Chapter 6

Field application of phosphate solubilizing microorganisms in organic farming

6.1Role of phosphate-solubilizing bacteria in organic farming

Organic farming is an eco-friendly system of farming which helps to maintain health of soil in terms of soil biological fertility and productivity. Crop production in organic farming mainly depends on nutrients released as a result of mineralization processes in soils. An active soil microflora and a considerable pool of accessible nutrients are, therefore, important priorities in organic farming. Organic farming avoids the input 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 system. A field experiment was conducted in which *Pantoea cypripedii* and *Pseudomonas plecoglossicida* isolates were inoculated alone or along with rock phosphate and compared with chemical fertilizer treatments. Maize and wheat was selected as test crops for field experiments. The site is situated at 31.65° N latitude and 76.26° E longitude and falls under sub-mountain undulating agroclimatic region of Punjab state. The region possesses dry sub humid type of climate. Soil was loamy sand (Typic Ustorthents), PH - 8.37, organic carbon - 0.42 %, organic matter - 0.7 mg, available P - 4.3 mg kg^{-1}, total P - 245 mg kg^{-1} and total nitrogen - 0.035 %.

6.1.1 Field study with Maize crop

A field experiment of maize was conducted during first year of field study in an organic farm (Plate 6.1). Inoculation of *P. cypripedii* and *P. plecoglossicida* in maize crop with and without rock phosphate fertilization were tested for the improvement in crop yield and soil fertility. The shoot height, shoot and root dry biomass and grain yield was significantly increased due to inoculation and inoculation along with RP fertilization compared to un-inoculation control soil. Total P in plant tissues (grains, shoots and roots) was increased significantly in inoculated and RP fertilized treatments compared to control soil (Table 6.2). It was observed that plant growth parameters, grain yield and total P uptake was significantly increased in all the inoculated and

RP fertilized soil treatments, but the results were more pronounced when inoculation was done along with RP fertilization than individual inoculation and control. Grain yield was increased to 17.9 % with inoculation, 15.8 % with RP fertilization and 20.7 % when inoculation was done along with RP fertilization. On the other hand di-ammonium phosphate (DAP) chemical P fertilizer treatment increased grain yield up to 13.0 % compared to control. Similarly, total P uptake in grains, shoots and roots was increased due to inoculation and RP fertilization treatments but the results were more pronounced and significant when RP fertilization was done along with seed inoculation.

Effect of inoculation alone or along with RP fertilization and in chemical fertilizer treatments was tested on soil physiochemical properties, which played an important role in improvement of soil fertility such as organic carbon, organic matter, total P, available P, total nitrogen and soil enzyme activities. Soil pH decreased slightly from its initial value in all the treatments compared to control soil treatments (Table 6.3). Organic carbon of soil was significantly improved in inoculation (44 %) and RP fertilization treatments along with inoculation (47 %) compared to RP fertilization alone (17 %) and chemical fertilizer treatment (20 %) compared to control soil (Table 6.4). There was a significant improvement in total P of soil with RP fertilization. Available P was increased up to 42 % with bacterial inoculation and it rose up to 113 % when RP fertilization was done along with bio-inoculation compared to control soil. A significant increased in total P was observed in soil with DAP treatment but increased in available P (23 %) due to DAP fertilization was not comparable with increased in available P in inoculation and RP fertilization treatments (113 %). Total nitrogen was not increased with DAP, bio-inoculation and RP fertilization treatments (Table 6.4). Soil enzyme activities such as acid phosphates, alkaline phosphatase, phytase and dehydrogenase enzyme activities were significantly improved in all the treatments, but the results were more pronounced with inoculation treatments along with RP fertilization (Table 6.5). Phytase enzyme activities in soil

were significantly higher than that of acid and alkaline phosphatase. Phosphate-solubilizing bacterial population density was significantly improved due to inoculation, RP fertilization and RP fertilization along with bacterial inoculation compared to DAP and control treatments. Population density of phosphate solubilizing-bacteria was tested in rhizospheric soil of each plot after harvesting of the crop and it was 1.8×10^{6} cfu g^{-1} in control treatment, $1.0\text{-}1.1\times10^{8}$ cfu g^{-1} in inoculation treatments, 3.5×10^{6} cfu g^{-1} in RP fertilization alone, $2.3\text{-}2.5\times10^{8}$ cfu g^{-1} in inoculation along with RP fertilization and 2.1×10^{6} cfu g^{-1} in DAP treatments. There was a significant increase in population density of phosphate-solubilizing bacteria in inoculation treatments along with RP fertilization compared to other treatments. Field study of maize crop in organic farming showed a significant improvement in crop yield, total P uptake and soil fertility in context to organic carbon, total P, available P, soil enzyme activities and population density of P-solubilizing bacteria in bio-inoculation treatments along with RP fertilization compared to other treatments.

a)

b)

c)

d)

Plate 6.1 Maize field experiment with different growth stages a) tillering b) stem extention c) heading d) ripening.

Table 6.1 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on the growth parameters of maize plants grown in an organic field.

Treatments	Shoot height (cm)	Shoot dry weight (g)	Root dry weight (g)	Grain yield (ton ha^{-1})
Soil	225 ± 3.3b	36 ± 1.9d	9.70 ± 1.0b	5.66 ± 0.14c
Soil + Pc	236 ± 4.7b	47 ± 1.3cd	11.3 ± 0.3b	6.61 ± 0.05ab
Soil + Pp	238 ± 3.1b	47 ± 10cd	11.3 ± 0.3b	6.66 ± 0.03ab
Soil + BC	239 ± 3.5b	51 ± 2.6bc	11.6 ± 0.9b	6.68 ± 0.05ab
Soil + RP	237 ± 3.0b	51 ± 5.1bc	10.0 ± 0.5b	6.55 ± 0.04ab
Soil + RP + Pc	**257 ± 4.7a**	**63 ± 6.6ab**	**13.7 ± 1.0a**	**6.74 ± 0.05ab**
Soil + RP + Pp	**265 ± 6.1a**	**69 ± 2.1a**	**14.2 ± 0.7a**	**6.79 ± 0.02ab**
Soil + RP + BC	**260 ± 4.7a**	**70 ± 2.5a**	**15.0 ± 0.81a**	**6.83 ± 0.41a**
DAP	239 ± 6.3b	49 ± 1.5cd	10.6 ± 0.8b	6.40 ± 0.04b
LSD (P<0.05)	9.74	8.12	1.26	0.26

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.2 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on P uptake of maize plants grown in an organic field.

Treatments	Total P (mg kg^{-1})		
	Grains	Shoot	Root
Soil	143 ± 10d	136 ± 9e	127 ± 10d
Soil + Pc	177 ± 9bcd	162 ± 4cd	165 ± 10bc
Soil + Pp	180 ± 17bc	165 ± 10bcd	180 ± 13b
Soil + BC	183 ± 17bc	166 ± 4bcd	186 ± 9b
Soil + RP	179 ± 13bcd	170 ± 11abcd	148 ± 6cd
Soil + RP + Pc	**198 ± 13ab**	**186 ± 6ab**	**220 ± 7a**
Soil + RP + Pp	**223 ± 13a**	**183 ± 4abc**	**226 ± 10a**
Soil + RP + BC	**227 ± 6a**	**188 ± 2a**	**230 ± 6a**
DAP	155 ± 13cd	150 ± 11de	138 ± 6d
LSD (P<0.05)	22	13	15

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.3 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of maize plants grown in an organic field.

Treatments	pH	EC ($mScm^{-1}$)	TDS (ppm)
Soil	8.33 ± 0.03a	0.18 ± 0.02c	0.12 ± 0.02c
Soil + Pc	7.84 ± 0.02cd	0.26 ± 0.03ab	0.17 ± 0.02ab
Soil + Pp	7.85 ± 0.01c	0.24 ± 0.03abc	0.16 ± 0.02abc
Soil + BC	7.81 ± 0.06cde	0.27 ± 0.02ab	0.17 ± 0.01ab
Soil + RP	7.94 ± 0.02b	0.19 ± 0.02bc	0.13 ± 0.01bc
Soil + RP + Pc	**7.78 ± 0.02cde**	0.27 ± 0.03a	0.18 ± 0.02a
Soil + RP + Pp	**7.76 ± 0.01de**	0.26 ± 0.03ab	0.17 ± 0.02ab
Soil + RP + BC	**7.75 ± 0.03e**	0.26 ± 0.01ab	0.17 ± 0.01ab
DAP	8.01 ± 0.04b	0.25 ± 0.02abc	0.17 ± 0.01abc
LSD (P<0.05)	0.047	0.04	0.028

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.4 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of maize plants grown in an organic field.

Treatments	Organic carbon (%)	Organic matter (%)	Total P (mg kg^{-1})	Available P (mg kg^{-1})	Total nitrogen (%)
Soil	0.39 ± 0.05d	0.66 ± 0.09d	237 ± 8b	4.01 ± 0.17c	0.034 ± 0.002a
Soil + Pc	0.52 ± 0.02abc	0.89 ± 0.04abc	241 ± 4b	5.62 ± 0.16b	0.039 ± 0.004a
Soil + Pp	0.55 ± 0.05ab	0.94 ± 0.08ab	256 ± 2b	5.63 ± 0.05b	0.040 ± 0.001a
Soil + BC	**0.56 ± 0.02a**	**0.96 ± 0.04a**	261 ± 6b	5.66 ± 0.10b	0.039 ± 0.013a
Soil + RP	0.46 ± 0.01cd	0.78 ± 0.02cd	465 ± 9a	5.29 ± 0.07b	0.042 ± 0.017a
Soil + RP + Pc	0.54 ± 0.01abc	0.92 ± 0.01abc	480 ± 35a	**8.07 ± 0.22a**	0.039 ± 0.009a
Soil + RP + Pp	**0.55 ± 0.02ab**	**0.94 ± 0.03ab**	**504 ± 17a**	**8.48 ± 0.33a**	0.048 ± 0.010a
Soil + RP + BC	**0.57 ± 0.03a**	**0.97 ± 0.05a**	**497 ± 6a**	**8.53 ± 0.10a**	0.041 ± 0.002a
DAP	0.47 ± 0.03bcd	0.79 ± 0.05bcd	487 ± 8a	4.94 ± 0.09b	0.039 ± 0.005a
LSD (P<0.05)	0.05	0.088	24	0.47	0.016

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.5 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on enzyme activities of rhizosphere soil of maize plants grown in an organic field.

Treatments	Acid phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Alkaline phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Phytase activity ($\mu M\ g^{-1}\ hr^{-1}$)	Dehydro-genase activity (ppm)
Soil	370 ± 1.2g	530±1.8d	6237±500c	8.63±0.92b
Soil + Pc	471 ± 2.8d	712 ± 13ab	11904 ± 1704a	10.41 ± 0.12ab
Soil + Pp	479 ± 3.6cd	699 ± 62b	11665 ± 1663a	9.82 ± 0.10ab
Soil + BC	481 ± 1.1c	710 ± 0.9ab	11913 ± 762a	10.14 ± 0.03ab
Soil + RP	425 ± 2.7e	703 ± 2.9ab	8735 ± 126b	9.94 ± 2.8ab
Soil + RP + Pc	490 ± 3.8b	746 ± 4.0ab	12446 ± 183a	12.14 ± 0.09a
Soil + RP + Pp	**523 ± 1.5a**	**762 ± 1.3a**	**12960 ± 287a**	**12.14 ± 0.07a**
Soil + RP + BC	521 ± 2.8a	753 ± 3.6ab	13052 ± 250a	12.44 ± 0.12a
DAP	411 ± 5.1f	615 ± 5.9c	8175 ± 203bc	9.24 ± 0.05b
LSD (P<0.05)	5.15	36	1483	1.71

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

6.1.2 Field study with Wheat crop

During the second year of field study wheat crop was grown and it was observed that bacterial inoculation alone and along with RP fertilization have significant effects on plant shoot height, shoot and root dry weight, and grain yield (Table 6.6). Inoculation along with RP fertilization showed more pronounce effects compared to individual inoculation treatments. Inoculation increased the grain yield of wheat 14 %, RP fertilization 11 %, and inoculations along with RP fertilization 20 % and DAP treatments increased the yield up to 6 % compared to control treatments. Total P in seeds, shoots and roots was increased 29 %, 41 % and 36 % in inoculation treatments; in RP fertilization, it was 48 %, 81 % and 33 %; in inoculation treatments along with RP fertilization it was increased 64 %, 194 % and 94 % and in DAP treatments 4 %, 23 % and 11 % compared to uninoculated control (Table 6.7).

With inoculation and RP fertilization treatments a slight decrease in soil pH was observed (Table 6.8). EC and TDS of soil were not affected. Organic carbon of rhizospheric soil was increased to 26 % in bio-inoculation, 33 % in RP fertilization along with inoculation, 3 % in RP fertilization and 13 % in chemical fertilizer treatments (Table 6.9). There was significant improvement in total P of soil with RP fertilization in soil. Available P was increased up to 41 % with bio-inoculation and it rose up to 86 % when RP fertilization was done along with inoculation compared to control soil. With DAP fertilization and RP fertilization, total P of the respective plots was significantly increased, but the available P was improved significantly only in RP fertilization and bio-inoculation treatments (86 %) compared to DAP treatments (12 %). Total nitrogen was not affected with any of the treatment (Table 6.9). Soil enzyme activities such as acid phosphates, alkaline phosphatase, phytase and dehydrogenase enzyme activities were significantly improved in all the treatments, but the results were more pronounced with inoculation treatments along with RP fertilization (Table 6.10). Phytase enzyme activities in soil were significantly higher than that of acid and alkaline phosphatase

(Table 6.10). After harvesting of the wheat crop phosphate-solubilizing bacterial population density was tested in rhizospheric soil of each plot and it was 2.0×10^{6} cfu g^{-1} in control treatment, $2.1\text{-}2.2\times10^{8}$ cfu g^{-1} in inoculation treatments, 4.1×10^{6} cfu g^{-1} in alone RP fertilization treatments, $3.6\text{-}4.1\times10^{8}$ cfu g^{-1} in inoculation along with RP fertilization and 2.1×10^{6} cfu g^{-1} in DAP treatments. Inoculation alone or along with RP fertilization significantly increased the population density of phosphate-solubilizing bacteria in rhizospheric soil compared to chemical fertilizer (DAP) treatments. Second year of field experiment in organic farming showed that RP fertilization done only once in previous maize experiment is still effective in improvement of second year crop yield, P uptake and soil fertility, in comparison to the chemical fertilization done on regular basis during each crop.

a)

b)

c)

d)

Plate 6.2 Wheat field experiment with different growth stages a) tillering b) stem extention c) heading d) ripening.

Table 6.6 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on the growth parameters of wheat plants grown in an organic field.

Treatments	Shoot height (cm)	Shoot dry weight (g)	Root dry weight (g)	Grain yield (ton ha^{-1})
Soil	99 ± 8.1b	1.44 ± 0.10b	0.66 ± 0.07d	3.85 ± 0.18b
Soil + Pc	104 ± 5.8ab	1.82 ± 0.03b	0.79 ± 0.07cd	4.17 ± 0.18ab
Soil + Pp	104 ± 2.0ab	1.86 ± 0.01b	0.84 ± 0.08bcd	4.27 ± 0.36ab
Soil + BC	109 ± 3.8ab	1.87 ± 0.07b	0.82 ± 0.07bcd	4.36 ± 0.31ab
Soil + RP	109 ± 3.8ab	1.70 ± 0.05b	0.79 ± 0.16cd	4.27 ± 0.18ab
Soil + RP + Pc	119 ± 0.9ab	2.56 ± 0.16a	0.93 ± 0.05abc	4.17 ± 0.18ab
Soil + RP + Pp	**120 ± 1.4a**	**2.54 ± 0.16a**	**1.03 ± 0.06ab**	**4.48 ± 0.18ab**
Soil + RP + BC	**122 ± 2.2a**	**2.55 ± 0.22a**	**1.12 ± 0.003a**	**4.63 ± 0.11a**
DAP	100 ± 1.1ab	1.58 ± 0.46b	0.75 ± 0.04cd	4.06 ± 0.31ab
LSD (P<0.05)	1071	0.33	0.131	0.408

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.7 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on P uptake of wheat plants grown in an organic field.

Treatments	Total P (mg kg^{-1})		
	Grains	Shoot	Root
Soil	227 ± 10e	38 ± 10d	222 ± 6e
Soil + Pc	263 ± 10cde	44 ± 6d	265 ± 9cd
Soil + Pp	294 ± 5ab	52 ± 6cd	301 ± 6b
Soil + BC	273 ± 15cd	54 ± 4cd	302 ± 6b
Soil + RP	336 ± 23ab	69 ± 6c	295 ± 8bc
Soil + RP + Pc	**372 ± 10a**	**109 ± 6ab**	**405 ± 10a**
Soil + RP + Pp	**350 ± 11a**	**93 ± 6b**	**429 ± 8a**
Soil + RP + BC	**366 ± 18a**	**112 ± 4a**	**430 ± 13a**
DAP	236 ± 20de	47 ± 6d	245 ± 26de
LSD (P<0.05)	25	11	21

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.8 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of wheat plants grown in an organic field.

Treatments	pH	EC ($mScm^{-1}$)	TDS (ppm)
Soil	8.30 ± 0.02a	0.14 ± 0.01c	0.09 ± 0.007c
Soil + Pc	7.67 ± 0.02d	0.14 ± 0.02c	0.09 ± 0.010c
Soil + Pp	7.61 ± 0.01ef	0.15 ± 0.01c	0.10 ± 0.007c
Soil + BC	7.65 ± 0.01de	0.22 ± 0.02a	0.15 ± 0.014a
Soil + RP	8.13 ± 0.02c	0.19 ± 0.01ab	0.13 ± 0.007ab
Soil + RP + Pc	**7.56 ± 0.03fg**	0.19 ± 0.02ab	0.13 ± 0.01ab
Soil + RP + Pp	**7.55 ± 0.01g**	0.16 ± 0.01bc	0.11 ± 0.004bc
Soil + RP + BC	**7.58 ± 0.03fg**	0.22 ± 0.01a	0.15 ± 0.007a
DAP	8.19 ± 0.03b	0.19 ± 0.02ab	0.13 ± 0.010ab
LSD (P<0.05)	0.032	0.023	0.015

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.9 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of wheat plants grown in an organic field.

Treatments	Organic carbon (%)	Organic matter (%)	Total P (mg kg^{-1})	Available P (mg kg^{-1})	Total nitrogen (%)
Soil	0.46 ± 0.04c	0.78 ± 0.06c	204 ± 7c	4.94 ± 0.18d	0.045 ± 0.005a
Soil + Pc	0.55 ± 0.02abc	0.94 ± 0.03abc	234 ± 25bc	6.72 ± 0.18b	0.050 ± 0.008a
Soil + Pp	0.57 ± 0.05abc	0.96 ± 0.08abc	245 ± 8bc	6.94 ± 0.18b	0.055 ± 0.011a
Soil + BC	0.58 ± 0.02abc	0.99 ± 0.08abc	250 ± 8b	6.88 ± 0.39b	0.051 ± 0.009a
Soil + RP	0.47 ± 0.08bc	0.80 ± 0.14bc	**455 ± 6a**	5.90 ± 0.18c	0.045 ± 0.010a
Soil + RP + Pc	0.57 ± 0.02abc	0.96 ± 0.03abc	**445 ± 8a**	**9.11 ± 0.11a**	0.061 ± 0.006a
Soil + RP + Pp	**0.60 ± 0.06ab**	**1.01 ± 0.10ab**	434 ± 28a	**8.81 ± 0.11a**	0.060 ± 0.016a
Soil + RP + BC	**0.61 ± 0.06a**	**1.04 ± 0.10a**	441 ± 8a	**9.18 ± 0.29a**	0.062 ± 0.028a
DAP	0.52 ± 0.02abc	0.88 ± 0.03abc	437 ± 19a	5.50 ± 0.11cd	0.046 ± 0.003a
LSD (P<0.05)	0.077	0.13	27	0.36	0.022

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.10 Effect of *Pantoea cypripedii* (Pc) and *Pseudomonas plecoglossicida* (Pp) alone or along with RP fertilization and chemical fertilizer (DAP) on enzyme activities of rhizosphere soil of wheat plants grown in an organic field.

Treatments	Acid phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Alkaline phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Phytase activity ($\mu M\ g^{-1}\ hr^{-1}$)	Dehydro-genase activity (ppm)
Soil	439 ± 1.4f	329 ± 0.9g	6485 ± 84e	12.3 ± 0.16e
Soil + Pc	540 ± 1.6c	467 ± 0.4d	8056 ± 97cd	14.0 ± 0.06c
Soil + Pp	541 ± 1.0c	466 ± 1.1d	8212 ± 99c	14.2 ± 0.05c
Soil + BC	539 ± 1.0c	469 ± 0.7d	8184 ± 55c	14.2 ± 0.16c
Soil + RP	465 ± 1.2e	403 ± 0.4f	8092 ± 89cd	13.9 ± 0.11c
Soil + RP + Pc	**575 ± 1.4b**	**491 ± 1.1c**	**9838 ± 55b**	**14.1 ± 0.06c**
Soil + RP + Pp	**574 ± 1.0b**	**495 ± 1.3b**	**9939 ± 136b**	**15.6 ± 0.07a**
Soil + RP + BC	**580 ± 1.7a**	**499 ± 0.7a**	**10664 ± 397a**	**15.1 ± 0.29b**
DAP	482 ± 1.2d	444 ± 2.5e	7679 ± 161d	13.4 ± 0.08d
LSD (P<0.05)	2.1	2.09	282	0.24

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

6.2 Phosphate-solubilizing fungi as bio-inoculants in organic farming

6.2.1 Maize experiment

Similar to the field study with bacterial inoculatnts, fungal bio-inoculation of *Aspergillus tubingensis* and *Aspergillus niger* in maize crop with and without rock phosphate fertilization was tested for the improvement of crop yield and soil fertility. It was observed that both fungal isolates, have stimulatory effect on growth parameters (shoot height, shoot and root dry biomass and on grain yield) of maize crop but the effects were more pronounced when RP fertilization was done along with inoculation compared to other treatments (Table 6.11). P uptake in plant tissues (grains, shoots and roots) was significantly increased in inoculated and RP fertilized treatments compared to control treatment (Table 6.12). Increase in grain yield was 19 % with fungal inoculation, 15.8 % with RP fertilization alone and it was increased up to 25.4 % when inoculation was done along with RP fertilization. On the other hand, DAP chemical fertilization increase grain yield up to 13.0 % compared to control treatment.

Soil pH decreased slightly from its initial value in all the treatments compared to control soil (Table 6.13). Organic carbon of the soil was significantly improved due to inoculation and RP fertilization compared to chemical fertilization and control (Table 6.14). Available P in soil was increased up to 87 % with fungal inoculation, and it rose up to 146 % when RP fertilization was done along with fungal inoculation compared to control soil. A significant increased in total P was observed in soil with DAP treatment but increase in available P was significantly higher in fungal inoculation and RP fertilization treatments (146 %) compared to DAP treatments (23 %). All treatments have no significant effect on improvement of soil total nitrogen (Table 6.14). Soil enzyme activities, such as acid phosphates, alkaline phosphatase, phytase and dehydrogenase enzyme activities were significantly improved in all the treatments (Table 6.15). Phytase enzyme activities in soil were significantly higher than

that of acid and alkaline phosphatase enzyme activities. Population density of P-solubilizing fungi in rhizospheric soil, after harvesting was 2.0×10^6 cfu g^{-1} in control treatment, 5.3-7.0×10^6 cfu g^{-1} in inoculation treatments alone, 3.3×10^6 cfu g^{-1} in RP fertilization alone, 3.0-4.7×10^7 cfu g^{-1} in inoculation treatments along with RP fertilization and 2.3×10^6 cfu g^{-1} in DAP treatments. Field study of maize crop in organic farming showed that there was a significant improvement in yield, P uptake and soil fertility in RP fertilization treatments along with fungal inoculation compared to individual inoculation and DAP treatments.

Aflatoxins in the maize grain samples were tested by HPLC analysis and the results revealed that aflatoxins such as B1, B2, G1, and G2 were absent in the grains.

Table 6.11 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on the growth parameters of maize plants grown in an organic field.

Treatments	Shoot length (cm)	Shoot dry weight (g)	Root dry weight (g)	Grain yield (ton ha^{-1})
Soil	225 ± 3.3d	36 ± 1.9g	9.70 ± 1.0b	5.66 ± 0.14d
Soil + At	258 ± 4.7ab	58 ± 1.3de	14.2 ± 0.9a	6.72 ± 0.06b
Soil + An	250 ± 3.0bc	64 ± 6.0cd	15.0 ± 0.9a	6.74 ± 0.08b
Soil + FC	252 ± 6.3bc	67 ± 1.0bc	14.8 ± 1.1a	6.73 ± 0.12b
Soil + RP	238 ± 3.0cd	51 ± 5.1ef	10.0 ± 0.5b	6.55 ± 0.04bc
Soil + RP + At	**271 ± 3.0a**	**74 ± 0.7ab**	**15.3 ± 0.4a**	**7.03 ± 0.07a**
Soil + RP + An	**270 ± 1.8a**	**75 ± 1.2ab**	**15.8 ± 0.9a**	**7.06 ± 0.07a**
Soil + RP + FC	**272 ± 4.7a**	**78 ± 2.6a**	**15.3 ± 1.2a**	**7.10 ± 0.10a**
DAP	239 ± 6.3cd	49 ± 1.5f	10.6 ± 0.8b	6.40 ± 0.04c
LSD (P<0.05)	9.44	5.19	1.56	0.149

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.12 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on P uptake of maize plants grown in an organic field.

Treatments	Total P (mg kg^{-1})		
	Grains	Shoot	Root
Soil	143 ± 10e	136 ± 9d	127 ± 10e
Soil + At	187 ± 11cd	172 ± 4bcd	176 ± 9cd
Soil + An	197 ± 17bc	177 ± 10abc	194 ± 24bc
Soil + FC	191 ± 4cd	176 ± 19abc	191 ± 4c
Soil + RP	179 ± 13cde	170 ± 11bcd	148 ± 6de
Soil + RP + At	**230 ± 15ab**	**212 ± 17a**	**229 ± 18ab**
Soil + RP + An	**238 ± 15a**	**197 ± 15ab**	**236 ± 10a**
Soil + RP + FC	**248 ± 17a**	**201 ± 17ab**	**244 ± 15a**
DAP	155 ± 13de	150 ± 11cd	139 ± 6e
LSD (P<0.05)	23	24	23

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.13 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of maize plants grown in an organic field.

Treatments	pH	EC ($mScm^{-1}$)	TDS (ppm)
Soil	8.33 ± 0.03a	0.18 ± 0.02c	0.12 ± 0.01c
Soil + At	7.77 ± 0.01de	0.25 ± 0.02abc	0.17 ± 0.02abc
Soil + An	7.74 ± 0.01def	0.23 ± 0.03bc	0.16 ± 0.02bc
Soil + FC	7.81 ± 0.01d	0.23 ± 0.05bc	0.15 ± 0.03bc
Soil + RP	7.94 ± 0.02c	0.19 ± 0.02bc	0.13 ± 0.01bc
Soil + RP + At	**7.67 ± 0.03g**	0.32 ± 0.04a	0.22 ± 0.02a
Soil + RP + An	**7.70 ± 0.02fg**	0.27 ± 0.04ab	0.18 ± 0.02ab
Soil + RP + FC	**7.73 ± 0.04efg**	0.33 ± 0.4a	0.22 ± 0.02a
DAP	8.01 ± 0.04b	0.25 ± 0.02abc	0.17 ± 0.01abc
LSD (P<0.05)	0.041	0.05	0.034

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.14 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of maize plants grown in an organic field.

Treatments	Organic carbon (%)	Organic matter (%)	Total P ($mg\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)	Total nitrogen (%)
Soil	0.39 ± 0.05c	0.66 ± 0.09c	237 ± 8d	4.02 ± 0.17e	0.035 ± 0.002a
Soil + At	**0.58 ± 0.04a**	**0.98 ± 0.06a**	248 ± 2d	7.51 ± 0.14b	0.045 ± 0.018a
Soil + An	**0.56 ± 0.02a**	**0.96 ± 0.03a**	250 ± 3d	7.23 ± 0.09b	0.042 ± 0.008a
Soil + FC	0.53 ± 0.02ab	0.91 ± 0.04ab	250 ± 4d	7.37 ± 0.07b	0.039 ± 0.005a
Soil + RP	0.46 ± 0.01bc	0.78 ± 0.02bc	465 ± 9c	5.29 ± 0.07c	0.042 ± 0.017a
Soil + RP + At	**0.56 ± 0.02a**	**0.96 ± 0.04a**	459 ± 9c	**9.96 ± 0.12a**	0.053 ± 0.010a
Soil + RP + An	**0.56 ± 0.03a**	**0.95 ± 0.05a**	**508 ± 8a**	**9.86 ± 0.05a**	0.050 ± 0.008a
Soil + RP + FC	**0.57 ± 0.01a**	**0.96 ± 0.01a**	469 ± 9bc	**9.91 ± 0.15a**	0.048 ± 0.013a
DAP	0.47 ± 0.03bc	0.79 ± 0.05bc	487 ± 8b	4.94 ± 0.10d	0.039 ± 0.005a
LSD (P<0.05)	0.049	0.084	12	0.20	0.019

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.15 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on enzyme activities of rhizosphere soil of maize plants grown in an organic field.

Treatments	Acid phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Alkaline phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Phytase activity ($\mu M\ g^{-1}\ hr^{-1}$)	Dehydro-genase activity (ppm)
Soil	370 ± 1.2e	530 ± 1.8g	6237 ± 500c	8.63 ± 0.92b
Soil + At	483 ± 1.0b	741 ± 0.7d	12272 ± 2063ab	10.5 ± 0.07ab
Soil + An	481 ± 5b	742 ± 0.9d	11968 ± 1496b	10.0 ± 0.05ab
Soil + FC	505 ± 1.2b	754 ± 1.3c	12529 ± 1224ab	11.3 ± 0.05ab
Soil + RP	425 ± 2.7d	703 ± 2.9e	8735 ± 126c	9.94 ± 2.8ab
Soil + RP + At	**567 ± 2.0a**	**792 ± 2.1b**	**14843 ± 97a**	**12.2 ± 0.09a**
Soil + RP + An	**569 ± 2.4a**	**802 ± 1.1a**	**14715 ± 83ab**	**12.3 ± 0.09a**
Soil + RP + FC	**578 ± 8.7a**	**796 ± 0.9ab**	**1457 7± 110ab**	**12.5 ± 0.1a**
DAP	411 ± 5.1c	615 ± 5.9f	8175 ± 203c	9.24 ± 0.05b
LSD (P<0.05)	4.77	4.54	1650	1.71

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

6.2.2 Wheat experiment

In wheat crop field, fungal inoculation alone and along with RP fertilization have significant effects on plant shoot height, shoot and root dry weight, and grain yield (Table 6.16). Effects of fungal inoculation along with RP fertilization were more pronounced compared to other treatments. Fungal inoculation treatments increased the yield of wheat 28 %, RP fertilization 11 %, inoculations along with RP fertilization 38 % and DAP treatments increased the yield 6 % compared to un inoculated control treatments. P uptake in seeds, shoots and roots was increased 44 %, 118 % and 59 % with inoculation, in RP fertilization alone it was 48 %, 81 % and 33 %, in inoculation treatments along with RP fertilization it was increased 76 %, 239 % and 109 % and in DAP treatments 4 %, 23 % and 11% increase in P uptake was observed compared to un-inoculated control (Table 6.17).

With inoculation and RP fertilization treatments, a slight decrease in soil pH was observed (Table 6.18). Organic carbon of soil was significantly improved due to inoculation treatments (39 %) and RP fertilization treatments along with inoculation (54 %) compared to RP fertilization alone (3 %) and chemical fertilizer treatment (13 %) and control soil (Table 6.19). Available P was increased up to 54 % with inoculation and it rose up to 127 % when RP fertilization was done along with inoculation compared to control soil. Increase in available P in inoculation and RP fertilization treatments was significantly higher (127 %) than increase in available P in DAP treatment (12 %) (Table 6.19). Soil total nitrogen was not significantly improved by any treatment. Acid phosphates, alkaline phosphatase, phytase and dehydrogenase enzyme activities were significantly improved in all the treatments but the results were more pronounced with inoculation treatments along with RP fertilization (Table 6.20). Phytase enzyme activities in soil were significantly higher than that of acid and alkaline phosphatase enzyme activities (Table 6.20). Alkaline phosphatase activities were decreased compared to alkaline phosphatase activities in maize rhizospheric soil. Population

density of phosphate-solubilizing fungi in rhizospheric soil after harvesting of the wheat crop was 2.3×10^6 cfu g^{-1} in control treatment, 6.0-7.3×10^6 cfu g^{-1} in fungal inoculation treatments alone, 4.3×10^6 cfu g^{-1} in RP fertilization treatments alone, 5.7-7.0×10^7 cfu g^{-1} in inoculation along with RP fertilization and 2.7×10^6 cfu g^{-1} in DAP treatments. Results showed that DAP treatment and RP fertilization alone was not increased the phosphate-solubilizing fungal population as much in soil, as it was significantly increased in fungal inoculation along with RP fertilization treatments.

Table 6.16 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on the growth parameters of wheat plants grown in an organic field.

Treatments	Shoot length (cm)	Shoot dry weight (g)	Root dry weight (g)	Grain yield (ton ha^{-1})
Soil	99 ± 8e	1.44 ± 0.10d	0.66 ± 0.07d	3.85 ± 0.18e
Soil + At	115 ± 1d	1.99 ± 0.12bcd	0.94 ± 0.05cd	4.50 ± 0.17bcd
Soil + An	119 ± 2bcd	1.92 ± 0.06cd	1.01 ± 0.05bc	4.30 ± 0.30bcde
Soil + FC	116 ± 2cd	2.15 ± 0.04abc	0.92 ± 0.05cd	4.92 ± 0.22ab
Soil + RP	109 ± 4de	1.70 ± 0.05cd	0.79 ± 0.16cd	4.27 ± 0.18cde
Soil + RP + At	**131 ± 3a**	**2.58 ± 0.20a**	**1.33 ± 0.14a**	**4.84 ± 0.16abc**
Soil + RP + An	**126 ± 4abc**	**2.54 ± 0.06ab**	**1.28 ± 0.18ab**	**5.30 ± 0.30a**
Soil + RP + FC	**129 ± 2ab**	**2.62 ± 0.25a**	**1.27 ± 0.07ab**	**5.25 ± 0.17a**
DAP	100 ± 1e	1.59 ± 0.46d	0.75 ± 0.04cd	4.06 ± 0.31de
LSD (P<0.05)	6.42	0.34	0.176	0.39

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.17 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on P uptake of wheat plants grown in an organic field.

Treatments	Total P (mg kg^{-1})		
	Grains	Shoot	Root
Soil	227 ± 10d	38 ± 10d	222 ± 6d
Soil + At	326 ± 17c	63 ± 6bcd	336 ± 17b
Soil + An	313 ± 17c	81 ± 9b	322 ± 10bc
Soil + FC	320 ± 15c	83 ± 11b	352 ± 6b
Soil + RP	336 ± 23bc	69 ± 6bc	295 ± 8c
Soil + RP + At	**373 ± 10ab**	**112 ± 4a**	**458 ± 8a**
Soil + RP + An	**384 ± 6a**	**120 ± 4a**	**463 ± 6a**
Soil + RP + FC	**400 ± 4a**	**129 ± 15a**	**459 ± 6a**
DAP	236 ± 20d	47 ± 6cd	245 ± 26d
LSD (P<0.05)	26	15	21

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.18 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of wheat plants grown in an organic field.

Treatments	pH	EC ($mScm^{-1}$)	TDS (ppm)
Soil	8.30 ± 0.02a	0.14 ± 0.01e	0.09 ± 0.007e
Soil + At	7.59 ± 0.04c	0.17 ± 0.02de	0.12 ± 0.010de
Soil + An	7.58 ± 0.02cd	0.22 ± 0.02abc	0.15 ± 0.01abc
Soil + FC	7.56 ± 0.03cde	0.17 ± 0.02de	0.12 ± 0.01de
Soil + RP	8.13 ± 0.02b	0.19 ± 0.02cd	0.13 ± 0.01cd
Soil + RP + At	**7.50 ± 0.05cde**	0.21 ± 0.02abcd	0.14 ± 0.01bcd
Soil + RP + An	**7.46 ± 0.05e**	0.24 ± 0.01ab	0.16 ± 0.01ab
Soil + RP + FC	**7.47 ± 0.08de**	0.25 ± 0.02a	0.17 ± 0.01a
DAP	8.19 ± 0.03b	0.19 ± 0.02bcd	0.13 ± 0.01bcd
LSD (P<0.05)	0.068	0.026	0.017

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.19 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on rhizosphere soil characteristics of wheat plants grown in an organic field.

Treatments	Organic carbon (%)	Organic matter (%)	Total P (mg kg^{-1})	Available P (mg kg^{-1})	Total nitrogen (%)
Soil	0.46 ± 0.04d	0.78 ± 0.06d	204 ± 7d	4.94 ± 0.18e	0.045 ± 0.005a
Soil + At	0.64 ± 0.02ab	1.09 ± 0.04ab	223 ± 6cd	7.23 ± 0.18bc	0.063 ± 0.013a
Soil + An	0.61 ± 0.03abc	1.05 ± 0.05abc	247 ± 9bc	7.59 ± 0.15b	0.056 ± 0.012a
Soil + FC	0.59 ± 0.01bc	1.00 ± 0.02bc	250 ± 8b	7.11 ± 0.18c	0.066 ± 0.011a
Soil + RP	0.47 ± 0.08d	0.80 ± 0.14d	**455 ± 6a**	5.90 ± 0.18d	0.045 ± 0.009a
Soil + RP + At	**0.66 ± 0.02ab**	**1.13 ± 0.04ab**	437 ± 8a	**10.95 ± 0.13a**	0.060 ± 0.012a
Soil + RP + An	**0.69 ± 0.03ab**	**1.18 ± 0.05ab**	434 ± 5a	**10.90 ± 0.09a**	0.059 ± 0.019a
Soil + RP + FC	**0.71 ± 0.04a**	**1.21 ± 0.06a**	443 ± 6a	**11.22 ± 0.18a**	0.064 ± 0.050a
DAP	0.52 ± 0.02cd	0.88 ± 0.03cd	437 ± 19a	5.51 ± 0.11d	0.046 ± 0.003a
LSD (P<0.05)	0.064	0.108	16	0.265	0.247

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.

Table 6.20 Effect of *Aspergillus tubingensis* (At) and *Aspergillus niger* (An) alone or along with RP fertilization and chemical fertilizer (DAP) on enzyme activities of rhizosphere soil of wheat plants grown an organic field.

Treatments	Acid phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Alkaline phosphatase ($\mu M\ g^{-1}\ hr^{-1}$)	Phytase activity ($\mu M\ g^{-1}\ hr^{-1}$)	Dehydro-genase activity (ppm)
Soil	439 ± 1.4d	329 ± 0.90g	6485 ± 84f	12.30 ± 0.16e
Soil + At	535 ± 37b	472 ± 00.55d	10600 ± 69c	14.48 ± 0.07b
Soil + An	564 ± 3.3ab	474 ± 0.75cd	10425 ± 84c	14.45 ± 0.05b
Soil + FC	575 ± 0.85a	477 ± 0.72c	10333 ± 110c	14.56 ± 0.06b
Soil + RP	465 ± 1.2cd	403 ± 0.36f	8092 ± 89d	13.94 ± 0.12c
Soil + RP + At	**585 ± 1.7a**	**528 ± 2.2b**	**12832 ± 115b**	**16.91 ± 0.02a**
Soil + RP + An	**590 ± 2.0a**	**526 ± 0.4b**	**12740 ± 42b**	**16.96 ± 0.07a**
Soil + RP + FC	**596 ± 0.85a**	**533 ± 2.2a**	**13181 ± 57a**	**16.99 ± 0.16a**
DAP	482 ± 1.2c	444 ± 2.5e	7679 ± 161e	13.45 ± 0.08d
LSD (P<0.05)	21	2.56	165	0.168

Values are Mean ± SD (n =10). Means sharing a common letter within the column are not significantly different at P<0.05. Pronounced results are represented in bold.