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Full Length Research Paper

Management of root rot of pea (*Pisum sativum* L.) through bioagents

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Root rot of pea is assuming the status of major disease in Kashmir. The diseased plants showed poor growth, yellowing and drying of foliage with partially or fully damaged root system. The pathogen associated with the disease was identified as *Fusarium solani* f.sp. *pisi* (Jones) Synder and Hansen. The dual culture studies of four biocontrol agents revealed that *Trichoderma harzianum* exhibited highest inhibition percentage of 78.60 followed by *Trichoderma viride* (75.72%), *Gliocladium virens* (69.52%) and *Pseudomonas fluorescence* (68.37%). *P. fluorescence* was the only biocontrol agent which showed zone of inhibition in dual culture. Seed treatment with biocontrol agents revealed the highest germination percentage in pots treated with *T. harzianum* and carbendazim (90% each) followed by *T. viride* (86%), *P. fluorescence* (83%) and *G. virens* (82%) while it was 61 per cent in control. *T. harzianum* treated seeds took a least 7.26 days to germinate followed by *T. viride* (7.32 days), carbendazim (7.34 days), *P. fluorescence* (7.37 days) and *G. virens* (7.53 days) as against 7.88 in control. Disease incidence and severity was lowest in the pots treated with carbendazim (14.64 and 4.98%) followed by *T. harzianum* (21.30 and 10.94%), *T. viride* (25.30 and 12.02%), *P. fluorescence* (29.28 and 14.98%) and *G. virens* (38.64 and 17.58%) whereas in control it was 54.64 and 32.02%, respectively.

Key words: Pea, *Fusarium solani* f.sp. *pisi*, root rot, biological control.

INTRODUCTION

Root rot disease complex is considered as the most destructive in pea as it affects its initial plant stand and more than 20 different pathogens have been reported to be associated with the disease from different parts of the world (USDA, 1960). Among these, *Fusarium solani* f.sp *pisi*, *Fusarium oxysporum* f.sp. *pisi, Aphanomyces euteiches* and *Pythium* spp. are highly destructive. The disease causes early inhibition of root growth, which affects uptake of nutrients and water and is thus manifested as stunted growth and the infected plants bear few partially filled pods, which mature early (Oyarzun, 1993). Under favourable environmental conditions, it can cause even complete failure of the crop (Tu, 1987). The disease has also been reported from Kashmir valley where pea is the only major pulse crop grown during *rabi* season with an incidence ranging between 14.8 to 64.7% (Masoodi et al., 2000).

Various fungicides have been effectively used in the management of this disease. However, development of fungicide-resistant phytopathogenic strains and adverse effect of pesticides on soil, plant health and crop products have compelled plant pathologists to look for ecofriendly strategies for plant disease management (Tu, 1997; Chattopadhyay et al., 2002). Further, the soil borne nature of the disease has rendered disease management through fungicides a difficult task. Therefore, thrust is laid on developing integrated management approach. Since biological control is one of the essential components of "Integrated Disease Management" and no work on this aspect of the disease has been carried out in Kashmir. Thus, present study was undertaken to characterise and identify the predominant pathogen associated with root rot of pea and to study the efficacy of various bio-agents against the pathogen.

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MATERIALS AND METHODS

Isolation of pathogen(s)

Root rot affected pea plants collected from fields were examined in the laboratory for typical symptoms of the disease. Pathogen (s) was isolated on potato dextrose agar (PDA) from discoloured root tissue of infected plants. Single spore isolation technique was employed for purification and the cultures were preserved at 4±1°C.

Pathogenicity

The isolates of the most frequently isolated fungus were multiplied on PDA. The isolate showing fastest growth on PDA was selected for further studies. The fastest growing isolate was used for pathogenicity test using rapid technique of Dyer and Ingram (1990). Seeds of cultivar "Bonnevellae" pea were surface sterilized for 1 min in 0.1% $HgCl_2$ solution, washed thoroughly with sterilized distilled water, sown in sterilized sand in trays and irrigated with sterilized half strength Hoagland's solution (Hoagland and Aron, 1950). Fourteen (14) days old pea plants (three to five nodal stage) were uprooted and roots were washed with sterilized water to remove sand particles. Uniform suspension of spores (10 to 12 spores per microscopic field) was prepared in ½ strength Hoagland's solution and was referred as "Standard inoculum". The pea seedlings were inserted into glass tubes (25 x 150 mm size) containing 40 ml of standard inoculum. Seedlings were held in position by cotton plugs in such a way that roots were immersed in spore suspension, leaving air gap of 2 cm between the plug and spore suspension. Seedlings in sterilized half strength Hoagland's solution served as control. Sterilized half strength Hoagland's solution was added in tubes after every 48 h to make up for the loss of water. Koch's postulates were followed to confirm pathogenicity.

Assay of biocontrol agents

The fungal and bacterial bioagents *viz*., *Trichoderma viride*, *Trichoderma harzianum, Gliocladium* and *Pseudomonas fluorescens* procured from Division of Plant Pathology SKUAST-Kashmir were evaluated against the pathogen in dual culture (Martyn and Stack, 1990; Tapwal et al., 2005). 5 mm discs of seven-day-old culture of pathogen as well as biocontrol agents were placed equidistantly (4 cm) apart in each Petri-plate containing PDA under aseptic conditions and incubated at 23 ± 2°C. The Petri plate containing PDA without biocontrol agents served as control. Observations on per cent radial growth of pathogen were recorded after 10 days of incubation. Based on the growth and mycoparasitic nature, biocontrol agents were grouped into various categories as per the scale given by Bell et al. (1982) with slight modification (Munshi and Dar, 2004).

I. Strong antagonist: Growth of biocontrol agent very fast, covering the entire medium surface and completely overgrew the pathogen,
II. Antagonist : Growth of biocontrol agent very fast, covering atleast ⅔rd medium surface but without showing mycoparasitic action,
III. Moderate antagonist: Growth of biocontrol agent very slow but rapidly overgrows the pathogen once in contact,
IV. Slow antagonist: Growth of biocontrol agent and pathogen similar in magnitude, none appeared to be dominant to other,
V. Poor antagonist: Growth of the pathogen is fast, it colonized atleast ⅔rd of the surface and appeared to withstand encroachment by the biocontrol agent,
VI. Non antagonist : Growth of the pathogen very fast, overgrew the biocontrol agent, covering the entire medium surface.

The radial mycelial growth of the pathogen was recorded 10 days after incubation and per cent inhibition in mycelial growth was calculated as:

Per cent inhibition = Control - Treatment/Control x 100

Pot culture experiment

The biocontrol agents were evaluated in pot culture experiments against the pathogen. Sterilized soil in pots was made sick by thoroughly mixing inoculum of the pathogen multiplied on sand maize meal at 1.0/20 kg of the garden soil (Mathew and Gupta, 1998). The pots were thoroughly moistened with water and covered with polythene sheets for 48 to 72 h for stabilization of the pathogen. Seed treatment with bio-agents and carbendazim was carried out as per Sofi et al. (2009). Surface sterilized seeds of pea (cv. Bonnvellae) were coated separately with each biocontrol agent. To allow proper adhesion of biocontrol agent with seed, carboxymethyl cellulose (CMC) was added to the suspension. The seeds were kept in moist chamber overnight before being sown. Twenty (20) seeds were sown per pot and the pots were kept in greenhouse. Ten (10) seedlings per pot were maintained after recording the germination (%) and days taken to germinate. Disease incidence and severity was recorded at 50% flowering stage. Seeds treated with Carbendazim 50 WP at 0.1% and sterile distilled water served as control and sham control. The disease incidence was recorded by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

Disease severity was recorded on 0 to 4 scale of Hwang and Chang (1989); (0 = healthy roots, 1= 1 to 9% root discolouration, 2 = 10 to 39% root discolouration, 3 = 40 to 69% root discolouration and 4 = 70% and above root discolouration). The root-rot index was calculated as follows:

$$\text{Root rot index} = \frac{\text{Sum of all ratings}}{\text{Total number of ratings} \times 4} \times 100$$

RESULTS AND DISCUSSION

Isolation of pathogen(s)

Isolations from diseased samples collected from various locations of Kashmir valley showed that *F. solani* f.sp *pisi* was obtained from 69 samples out of 80 samples (Table 1). The fungus produced scanty aerial mycelium on potato dextrose agar with a radial growth of 3.0 to 4.0 cm diameter after three days; aerial mycelium initially whitish becoming greyish and later having a puff-pink growth, covering almost the entire surface of the plate in seven to 10 days. On aerial mycelium the fungus produced abundant micro-conidia which were one celled, rarely two celled, hyaline, cylindrical, measuring 12 to 17.5 x 2.5 µm in size. Macroconidia were three septate, rarely two septate, hyaline and sickle shaped, measuring 31.5 to 37.5 x 3.5 to 5.5 µm in size.

Pathogenicity

In pathogenicity test, *F. solani* f.sp *pisi* proved to be a potential pathogen on pea plants and resulted in production of symptoms almost identical to those of

Table 1. Frequency of isolates from the root rot samples of pea.

District	Locality	Number of samples collected	Isolation frequency	
			Fusarium solani f.sp *pisi*	*Fusarium oxysporum* f.sp *pisi*
Anantnag	Sallar	5	4	1
	Daksun	5	4	1
	Dialgam	5	5	0
	Dooru	5	3	2
Baramulla	Delina	5	5	0
	Janbazpora	5	3	2
	Wadoora	5	5	0
	Tujar	5	4	1
Pulwama	Padgampora	5	5	0
	Awantipoa	5	4	1
	Noorpora	5	4	1
	Rajpora	5	5	0
Srinagar	Harwan	5	4	1
	Beehama	5	4	1
	Lar	5	5	0
	Zaznah	5	5	0
Grand total		80	69	11

Fusarium root rot borne under natural conditions. The artificially produced disease yielded the same fungus upon re-isolation. Sen and Majumdar (1970) and Kraft and Berry (1972) also observed the association of *F. solani* f.sp *pisi* with the root rot of pea and they also found this fungus very virulent on pea, producing root rot symptoms, which is in accordance with the present investigations.

Assay of biocontrol agents

The *in vitro* bioassay of bicontrol agents revealed that all the four biocontrol agents tested, significantly inhibited the radial mycelial growth (Table 2). *T. harzianum* was found to be a strong antagonist allowing minimum pathogen growth (19.20 mm) in dual culture followed by *T. viride* (21.80 mm), *G. virens* (27.40) and *P. fluorescens* (28.40 mm) compared to control (90.00 mm). The present findings are in conformity with Kumar and Dubey (2001). Similarly, Wang et al. (1995) also found that *Trichoderma* spp. had strong antagonistic activity towards *F. solani* f.sp *pisi* under *in vitro* conditions. Vimala et al. (1994) have screened different bioagents *in vitro* against *F. solani* and have reported that *T. harzianum* allowed minimum pathogen growth in dual culture followed by *T. viride* and *P. fluorescence*. The respective inhibitions over check ranged from 68.37 to 78.60%. *T. harzianum, T. viride* and *G. virens* showed strong mycoparasitic activity and completely overgrew the host mycelium once in contact with it. Rapid growth of *T. harzianum* covering the entire colonies of *F. solani* f.sp *pisi* have also been reported by Kapoor et al. (2006). It has been established that *Trichoderma* spp. inhibit pathogenic invasion through phenomena of mycoparasitism, antibiosis and competetion (Satyaprasad et al., 1998). Lysis of pathogen hyphae (Bell et al., 1982), coiling and penetration (Dennis and Webster, 1971), production of organic metabolite (Ahmad and Baker, 1988; Upadhyay and Mukhopadhyay, 1983) are wide range of phenomena attributed to biocontrol potential of *Trichoderma* spp. *P. fluorescens*. During the present study, it was found to cease the growth of pathogen leading to the conclusion that phenomenon of antibiosis was predominantly responsible in case of this biocontrol agent. Kapoor and Kumar (1991) have observed detoxification of fusaric acid by the antibiosis due to production of phenozine and some siderophores by *P. fluorescens*. Thus, in the present study *P. fluorescens* was found to act as moderate antagonist as per Bell's classification.

Pot culture experiment

Evaluation of seed treatments with various bio-agents and carbendazim 50 WP against *F. solani* f.sp *pisi* in pot culture revealed that all the treatments significantly improved seed germination over control (Table 3). The seed dressing with *T. harzianum* and carbendazim 50 WP resulted in maximum germination (90% each) followed by *T. viride* (86%), *P. fluorescens* (83%) and *G. virens* (82%) as compared to control (61%). Verma and

Table 2. Effect of various biocontrol agents on radial mycelial growth of *F. solani* f.sp. *pisi* in dual culture.

Treatment	Radial mycelia growth (mm) ±S.E(M)	Per cent inhibition in mycelial growth	Degree of antagonism	Zone of inhibition (+) zone of interaction (-)
Trichoderma harzianum	19.20±0.37	78.60 (62.45*±0.30**)	HA	-
Trichoderma viride	21.80±0.37	75.72 (60.48±0.27)	HA	-
Gliocladium virens	27.40±0.51	69.52 (56.49±0.36)	HA	-
Pseudomonas fluorescens	28.40±0.51	68.37 (55.78±0.35)	HA	+
Control	90.00±0.00	-	MA	
CD (p≤0.05)	1.18	0.96		

*Arc sin transformation; **standard error of mean; HA, highly antagonist; MA, moderately antagonist.

Table 3. Effect of seed dressing with various biocontrol agents on seed germination of pea (*Pisum sativum* L.) raised in soil inoculated with *F. solani* f.sp. *pisi.*

Treatment	Seed germination (%)	Days taken to germination
Trichoderma harzianum	90.00[ϕ](72.03*±1.97**)	7.26±0.07
Trichoderma viride	86.00 (68.08±0.77)	7.32±0.13
Gliocladium virens	82.00 (64.94±0.82)	7.53±0.08
Pseudomonas fluorescens	83.00 (65.70±0.82)	7.37±0.08
Carbendazim	90.00 (72.03±1.97)	7.34±0.10
Control	61.00 (51.37±0.98)	7.88±0.08
CD(p≤0.05)	3.89	0.28

[ϕ]Mean of five replications; *arc sin transformation; **standard error of mean.

Dohroo (2005) also reported the efficacy of biocontrol agents as seed treatment against *F. oxysporum* f.sp *pisi* pathogen causing wilt of pea. The present results are also in agreement with those of Kumar and Dubey (2001) and Xue (2003), who have also reported significant increase in seed germination of pea by treatment with biocontrol agents. The reason for increased germination of seeds could be attributed to the fact that antagonist was seed-borne in present case and *F. solani* f.sp *pisi* soil borne. The biocontrol agents and carbendazim seemed to have restricted the growth of pathogen near the seed either by the process of antibiosis or mycoparasitism and thus improved the seed germination, whereas, seed treatment with carbendazim is believed to exhibit systemic effects and remain in plant system to provide temporary resistance against any pathogen infection.

Seeds treated with *T. harzianum* took comparatively less time (7.26 days) for seed germination followed by *T. viride* (7.32 days) and carbendazim 50 WP (7.34 days), *P. fluorescens* (7.37 days) and *G. virens* (7.53 days) as compared to control, where seeds germinated after 7.88 days. Similar increase in seed germination of chickpea due to pre-sowing soil application of *T. harzianum* has been reported by Sharma et al. (1999). The stimulating effects on seed germination might be due to decreased incidence of pathogenic infection. Neelamegam and Govindarajalu (2002) demonstrated better results of plant stand and other growth parameters with biocontrol agents and farm yard manure (FYM) integration. Two major lines of research have focused attention on the role of the total microbial population acting as a

Table 4. *In vivo* efficacy of various biocontrol agents as seed treatment against Fusarium root rot incidence and severity in pea (*Pisum sativum* L.).

Treatment	Disease incidence (%)	Disease severity (%)	Per cent reduction in severity over control
Trichoderma harzianum	21.30 (27.32*±1.79**)	10.94 (19.30±0.38)	65.83 (54.25±0.8)
Trichoderma viride	25.30 (30.09±1.63)	12.02 (20.26±0.48)	62.46 (52.24±1.02)
Gliocladium virens	38.64 (38.40±1.47)	17.58 (25.07±0.43)	43.84 (41.45±1.05)
Pseudomonas fluorescens	29.28 (32.76±1.03)	14.98 (22.76±0.24)	53.21 (46.85±0.53)
Carbendazim	14.64 (22.15±2.16)	4.98 (12.86±0.44)	84.44 (66.83±0.83)
Control	54.64 (47.68±1.44)	32.02 (34.45±0.51)	-
CD(p≤0.05)	5.32	1.17	2.48

*Arc sin transformation; **standard error of mean.

nutrient sink, and thus restricting the opportunity for pathogenic *Fusarium* spp. to develop and the role of populations of specific antagonists that can inhibit the activity of pathogenic agents (Parker et al., 1985). Increased growth of plants by bio-agent treatments has been reported as an added advantage while pathogenic infection leads to the production of toxic metabolites which adversely affects plant growth (Mongia, 1996).

In pot experiment, all the seed treatments significantly reduced root rot incidence and severity of pea caused by *F. solani* f.sp *pisi* (Table 4). Carbendazim 50 WP proved most effective in restraining root rot incidence to 14.64%. Although *Trichoderma harzianum* (21.3%) and *Trichoderma viride*(25.3%) were at par with each other and superior over *G. virens* (38.64%) and *P. fluorescence* (29.28%) but proved inferior to carbendazim. Similarly, less root rot severity in the range of 4.98 to 17.58% was observed in carbendazim 50 WP, *T. harzianum*, *T. viride*, *P. fluorescens* and *G. virens* as compared to untreated check (32.02%). The decrease in root rot incidence and severity may be attributed to the inhibitory action of seed treatments on pathogenic growth and multiplication in the rhizosphere. The antagonists in rhizosphere are likely to compete with the pathogen for the host surface and nutrients as well as may inhibit pathogenic growth through antibiosis/mycoparasitism. This accordingly seems to have reduced/delayed the root rot incidence and severity. These speculations are substantiated by *in vitro* biocontrol studies on *F. solani* f.sp *pisi* as well as by the findings of Jha and Jalali (2006). The present findings are also in conformity with the findings of Lacicowa and Pieta (1994) and D'ella et al. (1998) who reported the effectiveness of various isolates of *Trichoderma* in controlling the root rot of pea caused by *F. solani* f.sp *pisi*. Similarly, Kapoor et al. (2006) also reported that *T. harzianum* had strong antagonistic activity against *Fusarium solani* f.sp *pisi*. Kumar and Dubey (2001) have achieved similar results while studying the management of collar rot of pea by the integration of biological and chemical methods. Prasad and Rangeshwaran (1999) noticed reduction in the incidence of wilt, root rot and collar rots by seed treatment with *Trichoderma* spp., *Glicladium* spp. and *P. fluorescens*. Thus, it can be concluded that sufficient antagonism was exhibited by bio-agents especially *Trichoderma* spp., *Gliocladium* sp. and *P. fluorescence* when applied against root rot pathogen of pea.

The possibility of these biocontrol agents to develop as effective means of biological management is high on account of their minor cost of production and multiplication on cheaper raw materials like sawdust, wheat-bran and cheaper seeds (Jeyarajan and Angappan, 1998). Due to their ease in application, low human toxicity, long lasting effect, environment safety and usage in eco friendly manner, biofungicides are entering into the progeny of industrial products and may compete with hazardous chemicals in the market thus opening a new chapter in the history of plant pathology. A meaningful merger of all cultural, protective and prophylactic measures can be adopted in integration with biological control for successful management of the disease.

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