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Biocontrol efficacy of indigenous *Trichoderma* isolates against root-rot pathogens of French bean in Uttarakhand hills

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ABSTRACT: Sixty cultures of *Trichoderma* were isolated from forty locations in Uttarakhand hills and screened for their ability to control root rot of French bean caused by *Rhizoctonia solani* (Kuhn) and *Fusarium solani* (Mart.) Sacc. Under laboratory screening by dual culture methodology, maximum inhibition of radial growth of *R. solani* (61.7%) was observed with isolate Tr-21, while isolate Tr-34 showed maximum inhibition of mycelial growth of *F. solani* (46.4%). Based on the results of *in vitro* screening, 7 isolates (Tr-5, 14, 17, 21, 28, 34 & 45) were selected for glasshouse studies. Talc formulation of the 7 isolates was prepared and isolates were applied as both seed and soil treatment in glasshouse studies. Although all the 7 isolates significantly reduced the incidence of root rot as compared to check, two isolates of *T. harzianum viz.*, Tr-34 and Tr-14 were found most effective in glasshouse evaluation showing only 20.8 and 23.4 per cent root- rot, respectively. These isolates were statistically at par with seed treatment with carbendazim, which showed 21.1 per cent root-rot. Treatment with isolate Tr-34 also resulted in significantly higher germination per cent and better plant vigour as compared to untreated control.

KEY WORDS: Biological control, French bean, root-rot, *Trichoderma*

INTRODUCTION

French bean is an important cash crop of the North Western Himalayan (NWH) region of India. However, the high incidence of various diseases is a major constraint in the profitable cultivation of in the Uttarakhand hills of N W H region. Root rot, in particular, is a very important disease, which affects the crop at various growth stages leading to poor plant stand and low yields. This disease complex, caused by the pathogens *Rhizoctonia solani* (Kuhn) and *Fusarium solani* (Mart.) Sacc. is difficult to control even with chemical fungicides due to its soil bone nature. Besides, a growing awareness of the deleterious impacts of chemical fungicides on the environment coupled with the impetus on organic farming in Uttarakhand state has focused attention on the biological control of diseases. *Trichoderma* species specially *T. viride*, *T. harzianum* and *T. virens*, alone or in combination with other bioagents or chemical fungicides, have been frequently used for biological control of several diseases like root rots, wilts, damping off, white rot and collar rot in a wide variety of crops (Samuels, 1996). However, inconsistent field performance is sometimes a problem with *Trichoderma* based biopesticides. Howell (2003) suggested that the inability of the introduced

bioagents to compete with the native microflora may be responsible for their poor activity and the use of *Trichoderma* strains, originally isolated from the areas where they are later expected to function, may help to overcome this problem. Therefore the present investigation was an attempt to isolate and characterize indigenous *Trichoderma* isolates from Uttarakhand hills and to screen them for management of root rot of French bean.

MATERIALS AND METHODS

Isolation of pathogens

The pathogens *R. solani* and *F. solani*, were isolated from the roots of infected French bean plants on potato dextrose agar (PDA) medium. The cultures were maintained on slants of PDA and stored at 4°C for further studies.

Isolation and characterization of *Trichoderma* isolates

Rhizosphere soil samples from French bean and other crops were collected from forty locations in the Uttarakhand hills and *Trichoderma* spp. were isolated from the soil samples using *Trichoderma* selective medium (TSM) (Elad *et al.*, 1981; Mukherjee, 1991). The soil was air-dried, diluted to 1: 1000 by serial dilution technique and 1 ml of this soil suspension was plated on TSM in Petri-dishes. The dishes were incubated at 28±2°C for 5 days and individual colonies were picked up and maintained in pure culture on PDA for further studies. The isolated cultures were identified as *Trichoderma* spp. on the basis of their morphological characteristics (Bisset, 1991).

The cultural characteristics and growth rates of thirty isolates (selected on basis of their antagonistic activity against *R. solani*) were determined on PDA medium. Petri-dishes containing 20 ml PDA were inoculated in triplicate, approximately 1.0 cm from the edge of the dish, with a 5-mm diameter mycelial disc and incubated at 25±1°C. Another set of similarly inoculated Petri-dishes was incubated at 35±1°C. Colony radius at both 25°C and 35°C was recorded after 72 hours and the time of first appearance of green conidia, pattern of conidiation and presence of odor or yellow pigmentation in the colony was also observed. For species identification, morphological observations on conidiophore and phialide arrangements in the isolates were made from cultures grown on 2% cornmeal dextrose agar at 25±1°C. Identification was performed using the keys provided by Bisset (1991a&b).

Antagonistic potential

All the isolates were first screened *in vitro* for their antagonistic potential against *R. solani* by the dual culture methodology (Dennis and Webster, 1971). Five millimeter diameter mycelial discs of pathogen and *Trichoderma* isolate were placed on solidified PDA in such a manner that they lie opposite to each other. The control plates were inoculated with only *R. solani*. All plates were incubated at 26±2 °C and observations on radial growth of pathogen in treatment and control were recorded after 4 days. On basis of screening of the isolates against *R. solani*, out of 60 isolates, 30 most effective isolates were selected and screened against *F. solani* following the methodology as explained above.

Screening under glasshouse conditions

Based on the *in vitro* studies on antagonistic potential of 30 *Trichoderma* isolates, 7 isolates were selected for glasshouse studies. Two isolates *viz.*, Tr-21 and Tr-34 were selected based on their high inhibitory activity against *R. solani* and *F. solani* respectively. The five other isolates selected (Tr-5, 14, 17, 28 & 45) exhibited good antagonistic potential (>55 % against *R. solani*, >35 % against *F. solani*) against both pathogens. All isolates were applied as both seed and soil treatment. Talc based formulations of the selected isolates were produced by inoculating 250 ml conical flasks containing 150 g barnyard millet grains (soaked overnight and autoclaved for 30 minutes at 15 p.s.i.) with agar discs of the isolates and incubating the flasks at 26 ± 2 °C for 12 days. Fully colonized grains were air-dried and ground to a fine powder followed by mixing of powder with talc to get the desired concentration (10^9 spores/ g). For soil application

the isolates were multiplied by inoculating 250 g of autoclaved FYM in 500 ml flasks with 10 ml spore suspension of individual *Trichoderma* isolate followed by incubation of flasks at 26±2 °C for 14 days (Zaidi and Singh, 2004). The pathogens were multiplied on autoclaved barnyard millet grains.

Colonized FYM was mixed with sterilized soil @ 6g/kg soil and filled in plastic pots. The pathogen inoculum was mixed in soil @ 1g/kg soil of each pathogen. Seeds of French bean variety VL Bauni bean-1 were surface sterilized with sodium hypochlorite (1%) and treated with talc formulation of the *Trichoderma* isolates @10g/kg seeds. Ten seeds were sown in each pot with 5 replications for each treatment. Seed treatment with carbendazim (0.15%) was taken as chemical control. Pots having uncolonized FYM or no FYM served as checks. Observations were recorded on germination, root rot incidence, root and shoot length.

Statistical Analysis

The data in percentage (root rot incidence and germination) was subjected to angular transformation prior to analysis while data on root/shoot length and radial growth in dual culture was analyzed directly. ANOVA was performed on the data and significant differences were established at p=0.05 using Microsoft Excel software.

RESULTS AND DISCUSSION

Isolation and characterization of *Trichoderma* isolates

A total of 60 isolates of *Trichoderma* were isolated from forty locations in Uttarakhand hills and studies on cultural characteristics of thirty selected isolates (based on high antagonistic potential) were carried out. All isolates except Tr-24, 25 & 44 showed a radial growth of 51 to 62 mm after 72 h at 25°C (Table1). Conidia were formed in an effuse pattern (Tr-5, Tr-45 etc.) or in tufts (Tr-24, 28, 34 & 44) on the medium. Reverse of the colonies were either colourless (Tr-28, Tr-29 etc.) or showed pigmentation in varying shades of yellow. First green conidia were visible after 72 h at 25°C but in some isolates they were visible within 48-72 h. At 35°C, the growth in all isolates was very slow and often the colonies were compact and deformed (Table1). Based on the morphological observations made on the 30 isolates, twenty-two isolates were identified as *T. harzianum*, four as *T. atroviride* (Tr-55, 56, 57 and 58) while one isolate was identified as *T. longibrachiatum* (Tr-20). Due to lack of distinguishable morphological characters, the remaining three isolates (Tr-11, 28, & 60) could not be assigned to species level (Table1).

Antagonistic potential of *Trichoderma* isolates

Rhizoctonia solani is a very fast growing and ubiquitous pathogen with a broad host range and therefore the 60 isolates of *Trichoderma* were first screened for their antagonistic potential against this pathogen *in vitro*. Isolates exhibiting >50 per cent inhibition in radial growth of *R. solani* were further screened against *F. solani*. All the tested isolates showed significant reduction in radial growth of both the pathogen (Table 2). Maximum inhibition of radial growth of *R. solani* was observed with isolate Tr-21 (61.7%), however, isolates Tr-22 and 28 also showed >59 per cent inhibition in radial growth of *R. solani*. Against *F. solani*, isolate Tr-34 exhibited maximum inhibition in radial growth (46.4%) followed by isolate Tr-45 (45.2%). The inhibition of several plant pathogens like *R. solani*, *S. rolfsii*, *Sclerotinia* sp., *Fusarium* spp., *Pythium* etc. by *Trichoderma* species has been frequently reported (Samuels, 1996). However, significant variation was observed in the antagonistic potential of the 30 isolates against *F. solani*. Although all 30 isolates had exhibited >50 per cent radial inhibition against *R. solani*, the range of radial inhibition against *F. solani* varied from 16.6 per cent (Tr-31) to 46.4 per cent (Tr-34). Some isolates also showed considerable variability in the antagonistic potential against the two pathogens. The *T. harzianum* isolate Tr-31 exhibited 51 per cent inhibition in radial growth of *R. solani* but showed only 16.6 per cent inhibition against *F. solani*. Similarly isolate Tr-15 of *T. harzianum* also showed high inhibitory potential against *R. solani* (58.3%) but had poor action against *F. solani* (17.8%). Even among the four *T. atroviride* isolates, it was observed that the radial inhibition values ranged from 28 to 40 per cent

Table 1. Cultural characteristics of selected *Trichoderma* isolates

Isolate no.	Radial growth (mm) after 72 h at 25°C*	Radial growth (mm) after 72 h at 35°C*	Conidiation pattern	Pigmentation	Time for formation of first green conidia
Tr-1 (*T. harzianum*)	53	8	Effuse	Light yellow	> 72 h
Tr-4 (*T. harzianum*)	51	7	Effuse	Light yellow	> 72 h
Tr-5 (*T. harzianum*)	61	1	Effuse	Light yellow	> 72 h
Tr-11 (UI)	60	9	Effuse	Light yellow	48-72 h
Tr-14 (*T. harzianum*)	58	10	Effuse	Dark yellow	>72 h
Tr-15 (*T. harzianum*)	51	14	Effuse	Light yellow	> 72 h
Tr-17 (*T. harzianum*)	60	10	Effuse	Light yellow	> 72 h
Tr-18 (*T. harzianum*)	59	11	Effuse	Light yellow	> 72 h
Tr-20 (*T. longibrachiatum*)	55	9	Effuse	Dark yellow	> 72 h
Tr-21 (*T. harzianum*)	59	10	Effuse	Light yellow	> 72 h
Tr-22 (*T. harzianum*)	59	12	Effuse	Light yellow	> 72 h
Tr-24 (*T. harzianum*)	42	10	Tufts	Light yellow	> 72 h
Tr-25 (*T. harzianum*)	49	7	Effuse	Light yellow	> 72 h
Tr-27 (*T. harzianum*)	58	9	Effuse	Light yellow	> 72 h
Tr-28 (UI)	56	9	Tufts	Colourless	48-72 h
Tr-29 (*T. harzianum*)	61	11	Effuse	Colourless	> 72 h
Tr-31 (*T. harzianum*)	51	12	Effuse	Light yellow	> 72 h
Tr-32 (*T. harzianum*)	56	9	Effuse	Light yellow	> 72 h
Tr-34 (*T. harzianum*)	61	10	Tufts	Light yellow	> 72 h
Tr-37 (*T. harzianum*)	62	14	Effuse	Light yellow	> 72 h
Tr-40 (*T. harzianum*)	55	13	Effuse	Light yellow	> 72 h
Tr-44 (*T. harzianum*)	45	14	Tufts	Light yellow	> 72 h
Tr-45 (*T. harzianum*)	62	11	Effuse	Light yellow	> 72 h
Tr-47 (*T. harzianum*)	62	10	Effuse	Light yellow	48-72 h
Tr-51 (*T. harzianum*)	58	12	Effuse	Light yellow	> 72 h
Tr-55 (*T. atroviride*)	56	14	Effuse	Colourless	> 72 h
Tr-56 (*T. atroviride*)	56	9	Effuse	Colourless	> 72 h
Tr-57 (*T. atroviride*)	57	11	Effuse	Colourless	> 72 h
Tr-58 (*T. atroviride*)	55	11	Effuse	Colourless	48-72 h
Tr-60 (UI)	54	13	Effuse	Light yellow	48-72 h

*Mean of three replications; UI=unidentified

against *F. solani*. This variability in the antagonistic activity of *T. harzianum* isolates as well as *T. atroviride* isolates implies that the antagonistic potential of *Trichoderma* isolates against a specific pathogen is not determined by its species. However, as observed in the present study it has

Table 2. *In vitro* inhibition of radial growth of *R. solani* and *F. solani* by *Trichoderma* isolates

Isolate no.	*Rhizoctonia solani*		*Fusarium solani*	
	Radial growth (mm)	Radial inhibition (%)	Radial growth (mm)	Radial inhibition (%)
Tr-1	36.0	57.6	22.3	20.2
Tr-4	41.3	51.4	17.3	38.0
Tr-5	36.3	57.2	16.3	41.6
Tr-11	37.3	56.1	20.0	28.5
Tr-14	36.0	57.6	16.6	40.4
Tr-15	35.3	58.3	23.0	17.8
Tr-17	36.3	57.3	17.6	36.9
Tr-18	39.0	54.1	15.6	44.0
Tr-20	38.3	54.9	15.6	44.0
Tr-21	32.7	61.7	19.0	32.1
Tr-22	34.7	59.2	19.0	32.1
Tr-24	38.3	54.9	22.0	21.4
Tr-25	41.7	51.0	18.0	35.7
Tr-27	38.3	54.9	19.0	32.1
Tr-28	34.7	59.2	16.3	41.6
Tr-29	38.7	54.5	18.0	35.7
Tr-31	41.7	51.0	23.3	16.6
Tr-32	39.7	53.3	17.6	36.9
Tr-34	37.0	56.5	15.0	46.4
Tr-37	37.0	56.5	18.6	33.3
Tr-40	37.3	56.1	18.6	33.3
Tr-44	41.0	51.8	16.0	42.8
Tr-45	37.7	55.7	15.3	45.2
Tr-47	39.3	53.7	17.0	39.2
Tr-51	41.0	51.8	18.3	34.5
Tr-55	38.0	55.3	17.3	38.0
Tr-56	41.7	51.0	18.3	34.5
Tr-57	42.0	50.6	16.6	40.4
Tr-58	41.7	51.0	20.0	28.5
Tr-60	39.7	53.3	18.3	34.5
Check	85.0		28.0	-
CD (P=0.05)	3.4	-	1.77	-

been previously reported that the antagonistic activity of biocontrol isolates of *Trichoderma* may be influenced by the target fungal pathogen (Grondona *et al.*, 1997; Hermosa *et al.*, 2000). In a study involving 45 isolates of *T. harzianum* and five plant pathogens, Grondona *et al.* (1997) showed that the antagonistic ability and behaviour of the 45 isolates varied according to the target fungus. Studies have also shown that *T. harzianum* relies on different mechanisms to control different pathogens (Woo *et al.*, 1999), which may be a major, factor contributing to its differential activity against different pathogens. This display of differential antagonistic activity by isolates of *Trichoderma* further underlines the need for broad spectrum screening of potential biocontrol isolates against several plant pathogens to obtain higher success under practical biocontrol situations.

Glasshouse studies

Out of the 8 treatments and 2 checks tested in the study, it was observed that seed and soil treatment with all the 7 *Trichoderma* isolates and chemical fungicide resulted in significant reduction in the incidence of root rot as compared to check. Lowest incidence of root rot was recorded in treatment with *T. harzianum* isolate Tr-34 (20.8%) followed by isolate Tr-14 (23.4%) (Table 3). Seed treatment with chemical fungicide resulted in 21.1 per cent root rot while in untreated check root rot incidence was 41.3 per cent. *Trichoderma* species have been successfully used for the management of several diseases caused by the pathogens *R. solani* and *F. solani* including root and foot rots (Dinakaran *et al.*, 1995; Hegde and Anahosur, 1998; Tewari and Singh, 2005). Hazarika and Das (1999) had reported the successful management of root rot of French bean caused by *R. solani* using biocontrol agents including *Trichoderma*.

Treatment with *Trichoderma* isolates also resulted in increased germination percentage and better plant growth. A maximum of 96 per cent germination was recorded in treatment with isolate Tr-34 followed by 94 per cent in Tr-14 and Tr-45. Significantly higher root length as compared to untreated check was observed in treatment with

Table 3. Effect of *Trichoderma* isolates on root rot disease and plant health of French bean

Treatment	Germination (%)*	Root rot incidence (%)*	Reduction in root rot (%)	Root length (cm)**	Shoot length (cm)**
Tr-5 (ST+SA)	88 (69.8)	27.2 (31.2)	34.1	14.8	19.5
Tr-14 (ST+SA)	94 (80.9)	23.4 (28.8)	43.3	16.4	22.5
Tr-17 (ST+SA)	92 (75.2)	28.2 (31.5)	31.7	15.3	20.4
Tr-21 (ST+SA)	90 (73.5)	26.2 (30.4)	36.4	15.2	20.1
Tr-28 (ST+SA)	90 (73.5)	24.8 (29.3)	39.9	16.6	20.1
Tr-34 (ST+SA)	96 (82.6)	20.8 (27.1)	49.4	18.0	25.5
Tr-45 (ST+SA)	94 (78.9)	25.5 (30.2)	38.2	15.3	20.1
Carbendazim @0.15per cent (ST)	90 (73.5)	21.1 (27.1)	48.9	13.4	17.8
Only FYM	86 (68.2)	38.2 (37.9)	7.5	12.9	18.8
Check	82 (63.6)	41.3 (39.9)	-	12.4	16.3
CD (P=0.05)	11.3	6.6	-	3.16	3.36

*Figures in parentheses indicate angular-transformed values. ** Root and shoot length 25 days after sowing.

three of the *Trichoderma* isolates (Tr-14, 28 and 34) while 6 isolates (Tr-14, 17, 21, 28, 34 and 45) showed significantly higher shoot length as compared to untreated check. It was observed that Tr-34 was significantly better than chemical fungicide treatment and numerically superior to all other *Trichoderma* isolates in improving germination and plant growth. No significant difference was observed between untreated check and chemical seed treatment in the germination percent or root and shoot length. Growth promotion is reported to be an important mechanism employed by *Trichoderma* spp. and is visible in the form of higher root and shoot length, improved plant health and higher productivity (Harman *et al.*, 2005).

The present study has identified two isolates of *T. harzianum* (Tr-14 and Tr-34), which have the potential to be used for management of root rot of French bean. The indigenous nature of these isolates and the improved plant growth response observed may provide an added advantage for their use in the NWH region.

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