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Evaluation of Native Biocontrol Agents against *Fusarium solani* f.sp. melongenae Causing Wilt Disease of Brinjal in Kashmir

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Summary

In the present study the native biocontrol agents (BCA) were isolated from rhizosphere viz. *Gliocladium roseum* from apple; *Paecilomyces varioti*, *Trichothecium roseum* and *Aspergillus flavus* from mulberry; *Trichoderma viride* (isolate-I&II) of mushroom and pea; *Trichoderma harzianum* (isolate-I &II) of pea and paddy while *Pseudomonas fluorescens* of rajmash and cultured on PDA/king's B medium. All the native BCA's were evaluated in vitro against *Fusarium solani* f.sp. *melongenae* causing wilt disease of brinjal. Except *Gliocladium roseum* and *Trichothecium roseum* all the fungal BCA's were more effective than bacterial BCA ie. *Pseudomonas fluorescens,* whereas, *Trichoderma harzianum* isolate-I caused maximum inhibition(70.79%) followed by *T. viride* isolate-I(68.57%), *T. harzianum* isolate-II(63.80%), *T.viride* isolate-II(60.0%), *Paecilomyces varioti*(53.33%) and *Aspergillus flavus*(46.34%) but *Trichothecium roseum* and *Gliocladium roseum* were the least effective and resulted 18.09 and 27.61 per cent inhibition of wilt pathogen respectively. The bacterial bioagent, *Pseudomonas fluorescens* was slightly more effective (28.17%) against wilt pathogen. Similarly, in vitro agar plug test, *T. viride*(isolate-I&II), *A.flavus, G.roseum* and *T.roseum* were created inhibition zones but rest fungal BCA's were advanced and occupied the entire plate within 10-20 days whereas *P. flourescens* did not show further advancement beyond the zone of contact.

Key Words: Evaluation, Native Bioagents, *Fusarium solani* f.sp. melongenae, Brinjal

Introduction

Brinjal yield is not so enough owing to a number of biotic constraints of which, the incidence of fungal diseases such as damping-off (*Pythium* spp.; *Phytophthora* spp.; and *Fusarium* spp.) ; root rot (*Rhizoctonia* spp. and *Sclerotium* spp.); blight (*Phomopsis* spp.) ; fruit rot (*Phomopsis vexans* and *Rhizopus stolonifer*) and wilt (*Verticillium* spp.; *Fusarium* spp.) are noteworthy and take a considerable proportion of the produce annually. Brinjal wilt-complex is known to be caused by number of fungal genera such as *Fusarium, Verticillium, Rhizoctonia, Sclerotium* and *Phytophthora* in different parts of the world (Rangaswami, 1979). In Jammu and Kashmir state, studies conducted by Lolpuri, 2002 have confirmed *Fusarium solani* f.sp. *melongenae* as causal agent of brinjal wilt wherever environmental conditions are conducive for cultivation in the state but consistent information regarding its management through biocontrol agents is not available so far to sustain crop production. Biological control assumes major importance especially soil-borne wilt disease. Rhizosphere competence of antagonists is a pre requisite for the biological control of soil borne plant pathogens and this phenomenon was associated with the production of higher amounts of cellulolytic enzymes and increased saprophytic ability and manipulate the environment around a crop plant to favor organisms that contribute to crop health and vigor, rather than pesticides which destroy a range of micro-organisms including the fungal pathogens. Domestic antagonists are most virulent strains to wilt pathogen because of their persistent capability under soil and local climatic conditions of Kashmir. Humus and organic rich soil of valley is favored to flourish the bioagents easily can control of wilt disease. Infact domestic antagonists are most virulent strains to the soil borne pathogens (Dohroo, 2001) because of their persistent capability under soil and local climatic conditions of the Kashmir. Therefore, local strains of bioagents were isolated and evaluated their effects against the wilt pathogen.

Materials and Methods

Distinct bioagents were isolated from the Rhizosphere of various hosts prevalent in Kashmir valley viz. *Gliocladium roseum* from apple; *Paecilomyces varioti* , *Trichothecium roseum* and *Aspergillus flavus* from mulberry; *Trichoderma viride* (isolate-I&II) from mushroom and pea respectively; *Trichoderma harzianum* (isolate-I &II) from pea and paddy while *Pseudomonas fluorescens* from rajmash were isolated and maintained on PDA/King's B medium. Isolated bioagents were mass multiplied on corn grain/ nutrient broth medium (Mathew and Gupta,1998) subsequently evaluation against wilt pathogen was followed by agar plug (Siddaramaiah et al.,1978) and dual culture (Prasad et al.,2000) methods. In agar plug method, spore suspension of $5X10^6$ ml^{-1} of the pathogen was added in PDA/King's B medium of 90 mm dia petriplates. 5mm disc of each isolated strains of bioagents were taken from actively and profusely growing culture and kept separately in the centre of the plates and incubated at 25-$^{+2}0$C for ten days. The inhibition zones formed as growth less

area around the periphery of the test bioagents agar plug was measured as an index of its efficacy against the wilt pathogen. In case where no conspicuous inhibiting zone was formed only relative growth of wilt pathogen and domestic antagonists was measured and classified according to Bell et al., 1982.

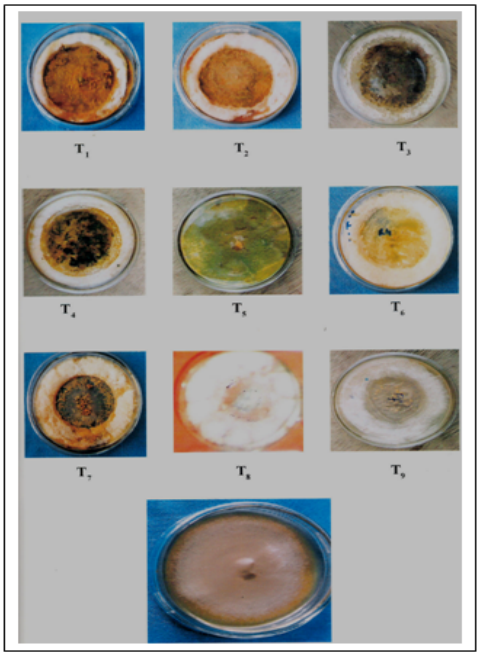
In dual culture method, 5mm discs of the test pathogen and bioagents were placed 70mm apart on freshly prepared PDA in plates and kept for incubation. PDA plates inoculated with the wilt pathogen not amended with the bioagents served as check. The radial growth was measured daily for ten days. Each treatment for both the methods had 4 replications and designing the experiment in CRD. Inhibition percentage of the wilt pathogen as a result of antagonist's application was calculated with formula given by Vincent (1947).

Results and Discussion

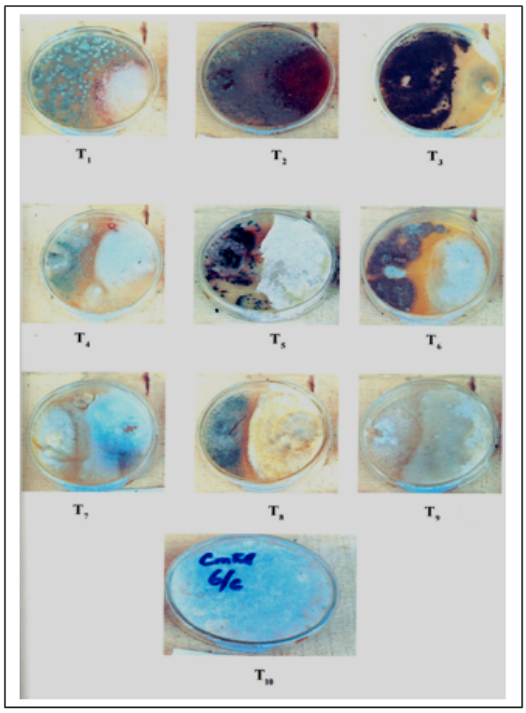
The results of dual culture tests depicted in Table-1 and compounded with fig.-1 which clearly showed that all the isolated bioagents significantly inhibited the mycelial growth of *F.solani* f.sp. *melongenae*. The data (Table-1) showed that these bioagents were able to inhibit the mycelial growth of *F.solani* f.sp. *melongenae* to an extent of 18.09 to 70.79 per cent and minimum pathogen growth of 2.30-2.48 cm in dual culture with *T. harzianum*(S.I) and *T. viride* (S.I). Maximum (70.79%) growth inhibition of *F.solani* f.sp. *melongenae* was attained in *Trichoderma harzianum* (strain-I) followed by *T. viride*-I, *T. harzianum*-II and *T viride*-II while. least inhibition (18.09%) was found to be in *Trichothecium roseum*. The strains –II of these bioagents were also produced inhibition zones of 63.8 and 60.0 per cent respectively which maximum than other bioagents those were produced the zones. The similar trend of the inhibition was also evident in the Fig.-1.

It was observed in agar plug method (Table-2) that *T.viride* strain-I made maximum zone of inhibition (0.52) followed by its strain-II (0.4).Other bioagents viz. *G. roseum, A. flavus* and *T. roseum* were also showed the inhibition zones but rest could not be produced inhibition zones with wilt pathogen in agar plug method. Both strains of *T. harzianum* and *P.varioti* were overgrew the wilt pathogen and occupied the entire medium surface within 10 and 20 days respectively whereas, minimum pathogen growth in dual culture with strain-I of both *T. harzianum and T. viride* was recorded with profuse growth and covered the plates very fastly than other ones (Fig.-2).

The results indicate that *T. harzianum* and *T. viride* are deleterious bioagents against wilt pathogen of brinjal. It has been established that *Trichoderma* spp. inhibit pathogenic invasion through phenomena of mycoparasitism, antibiosis and competition (Satyaparasad et al., 1998; Anwar et al.,2008. Lysis of pathogenic hyphae (Bell et al., 1982), coiling and penetration (Denis and Webster, 1971), production of organic metabolites-gliotoxin (Weindling, 1934), viridin (Weindling and Emerson, 1936) and volatile inhibitory substance-acetaldehyde (Upadhyay and Mukhopadhyay, 1983) are wide range of phenomena attributed to biocontrol potential of *Trichoderma* spp.



Agar plug method: Fig.-1
T_1: *T.harzianum* strain-I ,
T_2: *T.harzianum* strain-II
T_3: *T. viride* strain-I ,
T_4: *T. viride* strain-II ,
T_5: *Paecilomyces varioti*,
T_6:*Gliocladium roseum*
T_7: *A. flavus*
T_8: *T. rosueum*
T9: *P. fluorescens*
T_{10}: Check/Wilt pathogen



Dual Culture Method: Fig.-2

T_1: *T.harzianum* strain-I , T_6: *Gliocladium roseum* ,
T_2: *T.harzianum* strain-II T_7: *A.flavus* ,
T_3: *T. viride* strain-I , T_8: *T.roseum* ,
T_4: *T. viride* strain-II , T_9: *P.fluorescens* ,
T_5: *Paecilomyces varioti*, T_{10}: Check/wilt pathogen,

Table-1: Performance of domestic biocontrol agents in dual culture against *Fusarium solani* f.sp. *melongenae* causing wilt disease in brinjal.

Bio-agents	Radial growth of *F. solani* f.sp. *melongenae* (cm)	Inhibition per cent of *F. solani* f.sp. *melongenae*
Gliocladium roseum	5.70	27.61
Paecilomyces varioti	3.68	53.33
Trichothecium roseum	6.45	18.09
Aspergillus flavus	4.23	46.34
Trichoderma viride, isolate-I	2.48	68.57
Trichoderma viride, isolate II	3.15	60.0
Trichoderma harzianum, isolate-I	2.30	70.79
Trichoderma harzianum, isolate-II	2.85	63.80
Pseudomonas fluorescens	5.23	28.17
Control (Test fungus)	7.88	-
CD (P=0.05)	0.37	9.10

Table2: In vitro growth pattern of domestic bioagents in agar plug method against F*usarium solani* f.sp. *melongenae* causing wilt disease in brinjal.

Domestic bioagents	Host	Zone of inhibition(cm)	Substrate colour	Remarks
Gliocladium roseum	apple	0.38	rose	Similar growth pattern to *A. flavus*
Paecilomyces varioti	mulberry	-	green	Overgrows wilt pathogen
Trichothecium roseum	mulberry	0.28	rose	no further advancement beyond the zone of inhibition
Aspergillus flavus	mulberry	0.35	pink	Exhibits clear zone of inhibition
Trichoderma viride, isolate-I	mushroom	0.52	yellow	Exhibits clear zone of inhibition. Lysis and destroys the pathogen
Trichoderma viride, isolate II	pea	0.40	yellow	Exhibits clear zone of inhibition. Lysis and destroys the pathogen
Trichoderma harzianum, isolate-I	pea	-	chocholate	advanced and occupied the entire plate
Trichoderma harzianum, isolate-II	paddy	-	chocholate	advanced and occupied the entire plate
Pseudomonas fluorescens	rajmash.	-	green	No further advancement beyond zone of contact

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