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RESEARCH ARTICLE

BIO-MANAGEMENT OF ROOT ROT OF PEA (*PISUM SATIVUM* L.) CAUSED BY *USARIUM SOALNI* F. SP.*PISI*

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ABSTRACT

The efficacy of three fungal and one bacterial bioagents *viz., Trichoderma harzanium, Trichoderma viride, Gliocladium verns and pseudomonas fluorescens* were evaluated *in vitro* conditions against pea root rot pathogen. Among the bio-agents, *Trichoderma harzanium* proved superior over other bio-control agents by exhibiting a maximum of 17.28 radial mycelial growths as against 89.00 recorded in check. *Trichoderma harzanium* maximum mycelial growth inhibition of 76.33 and minimum of 66.33 per cent by *pseudomonas fluorescens*. Zone of inhibition was observed only in *Pseudomonas fluorescens* within a week's incubations at 28±2 ^{0}C. Soil application based formulation of *Trichoderma harzanium, Trichoderma viride, Gliocladium verns and pseudomonas fluorescens* effectively control the root rot of pea plant under field condition.

INTRODUCTION

Pea (*Pisum sativum* L.) is an important vegetable and pulse crop of India. It is relished both as fresh vegetable as well as pulse. Being a legume, it plays an important role in the eco-build up of Agriculture, as it enriches the soil by fixing the atmospheric nitrogen (Goswami, and Pareek, 1976). Among various pathogens fungi constitute an important group as they inflect damage to crop at different stages (Agrios, 2000). Among the fungal diseases, the root rot caused by *Fusarium solani* f.sp.*pisi* remains to be a challenging task in terms of management, since it is soil borne in nature. Various disease management methods have been implemented to combact and eradicate pathogenic fungi. These include cultural, regulatory, physical, chemical and biological methods. All the methods are effective only when employed well in advance as precautionary measures (Sharma, 1996, Kata, 2000). Once the disease has appeared, these methods become impractical / ineffective. In such situation, chemical control offers a good choice to grower to control the diseases. Chemical pesticides have been in use since long as they provide quick, effective and economic management of plant disease.

It has realized that use of chemicals in Agriculture is not a beneficial as it was visualized. Chemicals pose serious health hazards to the applicator as well as to a consumer of the treated material. In addition to target organism, pesticides also kill various beneficial organisms including nitrogen fixing bacteria. Their toxic form persists in soil and contaminates the whole environment (Hayes and Laws, 1991). However, development of fungicide-resistant phytopathogenic strains and adverse effect of pesticide on soil, plant health and crop products have compelled plant pathologists to look for eco-friendly strategies for plant disease management (Tu 1997, Chattopadhyay. et al 2002). Increasing awareness of human kind towards the ecosystem and environment has made a marked shift from synthetic material to bio-products. Fungi and bacteria constitute a major group of bio-agents against various kinds of pathogens. A good number of fungal and bacterial bio-agents such as *Trishoderma harzianum, Trichoderma viride, Gliocladium virens* and *Pseudomonas fluorescens* can suppress the parasitism of *Fusarium* spp., *Rhizocotina* spp. (Rajappan, and Ramaraj, 1999, Lifshitz, et al 1986).

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MATERIALS AND METHODS

The bio-agents used in the present study were obtained from Division of Mycology and Plant Pathology, IARI New Delhi and SKUAST-K Shalimar (Jammu and Kashmir). The fungal bio-control agents were maintained on Potato Dextrose Agar (PDA) by periodic subculture at monthly intervals (Appendix-1). The bacterial bio-control agent was maintained and mass multiplied on Kings B media (Appendix-1). The bacteria were sub-cultured on Kings B media at monthly intervals (plate-8) were used for the present study. Antagonistic activities of these bio-agents were determined by Dual Culture Technique (Dennis and Webster, 1971). Each treatment was replicated three times and incubated at 32± 2^{o}C. The experiment was laid out in a

completely randomized design. Observations on per cent radial growth of pathogen were recorded after 10 days of incubation. The radial mycelial growth of the pathogen was measured with the help of an ordinary millimeter scale.

$$\text{Per cent inhibition in mycelial growth} = \frac{C-T}{C} \times 100$$

Where, C= mycelial growth in control.
T= mycelial growth in treatment.

In vivo evaluation of bio-control agents

A field trial was conducted in the sick pots at Amar Singh College Srinagar (University of Kashmir), during march-June 2011. The bio-control agents were evaluated in the pot culture experiments against the target pathogen. The garden soil was collected in pots. The pots were given light irrigation after 24hrs. Fumigated with 4 per cent formaline and covered with polythene for 48hrs. So that the fumes sterilize the soil completely. Sterilized soils in pots were made sick by thoroughly mixing inoculums of the pathogen multiplied on sand maize@ 1.0 kg/20kg of garden soil (Mathew and Gupta, 1998). Apparently healthy and fresh seeds of the susceptible pea cultivar "Bonnvellae" were surface sterilized with 0.1% HgCl2 and used for the experiment. For treatment with bio-control agents, culture of *Trichiderma harzanium, Tricoderma viride, Gliocladium verns* were grown on PDA in petriplates and *Pseudomonas fluorescens* on Kings B media. Seeds were coated separately with each bio-agents using 1ml spore suspension. In control treatment, distilled water was used for dip. Twenty seeds were sown per pot and the pots were kept in green house. Fifteen seedlings per pot were maintained after recording the germination percentage and the days taken to germinate. Per cent infected seedlings and the disease severity was recorded at 50 per cent flowering stage. Each treatment was replicated five times in Completely Randomized Design (CRD). Data on disease were recorded on 0-4 scale (Hwang, and Chang, 1989.). Observation on root rot incidence was recorded and analyzed statistically using analysis of variance as per the method (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

All the four bio-control agents, which proved effective against *Fusarium soalni* f.sp.*pisi* were assessed as seed treatment against *Fusarium soalni* f.sp.*pisi* in pot culture experiment. Seeds treated with Carbendazim @0.1 % and sterile distilled water used separately. Table-1 revealed that all the treatments significantly increase the seed germination as compared to untreated check. Highest seed germination of 80.00 per cent was recorded in *Trichoderma harzanium* which was statistically par with Carbendazim 50 WP (80%). This was followed by *Trichoderma viride* with seed germination of 75 per cent. Pseudomonas fluorescens treated seeds gave 70-73 per cent germination and were statistically at par with each other but significantly superior over untreated check (59%). Data on days taken to germinate revealed that all seeds treated with *Trichoderma harzanium* took comparatively less time (6.92 days) for germination, followed by *Trichoderma viride* (6.94 days), while seed treated with Carbendazim 50WP took 7.01 days followed by *Pseudomonas fluorescens* (6.98 days) and *Gliocladium virens* (7.21 days). All the above seed treatments were statistically different as compared to untreated check. The data on root rot incidence of pea recorded at flowering time is presented in table-8. All the seed treatments proved significantly effective in reducing the root rot incidence. Least root rot incidence of 12.62 per cent was recorded in case of seeds treated with Carbendazim 50WP @ 0.1 per cent. This was followed by *Trichoderma harzanium* with 12.98 per cent incidence, which was statistically at par with *Trichoderma viride* with 23.28 per cent root rot incidence. *Pseudomonas fluorescens* proved to be the next best treatment by exhibiting 27.26 root rot incidence followed by *Gliocladium verns* with root rot incidence of 36.62 per cent. Highest incidence of 52.62 per cent observed in untreated check. Observations were also recorded for disease severity on the roots of pea plants at flowering time. Least disease severity of 2.96 per cent was recorded in Carbendazim 50WP treated seeds and highest severity of 30.00 per cent was recorded in untreated check. Other treatments, which followed increasing order of disease severity, were *Trichoderma harzanium* (8.92%) and *Trichoderma viride* (10.00%) which was statistically at par with each other. *Pseudomonas fluorescens* (12.96%) and *Gliocladium verns* (15.56%) treated seeds differed significantly from one another and were inferior to *Trichoderma harzanium* but superior over untreated check-(Fig-2)

Data on per cent disease reduction over control is presented in Table-2. The highest reduction in disease severity over control (82.44%) was recorded in case of Carbendazim 50 WP treated seeds followed by *Trichoderma harzanium* (63.81 %), *Trichoderma viride* (60.44%), *Pseudomonas fluorescens* (51.19%) and *Gliocladium verns* (41.82%). *Trichoderma harzanium* and *Trichoderma viride* were equally superior over rest of the bio-agents in controlling the disease but were statistically inferior to Carbendazim, which gave best control of the disease. The seeds treated with *Trichoderma harzanium* resulted in maximum germination (80%), which was at par with Carbendazim 50 WP (80%), followed by *Trichoderma viride* (75%), *Pseudomonas fluorescens* (73%) and *Gliocladium virens* (70%) as compared to untreated check (59%). The highest reduction in disease severity over control (82.44%) was recorded in case of Carbendazim 50 WP treated seeds followed by *Trichoderma harzanium* (63.81 %), *Trichoderma viride* (60.44%), *Pseudomonas fluorescens* (51.19%) and *Gliocladium verns* (41.82%). *Trichoderma harzanium* and *Trichoderma viride* were equally superior over rest of the bio-agents in controlling the disease but were statistically inferior to Carbendazim, which gave best control of the disease. The seeds treated with *Trichoderma harzanium* resulted in maximum germination (80%), which was at par with Carbendazim 50 WP (80%), followed by *Trichoderma viride* (75%), *Pseudomonas fluorescens* (73%) and *Gliocladium virens* (70%) as compared to untreated check (59%). The present results were also in agreement with those of (Kumar and Dubey, 2001, 15 Xue, 2003) who have reported significant increase in seed germination of pea by treatment with bio-control agents. In pot experiment, all the seed treatment significantly reduces root rot incidence and severity of pea caused by *Fusarium solani* f.sp.*pisi*. The present findings are also in conformity with the findings of (Lacicowa and Pieta, 1994, Della et al .,1998) who reported

Table 1: Effect of seed dressing with various biocontrol agents on seed germination of pea (Pisum *sativum* L.) In soil inoculated with *F. solani* f. sp. *pisi.*

Treatment	Seed germination (%)	Days taken to germinate
T1	80†.00	
Trichoderma harzianum	73.03* ± 3.97**	6.92 ± 0.05
T2	75.00	
Trichoderma virid	67.06 ± 0.66	6.94 ± 0.08
T3	70.00	
Gliocladium virens	62.81 ± 0.75	6.98 ± 0.06
T4	73	
Pseudomonas fluorescens	64.50 ± 0.76	6.98 ± 0.07
T5	80.00	
Carbendazim	73.03 ± 3.97	7.01 ± 0.08
T6	59.00	
Control	49.62 ± 0.87	7.21± 0.06
CD(P≤ 0.05	2.97	0.20

† Mean of five replicates; *= Arc sin transformation ** = Standard error of mean

Table-2. *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 (%)	Percent reduction in severity over control
T1*Trichoderma harzianum*			
	19.28	8.92	63.81
	25.91* ± 1.68**	17.98 ± 0.28	52.23 ± 0.6
T2			
Trichoderma viride	23.28	10.00	60.44
	27.08 ± 1.53	19.29 ± 0.40	50.22 ± 1.00
T3			
Gliocladium virens	36.62	15.56	41.82
	36.42 ± 1.36	23.99 ± 0.38	39.43 ± 1.03
T4			
Pseudomonas fluorescens	27.26	12.96	51.19
	29.71 ± 1.01	20.86 ± 0.14	44.83 ± 0.51
T5			
Carbendazim	12.62	2.96	82.42
	19.27 ± 1.9885	11.96 ± 0.36	64.81 ± 0.81
T6			
Control	52.26	30.00	
	44.98 ± 1.34	32.43 ± 0.41	
CD(P≤ 0.05	4.23	1.11	1.46

*= Arc sin transformation ** = Standard error of mean

the effectiveness of various isolates of *Trichoderma* in controlling the root rot of pea caused by *Fusarium solani* f.*sp.pisi*. The present results are also in agreement with those of (Jha and Jalali, 2006).

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