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Effects of PGPR and antagonistic fungi on the growth, enzyme activity and *Fusarium* root-rot of pea

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The effects of plant growth-promoting rhizobacteria (PGPR) (*Bacillus pumilus* and *Pseudomonas putida*) and antagonistic fungi (*Aspergillus awamori, Aspergillus niger* and *Trichoderma harzianum*) were studied alone and in combination in glasshouse experiments on the growth, chlorophyll catalase and peroxidase activity and on the *Fusarium* root-rot of pea caused by *Fusarium solani* f. sp. *pisi*. Application of PGPR and antagonistic fungi caused a significant increase in growth, chlorophyll, catalase and peroxidase activities of both root-rot fungus inoculated and un-inoculated pea plants. Use of *P. putida* was more effective in reducing disease severity and improving the growth of root-rot fungus-inoculated plants than *A. niger* and *T. harzianum*. The greatest increase in growth, chlorophyll, catalase and peroxidase activities of root-rot fungus-inoculated plants and reduction in disease severity was achieved when *A. awamori* or *B. pumilus* was used with *P. putida* compared to other tested combinations.

Keywords: catalase; *Fusarium solani*; peroxidase; *Pisum*

Introduction

Pea (*Pisum sativum* L.) is grown as an annual tender and vigorous knee-high vines and are the important source of dietary protein for human and livestock consumption. The mature seed contains protein (19–27%) and is relatively free of anti-nutritional substances (Saharan & Khetarpaul 1994). Pea contributes significantly to yield and protein content of the succeeding cereal crop in rotation by improving nitrogen status of the soil (Rowland et al. 1994). India is the largest grower of pea after Canada and Russia (Khan & Dixit 2001) with a cultivated area of about 0.81 million hectare, out of which 65% area lies in the state of Uttar Pradesh. Several soil fungal pathogens, such as *Alternaria, Aphanomyces, Ascochyta, Colletotrichum*, *Erysiphe, Fusarium, Mycosphaerella, Peronospora, Pythium,* and *Sclerotinia,* have been recognised as causal agents of pea worldwide. Amongst the various soil funguses, *Fusarium solani* f. sp. *pisi* is the most frequent causal agent of root-rot of many host plants, including pea (Ondrej et al. 2008). It is revealed from the survey conducted in Aligarh, Bulandshahar, Hathras and Mathura districts of Uttar Pradesh that frequency of occurrence of this fungus associated with roots of pea is about 63.8% and the annual yield loss is about 30–36%. Pea roots infected with

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F. solani f. sp. *pisi* showed clear reddish-brown streaks on primary and secondary roots. The plants are stunted with wedge-shaped bark brown-coloured lesions on the stems with yellowish leaves showing distortion in chlorophyll pigments.

The rhizosphere is the zone of intense microbial activity and harbours a lot of microorganisms. This zone is relatively rich in nutrients due to the loss of up to 40% of plant photosynthates from roots, and the rhizosperic microorganisms have the capacity to utilise the compounds and materials released from the crop roots as nutrition source. Consequently, the rhizosphere supports large and active microbial populations capable of exerting beneficial, neutral or detrimental effects on plant growth. Among the rhizosphere organisms, *Aspergillus* species have been reported to produce a variety of secondary metabolites and are useful in the biocontrol of root-rot of chickpea (Akhtar & Siddiqui 2006). Rashid et al. (2004) reported that *Aspergillus* sp. possess the ability to solubilise the insoluble phosphates in soil into solublised forms by secreting organic acids, such as formic, acetic, propionic, lactic, glycolic, fumaric, and succinic acids, while Khan and Khan (2001) reported that application of *Aspergillus awamori* and *Aspergillus niger* resulted in an 80 and 58% increase in the yield of pathogen-inoculated tomato plants. Siddiqui and Akhtar (2007) found that the application of *A. awamori* significantly increases the plant growth, chlorophyll and NPK contents, and also reduced root-rot disease on chickpea. It has been also reported that inoculation of *A. awamori* produced phenyl ethanol, phenyl acetic acid and phenoxy acetic acid (Nair & Burke 1988), which may have reduced the disease severity in crop plants (Palakshappa et al. 1989). Similarly, *Trichoderma* spp., are free-living fungi that are highly interactive in root, soil and foliar environments. *Trichoderma harzianum* has been recognised for its biocontrol activity against several plant pathogenic fungi through various mechanisms: antibiosis, competition, mycoparasitism and enzymatic hydrolysis (Harman et al. 2007). Moreover, this fungus may also promote plant growth and induce systemic resistance in plants (Yedidia et al. 1999).

Some of rhizospheric bacteria, especially *Pseudomonas* and *Bacillus* spp., have considerable potential for the biocontrol of plant pathogens. Rhizobacteria have the ability to improved seed germination, root development, mineral nutrition and water utilisation and can also suppress plant diseases. The manipulation of crop rhizosphere by inoculation with plant growth-promoting rhizobacteria (PGPR) for biocontrol of plant diseases has shown considerable promise (Akhtar & Siddiqui 2009). Applications of two or more biocontrol agents at the same time provide a better management against a single pathogen (Wilson & Backman 1999) because mixtures of biocontrol agents with different plant colonisation patterns may be useful for biocontrol of different plant pathogens via different mechanisms of disease suppression (Siddiqui & Akhtar 2008). Moreover, mixtures of biocontrol agents require different optimum temperature, pH and moisture conditions that may colonise roots more aggressively, improve plant growth and efficiency of biocontrol. Dual inoculation with biocontrol agents having different mechanisms of action is known to provide greater biocontrol against plant pathogens on different crops than inoculation with a single agent (Guetsky et al. 2002).

Several investigations of enzymes in fungus-infected plants have been conducted. Catalase and peroxidase are the important groups of enzymes, which play a very crucial role in the defence mechanism of plants against the pathogens. Catalase is a soluble haemeprotein. It breaks the hydrogen peroxide into hydrogen and water. The role of catalase in pathogen defence has been investigated by earlier workers (Mittler et al. 1999; Vandenabeele et al. 2004). Generally, it has been assumed that the suppression in catalase activity results in plants that have reactive oxygen species and are primed to

resist pathogen attack (Magbanua et al. 2007), while peroxidase catalyses the polymerisation of phenolic compounds and forms cross-links between extensin, lignin and feruloylated polysaccharides (Espelie et al. 1986). Das et al. (2003) reported that peroxidase plays a key role in the phenyl propanoid pathway, it will be activated in response to pathogens. It has been also reported by Mehrotra and Aggarwal (2003) that changes in the peroxidase activity may also regulate the metabolic pathways in diseased or injured tissues, while the lower peroxidase activity was recorded in wheat seedlings inoculated with *Gaeumannomyces graminis* var. *tritici* (Monfort et al. 2005).

In the present study, an attempt was made to examine the effects of PGPR (*Bacillus pumilus* and *Pseudomonas putida*) alone and in combination with antagonistic fungi (*A. awamori, A. niger* and *T. harizianum*) on growth, chlorophyll, catalase and peroxidase activities and *Fusarium* root-rot of pea**.**

Materials and methods

The root-rot fungus *F. solani* f. sp. *pisi* was the tested pathogen. PGPR (*B. pumilus* and *P. putida*) and antagonistic fungi (*A. niger, T. harzianum* and *A. awamori*) were applied alone and in combination to pea (*Pisum sativum* cv. Arkil). The influence of these treatments on chlorophyll, catalase and peroxidase activity was determined at the 45 days (flowering stage) after inoculation, while the plant growth was assessed 90 days after inoculation in glasshouse experiments.

Preparation and sterilisation of soil mixture

The soil used in the study was sandy loam (pH 7.3, porosity 36%, water holding capacity 42%, electrical conductivity 0.68 (ds/m), available N 212 kg/ha, P 25 kg/ha and K 362 kg/ha). Appropriate amount of water was added into each bag, in order to moist the soil before transferring to an autoclave for sterilisation at 137.9 kPa for 20 min. After autoclaving, the soil was allowed to cool to room temperature and about 1 kg of soil was filled in each 15 cm diameter clay pots.

Raising and maintenance of test plants

Seeds of pea were surface sterilised with 0.1% sodium hypochlorite (NaOCl) for 2 min and then rinsed three times with distilled water. Five healthy pea seeds of similar sizes were sown in each pot and after germination, thinning was done to maintain one healthy seedling per pot. Two days after thinning, seedlings received the treatments. The pea seedlings grown in pot were inoculated with antagonistic fungi (*A. awamori*, *A. niger* and *T. harzianum* @10 ml of 1.2×10^5 cfu/ml) and PGPR (*B. pumilus* and *P. putida* @10 ml of 1.5×10^7 cfu/ml) were added around each seedling in the pots, while un-inoculated plants served as a control. All the pots were kept in a glasshouse at 22 ± 2 °C and watered as needed.

Inoculum production of microorganisms

B. pumilus and P. putida was sub-cultured on nutrient broth (Hi-Media Laboratories, Mumbai, India). One millilitre of nutrient broth suspension contained about 1.5×10^7 cfu/ml. Ten millilitre of this suspension was used to inoculate the respective pots around the pea seedlings. *A. awamori, A. niger* and *T. harzianum* were cultured in

Richard's medium at 25 ± 1 °C for 15 days. Ten millilitre (1.2×10^5 cfu/ml) suspension of each was used to inoculate the respective pots around the pea seedlings.

Inoculation procedure

For inoculation of *A. awamori, A. niger, T. harzianum, B. pumilus* and *P. putida,* soil around the root was carefully removed without damaging the roots. The inoculum suspensions were poured or placed around the roots and the soil was replaced. An equal volume of sterile water was added to control treatments.

Experimental design

The experiment was carried out in a completely randomised blocked design with three experimental variables: (a) Control, (b) *B. pumilus* and (c) *P. putida*. Each set was inoculated with the following four treatments: (1) Control, (2) *A. awamori,* (3) *A. niger,* and (4) *T. harzianum* ($3 \times 4 = 12$ treatments). These 12 treatments were tested both in presence and absence of fungus ($12 \times 2 = 24$ treatments). Each treatment was replicated five times ($24 \times 5 = 120$ pots) and the experiment was repeated once. The data in this paper represents the pooled data of both the experiments.

Parameter assessment

The plants were harvested 90 days after inoculation. Data on dry shoot weight and root-rot indices were recorded. Chlorophyll content was estimated by method of Arnon (1949), while the catalase and peroxidase activities in leaves were determined by Chance and Maehly (1995) method at flowering stage. Plants were kept in envelopes at 60 °C for 2–3 days before dry weight measurements. The root-rot of plant inoculated with *F. solani* f. sp. *pisi* was recorded by scoring disease severity on a scale ranging from 0 (no disease) to 5 (severe root-rot).

Statistical analysis

The data were analysed statistically using multifactorial analysis (*F. solani* × PGPR × antagonistic fungi) of variance $p = 0.05$. Effects of *F. solani* × PGPR × antagonistic fungi were analysed individually and also using the interactions of two and three factors. Duncan's multiple range test (DMRT) was employed to distinguish differences between treatments.

Results

Effects of root-rot fungus (*F. solani*), PGPR (*B. pumilus* and *P. putida*) and antagonistic fungi (*A. awamori, A. niger* and *Trichoderma harizianum*) individually and their interactions were found to be significant on dry shoot weight except for the interaction of fungus and antagonistic fungi and interaction of all the three factors together. Inoculation of PGPR and antagonistic fungi alone caused a significant increase in dry shoot weight of plants without root-rot fungus over inoculated ones (Table 1). *P. putida* alone caused a greater increase in the dry shoot weight of the plants without root-rot fungus followed by *A. niger* or *T. harizianum*. However, increase in dry shoot weight caused by *A. awamori* or *B. pumilus* was similar to that caused by *P. putida*. Use of *P. putida*

Table 1. Effects of PGPR and antagonistic fungi alone and in combination on dry shoot weight in *F. solani* f. sp. *pisi*-inoculated and un-inoculated pea plants.

	Shoot dry weight (g)					
	Without *F. oxysporum*			With *F. oxysporum*		
Treatments	C	Bp	Pp	C	Bp	Pp
C	3.92 ± 0.35^{no}	5.28 ± 0.25^{e}	5.38 ± 0.22^{de}	3.20 ± 0.16^{p}	4.02 ± 0.27^{lmn}	4.12 ± 0.17^{klmn}
An	4.92 ± 0.42^{fg}	5.72 ± 0.34^{c}	6.02 ± 0.33^{ab}	3.94 ± 0.37^{mno}	4.46 ± 0.35^{ij}	4.66 ± 0.28^{ghi}
Aw	5.20 ± 0.44^{ef}	6.10 ± 0.33^{a}	6.16 ± 0.38^{a}	4.22 ± 0.41^{klm}	4.62 ± 0.37^{hi}	4.76 ± 0.24^{gh}
Th	4.76 ± 0.36^{gh}	5.66 ± 0.29^{cd}	5.78 ± 0.26^{bc}	3.70 ± 0.37^{o}	4.28 ± 0.36^{jkl}	4.40 ± 0.38^{ijk}

*Values within the same column followed by different letters are significantly different at $p < 0.05$, DMRT. C = Control; An = *Aspergillus niger*; Aw = *A. awamori*; Th = *Trichoderma harzianum*; Bp = *Bacillus pumilus*; Pp = *Pseudomonas putida*. ± = standard deviation.

with *A. awamori* resulted in greater increase in dry shoot weight of plants without root-rot fungus compared with any other tested combination. However, inoculation of *B. pumilus* plus *A. awamori* caused a similar increase in dry shoot weight of plants without root-rot fungus to that caused by *P. putida* with *A. awamori* (Table 1).

Inoculation of root-rot fungus caused a significant reduction in dry shoot weight of plants over uninoculated ones. Inoculation of *B. pumilus, P. putida* and antagonistic fungi alone resulted in significant increase in dry shoot weight of root-rot fungus-inoculated plants. *P. putida* alone caused a greater increase in dry shoot weight in root-rot fungus-inoculated plants than caused by *T. harzianum* (Table 1).

Root colonisation by PGPR was greater when inoculated with antagonistic fungi than when used singly (Table 1). Root colonisation caused by PGPR was increased in the presence of antagonistic fungi. *A. awamori* caused a higher increase in root colonisation followed by *A. niger* and *T. harzianum*. Inoculation of root-rot fungus had an adverse effect on the root colonisation by PGPR (Figure 1).

Plants inoculated with root-rot fungus alone had a rotting index 4. The root-rot index was reduced to 3 when fungus-inoculated plants were treated only with *A. niger* or *T. harzianum* or *B. pumilus*. The root-rot index was reduced to 2 when fungus-inoculated plants were treated with *A. awamori* or *P. putida* alone; or *B. pumilus* plus *A. niger;* or *B. pumilus* plus *T. harzianum;* or *P. putida* plus *T. harzianum*, while in the other treatments, the root-rot index reduced to 1 (Figure 2).

Effects of root-rot fungus, PGPR and antagonistic fungi individually and their interactions were found to be significant on chlorophyll content except for the interaction of PGPR, antagonistic fungi and interaction of all the three factors together. Inoculation of PGPR and antagonistic fungi alone caused a significant increase in chlorophyll content of plants without root-rot fungus over inoculated ones (Table 2). *P. putida* alone caused a greater increase in the chlorophyll content of the plants without root-rot fungus followed by *A. niger* or *T. harizianum*. However, increase in chlorophyll content caused by *A. awamori* or *B. pumilus* was similar to that caused by *P. putida*. Use of *P. putida* with *A. awamori* resulted in greater increase in chlorophyll content of plants without root-rot fungus compared with any other tested combination. However, inoculation of *B. pumilus* plus *A. awamori* caused a similar increase in chlorophyll content of plants without root-rot fungus to that caused by *P. putida* with *A. awamori* (Table 2).

Inoculation of root-rot fungus caused a significant reduction in chlorophyll content of plants over un-inoculated ones. Inoculation of *B. pumilus, P. putida* and antagonistic fungi alone resulted in significant increase in chlorophyll content of root-rot fungus-

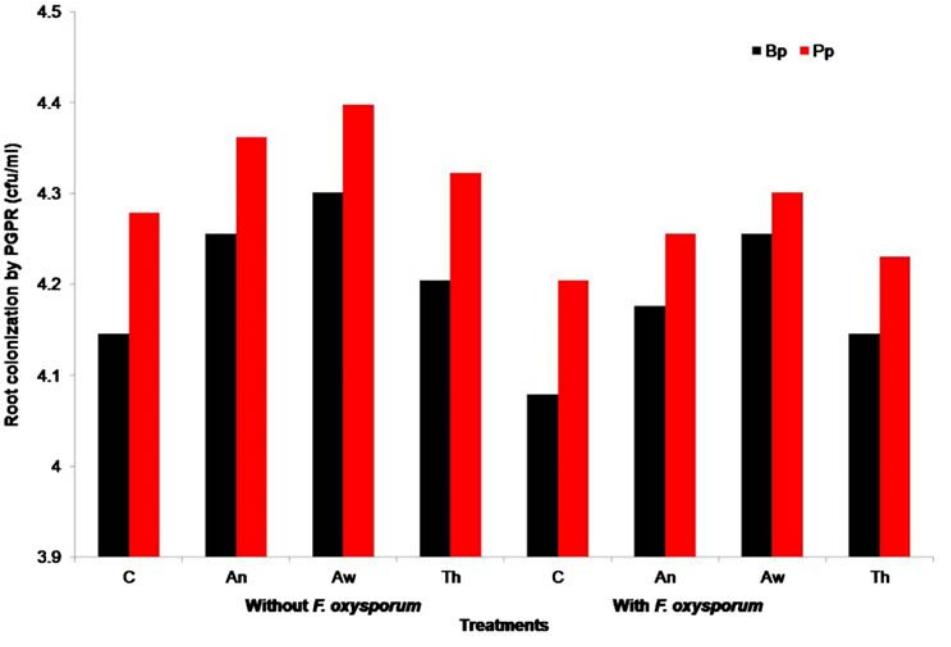

Figure 1. Effects of antagonistic fungi on root colonisation by PGPR in *F. solani* f. sp. *pisi*-inoculated and uninoculated pea plants. C = Control; An = *Aspergillus niger*; Aw = *A. awamori*; Th = *Tricoderma harzianum*; Bp = *Bacillus pumilus*; Pp = *Pseudomonas putida.* (Data are in log (x + 1)-transformed values).

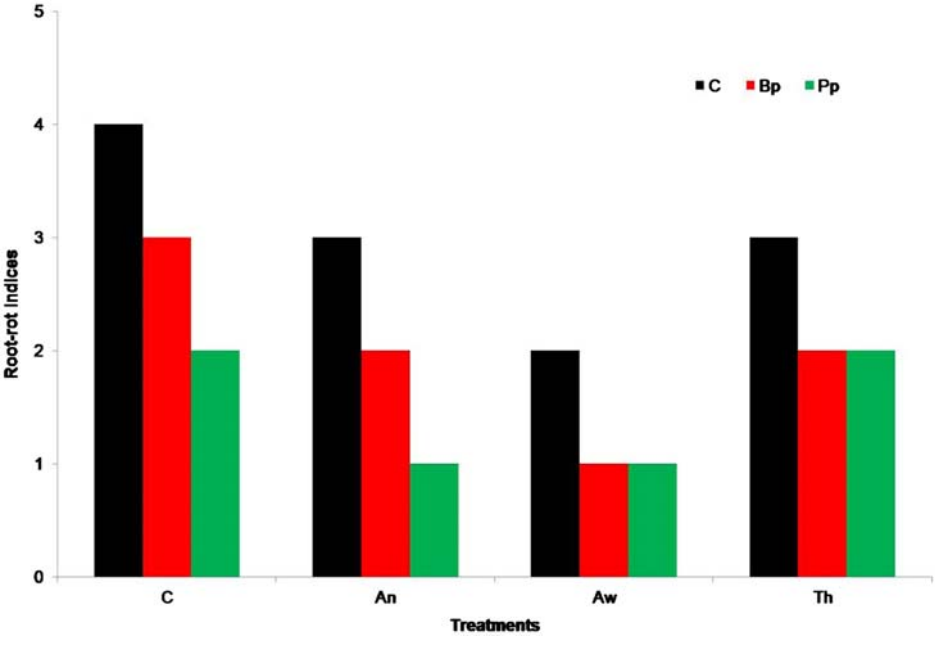

Figure 2. Effects of PGPR and antagonistic fungi alone and in combination on root-rot indices. C = Control; An = *Aspergillus niger*; Aw = *A. awamori*; Th = *Tricoderma harzianum*; Bp = *Bacillus pumilus*; Pp = *Pseudomonas putida.*

Table 2. Effects of PGPR and antagonistic fungi alone and in combination on chlorophyll content in *F. solani* f. sp. *pisi*-inoculated and uninoculated pea plants.

	Chlorophyll content (mg/g)					
	Without *F. oxysporum*			With *F. oxysporum*		
Treatments	C	Bp	Pp	C	Bp	Pp
C	2.318 ± 0.11^{kl}	2.628 ± 0.08^{def}	2.674 ± 0.08^{de}	1.672 ± 0.14^{p}	2.160 ± 0.08^{mn}	2.252 ± 0.11^{lm}
An	2.480 ± 0.06^{hij}	2.806 ± 0.09^{c}	2.936 ± 0.08^{b}	2.144 ± 0.05^{no}	2.478 ± 0.14^{hij}	2.538 ± 0.09^{fgh}
Aw	2.596 ± 0.05^{efg}	2.918 ± 0.08^{b}	3.092 ± 0.18^{a}	2.176 ± 0.07^{mn}	2.514 ± 0.11^{ghi}	2.654 ± 0.06^{de}
Th	2.432 ± 0.05^{ij}	2.718 ± 0.10^{cd}	2.788 ± 0.09^{c}	2.054 ± 0.06^{o}	2.382 ± 0.34^{jk}	2.472 ± 0.08^{hij}

*Values within the same column followed by different letters are significantly different at $p < 0.05$, DMRT. C = Control; An = *Aspergillus niger*; Aw = *A. awamori*; Th = *Tricoderma harzianum*; Bp = *Bacillus pumilus*; Pp = *Pseudomonas putida*. ± = standard deviation.

inoculated plants. *P. putida* alone caused a greater increase in root-rot fungus-inoculated plants than caused by *A. niger* or *T. harzianum* (Table 2).

Effects of root-rot fungus, PGPR and antagonistic fungi were significant on catalase and peroxidase activity, while the interaction of two factors and the interaction of all the three factors together were non-significant except the interaction of PGPR and antagonistic fungi on peroxidase activity. Inoculation of PGPR and antagonistic fungi alone caused a significant increase in catalase and peroxidase activity of plants without root-rot fungus over inoculated ones (Tables 3 and 4). Inoculation of *P. putida* alone caused a significant increase in catalase activity than caused by antagonistic fungi or *B. pumilus* (Table 3), while the inoculation of *P. putida* alone caused a greater increase in peroxidase activity of the plants without root-rot fungus than caused by any antagonistic fungi (Table 4). Inoculation of *P. putida* with *A. awamori* caused a greater increase in catalase and peroxidase activity of plants without root-rot fungus compared to that caused by other treatments (Tables 3 and 4). However, inoculation of *A. awamori* and *P. putida* caused almost similar increase in peroxidase activity of the plants without root-rot fungus than caused by *P. putida* plus *A. niger*.

Inoculation of root-rot fungus caused a significant reduction in catalase and peroxidase activity of plants over un-inoculated ones (Tables 3 and 4). Inoculation of PGPR and antagonistic fungi caused a significant increase in catalase and peroxidase activity of root-rot fungus-inoculated plants. *P. putida* alone caused almost similar increase in peroxidase activity in root-rot fungus-inoculated plants than caused by antagonistic fungi/*B. pumilus* (Table 4), while the inoculation of *P. putida* alone caused a significant

Table 3. Effects of PGPR and antagonistic fungi alone and in combination on catalase activity in *F. solani* f. sp. *pisi*-inoculated and uninoculated pea plants.

	Catalase activity (H_2O_2/min)					
	Without *F. oxysporum*			With *F. oxysporum*		
Treatments	C	Bp	Pp	C	Bp	Pp
C	3.48 ± 0.10^{kl}	4.18 ± 0.09^{fg}	4.30 ± 0.22^{de}	3.02 ± 0.13^{n}	3.56 ± 0.09^{kl}	3.78 ± 0.09^{j}
An	3.96 ± 0.18^{hi}	4.62 ± 0.12^{c}	4.82 ± 0.33^{b}	3.44 ± 0.08^{l}	3.98 ± 0.14^{hi}	4.20 ± 0.19^{ef}
Aw	4.08 ± 0.22^{gh}	4.76 ± 0.11^{b}	5.06 ± 0.38^{a}	3.58 ± 0.08^{k}	4.16 ± 0.13^{m}	4.34 ± 0.13^{d}
Th	3.78 ± 0.12^{j}	4.36 ± 0.09^{d}	4.54 ± 0.26^{c}	3.28 ± 0.08^{m}	3.74 ± 0.09^{j}	3.92 ± 0.13^{i}

*Values within the same column followed by different letters are significantly different at $p < 0.05$, DMRT. C = Control; An = *Aspergillus niger*; Aw = *A. awamori*; Th = *Tricoderma harizianum*; Bp = *Bacillus pumilus*; Pp = *Pseudomonas putida*. ± = standard deviation.

Table 4. Effects of PGPR and antagonistic fungi alone and in combination on peroxidase activity in *F. solani* f. sp. *pisi*-inoculated and uninoculated pea plants.

	Peroxidase activity (Purpurogallin/mg/min)					
	Without *F. oxysporum*			With *F. oxysporum*		
Treatments	C	Bp	Pp	C	Bp	Pp
C	0.221 ± 0.008^{mn}	0.272 ± 0.008^{ef}	0.278 ± 0.010^{de}	0.198 ± 0.007^{o}	0.238 ± 0.27^{lmn}	0.242 ± 0.17^{klmn}
An	0.252 ± 0.005^{ij}	0.296 ± 0.013^{bc}	0.310 ± 0.011^{a}	0.222 ± 0.006^{m}	0.258 ± 0.35^{ij}	0.266 ± 0.28^{ghi}
Aw	0.266 ± 0.010^{fg}	0.302 ± 0.007^{b}	0.316 ± 0.005^{a}	0.236 ± 0.010^{l}	0.264 ± 0.37^{hi}	0.278 ± 0.24^{gh}
Th	0.240 ± 0.008^{l}	0.284 ± 0.006^{d}	0.292 ± 0.010^{c}	0.214 ± 0.010^{n}	0.248 ± 0.36^{jkl}	0.254 ± 0.38^{ijk}

*Values within the same column followed by different letters are significantly different at $p < 0.05$, DMRT. C = Control; An = *Aspergillus niger*; Aw = *A. awamori*; Th = *Tricoderma harizianum*; Bp = *Bacillus pumilus*; Pp = *Pseudomonas putida*. ± = standard deviation.

increase in catalase activity of root-rot fungus-infected plants than caused by *B. pumilus* or any tested antagonistic fungi (Table 3). However, inoculation of *P. putida* with *A. awamori* caused a greater increase in catalase and peroxidase activity of plants infected with root-rot fungus compared with other treatments (Tables 3 and 4).

Discussion

The antagonistic effect of rhizoshperic bacteria against a wide range of pathogenic fungi is mediated by a variety of compounds of microbial origin, such as antibiotics, enzymes, siderophores, HCN, catalase, bacteriocins, volatiles and toxic substances. *Pseudomonas* spp. had the ability to improve the seed germination, root colonisation, stimulate the mineral and water uptake and also reduced the disease severity (Whipps 2001; Akhtar et al. 2010). Egamberdieva (2008) observed the positive effect of PGPR on the growth of wheat and pea, while seed inoculation improved seedling growth in maize (Gholami et al. 2009). Akhtar et al. (2010) demonstrates that combined application of PGPR improved the growth of *F. oxysporum*-inoculated lentil plants. Moreover, *Pseudomonas* spp. can indirectly protect plants by inducing systemic resistance against various plant pathogens (Van Loon et al. 1998; Ramamoorthy et al. 2001). Similarly, *P. putida* have been reported to promote the growth of a wide range of plants (Akhtar & Siddiqui 2009). These responses may be due to the production of siderophores, antibiotics, wall appositions and defence enzymes which adversely affect on the pathogens.

Aspergillus species are commonly found in soils-decaying plant material and stored grains and had the ability to produce a range of secondary metabolites (Domsch et al. 1980). Some *Aspergillus* species have been widely used for their biocontrol potential against plant pathogenic fungi. Khan and Khan (2001) reported that use of *A. awamori* and *A. niger* were effective against the *Fusarium* wilt of tomato. Similarly, Najar et al. (2011) reported that *A. flavus* had the ability to reduce the disease severity of *F. solani* f. sp. *melongenae* on eggplant. These results clearly indicated that *Aspergillus* species isolated from the rhizosphere of crop plants produce number of secondary metabolites that are soluble in ethyl acetate and can potentially influence the efficacy of the biocontrol strains. Thus, the growth promoting attributes by *A. awamori* may be due to its secondary-metabolites-producing capacity and the increase in catalase activity may be responsible for it.

The results of the present study showed that the use of PGPR and antagonistic fungi alone and in combination significantly improved the plant growth and reduced the disease severity of *Fusarium* root-rot. This study clearly indicates that the use of suitable combination of biocontrol agents were effective against the *Fusarium* root-rot caused by *F. solani* f. sp. *pisi*. Combined application of suitable and compatible microorganisms having different mechanisms of action is known to provide greater biocontrol against plant pathogens on different crops than an inoculation with a single agent (Guetsky et al. 2002; Akhtar et al. 2010). The effectiveness of PGPR and antagonistic fungi may vary against different fungal pathogens. In our experiment, the combined application of *P. putida* either with *B. pumilus* or with either *A. awamori* caused a greater decrease disease severity as compared with other combinations or individual inoculation. Application of *A. awamori* with *P. putida* had an additive effect in reducing rotting symptoms and acceleration in enzyme activity and secretion of secondary metabolites may develop an induced systemic resistance against applied pathogens.

It was also evident from our results that the chlorophyll, catalase and peroxidase activities were less in fungus-infected plants as compared with uninoculated plants. The increase in enzyme activity was observed when PGPR and antagonistic fungi were inoculated alone or in combination. Magbanua et al. (2007) reported the suppression in the catalase activity due to generation of reactive oxygen species which may protect the plants against the pathogen attack, while the lower peroxidase activity was recorded in the wheat seedlings inoculated with *G. graminis* (Monfort et al. 2005).

The experiment described in the present study was carried out in pots under greenhouse condition. When these microorganisms will be applied in field condition, they have to compete with other soil microorganisms present in same ecological niche, which may influence the performance of used biocontrol agents. Our study suggested that use of *A. awamori* and *P. putida* have the greater potential to reduce the disease severity of *Fusarium* root-rot on pea. However, further studies are needed to confirm these results under different pathosystems.

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