 6b	330 ± 17c
An	119 ± 2abc	1.92 ± 0.06b	1.01 ± 0.05ab	4.30 ± 0.30bc	322 ± 10b	81 ± 8b	313 ± 17c
RP	109 ± 4 cd	1.70 ± 0.05bc	0.79 ± 0.16bc	4.27 ± 0.18bc	295 ± 8b	69 ± 6b	335 ± 21bc
RP + At	131 ± 3a	2.58 ± 0.20a	1.33 ± 0.14a	4.84 ± 0.16ab	458 ± 8a	112 ± 4a	373 ± 10ab
RP + An	126 ± 4ab	2.54 ± 0.06a	1.28 ± 0.18a	5.30 ± 0.30a	463 ± 6a	120 ± 4a	384 ± 6a

Means sharing a common letter within the column are not significantly different at $P \leq 0.05$. Values are Mean ± SD ($n = 10$).

soils than other treatments. Acid phosphatase, alkaline phosphatase, phytase and dehydrogenase enzyme activities were higher due to fungal inoculation. However, the effect was more pronounced in the combined treatment of fungal inoculation and RP fertilization (Table 5). Aflatoxins such as B1, B2, G1 and G2 were absent in the grains of wheat. There was no significant increase in the total nitrogen levels in both crops. Present study results suggested that both *A. tubingensis* and *A. niger* are effective in improving the yield of maize and wheat and soil fertility in both seasons.

Discussion

All the isolates significantly solubilized RP, ferric phosphate and aluminium phosphate while showing maximum P solubilization by *A. tubingensis* (PSF-4) and *A. niger* (PSF-7). Hence these isolates (PSF-4 and PSF-7) were further tested for field trials. Increase in solubilization of P was observed with reduction in pH of culture medium. A significant reduction in the pH of the culture filtrates containing various inorganic phosphates suggested secretion of organic acids by the fungal strain (Nahas 1996; Pradhan & Sukla 2005; Singh & Reddy 2012). The selected fungal isolates were producing organic acids in RP, ferric phosphate and aluminium phosphate supplemented PKV broth. Relwani et al. (2008) suggested that acid phosphatase enzyme plays a major role in P-solubilization, apart from other phosphate solubilization mechanisms.

The inoculation of P-solubilizing fungi is a promising technique because it can increase the available P in soils fertilized with RP (Reyes et al. 2002). Mittal et al. (2008) studied the effect of six phosphate-solubilizing fungi, including two strains of *A. awamori*, and four of *Penicillium citrinum*, on growth and seed production of chickpea. They found that inoculation of two *A. awamori* strains showed maximum stimulatory effect on chickpea plants with increase in shoot height seed number and seeds weight as compared to the control plants. Shin et al. (2006) found that inoculation of *P. oxalicum* used either alone or fused with RP increased the growth, N and P accumulation in maize plants compared to control. In addition, a substantial increase in plant height, plant weight and root length of maize following inoculation of P-solubilizing fungus *Penicillium* sp. was reported (Kang & Choi 1999). Accordingly, Many authors have reported a profound increase in yield of several crops through inoculation of P-solubilizing fungi (Khan et al. 2010 and references therein).

Table 4. Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) on rhizosphere soil (5–10 cm depth) characteristics of maize (July–October 2011).

Treatments	pH	Organic C (%)	Available P (mg kg^{-1})	Total P (mg kg^{-1})	Total N (%)	Acid phosphatase	Alkaline phosphatase	Phytase	Dehydrogenase
						(μM g^{-1} h^{-1})			(μM *p*-NPP g^{-1} h^{-1})
Control	8.33 ± 0.03a	0.39 ± 0.05b	4.02 ± 0.17d	237 ± 8c	0.035 ± 0.002a	370 ± 1.2d	530 ± 1.8e	6237 ± 500b	8.63 ± 0.92b
At	7.77 ± 0.01c	0.58 ± 0.04a	7.51 ± 0.14b	248 ± 2c	0.045 ± 0.018a	483 ± 1.0b	741 ± 0.7c	12272 ± 2063a	10.5 ± 0.07ab
An	7.74 ± 0.01 cd	0.56 ± 0.02a	7.23 ± 0.09b	250 ± 3c	0.042 ± 0.008a	481 ± 5b	742 ± 0.9c	11968 ± 1496a	10.0 ± 0.05ab
RP	7.94 ± 0.02b	0.46 ± 0.01b	5.29 ± 0.07c	465 ± 9b	0.042 ± 0.017a	425 ± 2.7c	703 ± 2.9d	8735 ± 126b	9.94 ± 2.8ab
RP + At	7.67 ± 0.03e	0.56 ± 0.02a	9.96 ± 0.12a	459 ± 9b	0.053 ± 0.010a	567 ± 2.0a	792 ± 2.1b	14843 ± 97a	12.2 ± 0.09a
RP + An	7.70 ± 0.02de	0.56 ± 0.03a	9.86 ± 0.05a	508 ± 8a	0.050 ± 0.008a	569 ± 2.4a	802 ± 1.1a	14715 ± 83a	12.3 ± 0.09a

TPF*: Triphenyl tetrazolium formazan; Means sharing a common letter within the column are not significantly different at $P \leq 0.05$. Values are Mean ($n = 10$).

Table 5. Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) on rhizosphere soil (5–10 cm depth) characteristics of wheat (November 2011–April 2012).

Treatments	pH	Organic C (%)	Available P (mg kg^{-1})	Total P (mg kg^{-1})	Total N (%)	Acid phosphatase	Alkaline phosphatase	Phytase	Dehydrogenase
						(μM g^{-1} h^{-1})			(μg TPF* g^{-1} h^{-1})
Control	8.30 ± 0.02a	0.46 ± 0.04b	4.94 ± 0.18d	204 ± 7e	0.045 ± 0.005a	439 ± 1.4c	329 ± 0.90d	6485 ± 84d	12.30 ± 0.16d
At	7.59 ± 0.04c	0.64 ± 0.02a	7.23 ± 0.18b	223 ± 6d	0.063 ± 0.013a	535 ± 37b	472 ± 0.55b	10600 ± 69b	14.48 ± 0.07b
An	7.58 ± 0.02c	0.61 ± 0.03a	7.59 ± 0.15b	247 ± 9c	0.056 ± 0.012a	564 ± 3.3ab	474 ± 0.75b	10425 ± 84b	14.45 ± 0.05b
RP	8.13 ± 0.02b	0.47 ± 0.08b	5.90 ± 0.18c	455 ± 6a	0.045 ± 0.009a	465 ± 1.2c	403 ± 0.36c	8092 ± 89c	13.94 ± 0.12c
RP + At	7.50 ± 0.05 cd	0.66 ± 0.02a	10.95 ± 0.13a	437 ± 8ab	0.060 ± 0.012a	585 ± 1.7a	528 ± 1.2a	12832 ± 115a	16.91 ± 0.02a
RP + An	7.46 ± 0.05d	0.69 ± 0.03a	10.90 ± 0.09a	434 ± 5b	0.059 ± 0.019a	590 ± 2.0a	526 ± 2.4a	12740 ± 42a	16.96 ± 0.07a

TPF*: Triphenyl tetrazolium formazan; Means sharing a common letter within the column are not significantly different at $P \leq 0.05$. Values are Mean ± SD ($n = 10$).

Wahid and Mehana (2000) reported that inoculation of *P. pinophilum* increased the yield of wheat grains by 28.9 and 32.8% in the soil treated with RP and superphosphate, respectively. Many of these studies are confined to a single crop grown in a particular season. Not many reports are available on the efficacy of the inoculation of these fungi to improve the crop yield grown in the same field in different seasons. Singh and Reddy (2011) reported that inoculation of *P. oxalicum* significantly increased the growth and yield of wheat and maize compared to the control in alkaline soil. Inoculation significantly increased P content in the plants and also soil fertility such as available P and organic carbon levels. In the present study, significant increase in plant height, shoot biomass and root dry biomass and grain yield in maize and wheat was achieved due to inoculation of P-solubilizing fungi.

The field experiments showed highly significant interaction between RP amendment and inoculation with RP-solubilizing fungus. The growth parameters were higher in RP fertilized soil inoculated with fungus compared to control soil. As phosphorus is known to initiate cell division and enlargement processes, the increase in shoot height could be probably due to increased mobilization of phosphorus made soluble by P-solubilizing fungi from soil reserves and RP (Reyes et al. 2001). The reduction in the soil pH, increase in available P and organic carbon was higher in inoculated treatments as compared to control ones which may be attributed to ability of such microorganisms to excrete organic acids, thereby decrease the pH and increase the concentration of phosphorus in soil by mechanisms involving chelation and exchange reactions (Vassilev et al. 1996).

This could be attributed to a greater absorption of nutrient, particularly P. The application of RP along with P-solubilizing fungi further improve the plant growth parameters, total P in seeds, shoot and roots and grain yield. Soil enzyme activities such as acid phosphatase, alkaline phosphatase, phytase and dehydrogenase were significantly improved in all the treatments but the results were more pronounced in inoculation along with RP fertilization treatments compared to control. Soil enzymes have been suggested as potential indicator of soil quality because of the irrelationship to soil biology, ease of measurements and rapid response to change in soil management (Dick et al. 1996).

Inorganic P is released from organic matter by hydrolysis of C–O–P ester bonds by phosphatases, which are, therefore, important in the P nutrition of plants. Dehydrogenases represent a class of enzymes that give us information about the influence of natural environmental conditions on microbial activities of the soil (Schaffer 1993). Higher enzyme activities in soils indicated the potential of soil to affect the biochemical transformations necessary for the maintenance of soil fertility (Rao et al. 1990). Activities of enzymes such as dehydrogenase, acid phosphatase, alkaline phosphatase and phytase in all the treatments were higher than the control soil. In all the treatments, the phytase activity was observed to be higher than the phosphatase activity. This may be due to the higher extracellular phytase enzyme activity of selected fungal isolates as compared to extracellular phosphatase enzyme activity, a result that compares well to Aseri et al. (2009) who found that microbes execute extracellular phytase activity many times more than extracellular phosphatase activity. The alkaline phosphatase and phytase activities were slightly decreased after wheat harvest as compared to maize rhizosphere soil after harvest. This may be due to the slight reduction in soil pH, a fact which has been also observed by Richardson et al. (2005).

Conclusions

From these results it was concluded that RP can be used as a crude phosphate fertilizer by direct application to organic farm along with P-solubilizing fungi to improve the growth and yield of maize and wheat. Thus, the application of P-solubilizing fungi is recommended as a sustainable way for increasing crop yield and also improving the physico-chemical properties of the organic farm.

Disclosure statement

No potential conflict of interest was reported by the authors.

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