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Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato

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Abstract

The general inadequacy of chemical fungicides to tackle *Rhizoctonia solani* and *Fusarium oxysporum* diseases in tomato has led to the search for biocontrol solutions to these maladies. Twenty-six local isolates of *Trichoderma* spp. and 56 isolates of fluorescent pseudomonads from Kerala were evaluated for their antagonistic activity against *R. solani* and *F. oxysporum* under *in vitro* conditions. Different isolates showed varying degrees of antagonism. The two most antagonistic isolates against *R. solani* were *T. pseudokoningii* TR17 and *T. harzianum* TR20. Likewise, *T. viride* TR19 and TR22 formed the most effective isolates against *F. oxysporum*. Production of volatile and non-volatile antibiotic compounds varied among these isolates. Of the fluorescent pseudomonads, *Pseudomonas fluorescens* isolates P28 and P51 showed the greatest inhibition against *R. solani* whereas against *F. oxysporum*, P20 and P28 were most effective. Isolates obtained from the phylloplane were generally unsuccessful. Inhibitory property of the antagonistic bacteria was also media-dependent. Many of the pseudomonads, which inhibited the pathogens on KMB agar, failed to retard the pathogen's growth on the PDA medium. The bacterial and fungal antagonists were also not mutually antagonistic as their co-inoculation hardly inhibited each other.

Keywords: Antagonists, Biocontrol, Diffusible metabolites, Phylloplane bacteria, Volatile antibiotic compounds.

Introduction

Rhizoctonia solani and *Fusarium oxysporum* are major pathogens of both greenhouse and field grown tomatoes (*Lycopersicon esculentum* Mill.) in the warm vegetable growing areas of the world. Fungicidal application as seed or soil treatment, however, has been found to be ineffective against these pathogens as the propagules are capriciously distributed in the soil and often beyond the reach of chemicals (Campbell, 1989). Biological control, therefore, holds promise as a strategy for disease management and it is environment friendly too. Antagonistic fungi especially *Trichoderma* spp. and the bacteria, fluorescent pseudomonads have been widely used against a number of phytopathogens (Bell et al., 1982; Rini and Sulochana, 2006). In recent years, attempts were also made to use a consortium of biocontrol agents to get persistent control of plant pathogens (Chaube and Sharma, 2002). Keeping this in view and the growing importance of biological control agents, the present study was carried out. The main objective was to evaluate the biocontrol efficiency of native isolates of *Trichoderma* and fluorescent pseudomonads against *R. solani* and *F. oxysporum* and to study their nature of action. An attempt was also made to evaluate the compatibility of selected strains of fluorescent pseudomonads and *Trichoderma* spp. to be used as mixed inoculum.

Materials and Methods

Trichoderma spp. and fluorescent pseudomonads were isolated from the rhizosphere of healthy tomato plants collected from the major vegetable growing tracts of Idukki district and the forest soils of Nelliyampathi, Parambikulam, and Nenmara in Kerala. Twelve soil samples from the forest area and 35 plant samples from

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different vegetable fields were collected during August to October 2003 and isolation done following dilution plate technique. For *Trichoderma*, the fungal colonies with white mycelium, which later changed into different shades of green on the culture medium, were examined, purified, and transferred to potato dextrose agar (PDA) slants. Bacterial colonies showing the characteristic fluorescence in King's medium B (KMB) were picked up, purified, and maintained on KMB slants.

Endophytic and epiphytic bacteria were isolated from disease-free leaves of field-grown tomato plants following the dilution plate technique. To isolate the epiphytic bacteria, the leaf samples (10 g) were dipped in 100 ml sterile distilled water and agitated for 10 min. on a rotary shaker. An extract made by crushing the leaf samples using a sterile mortar and pestle was added to this and diluted to obtain 10^{-3} concentration. From this an aliquot of 1 ml was transferred to sterile plates, KMB poured, mixed well, and incubated for 24 to 48 h at room temperature (28±2ºC). The colonies showing fluorescence were selected, purified, and maintained on KMB slants.

R. solani and *F. oxysporum* were isolated from root rot and wilt infected tomato plants respectively. Pathogenicity was tested by placing the pathogen culture over the roots after giving fine pinpricks. The pathogen cultures were purified and maintained on PDA slants. For the characterization studies, *Rhizoctonia* and *Fusarium* were grown on PDA and potato sucrose agar (PSA) media respectively. Based on the culture and morphology, the pathogens were initially identified and further confirmed at the Agharkar Research Institute, Pune, India.

Pathogen suppression by Trichoderma *spp*.

All the *Trichoderma* isolates were evaluated for their antagonistic activity against the pathogens under *in vitro* conditions following the dual culture technique (Skidmore and Dickinson, 1976). The culture plates were observed constantly, the radial growth of the pathogen recorded on the sixth day of inoculation, and the percent inhibition worked out as follows.

$$PI = \frac{C-T}{C} \times 100$$

Where, PI = Percent inhibition of mycelial growth
C = Radial growth of pathogen in control plates (cm)
T = Radial growth of pathogen in dual culture (cm)

Eleven isolates found antagonistic against *R. solani* and eight isolates against *F. oxysporum* in the preliminary screening were documented based on the cultural and morphological characters. In order to select the most efficient isolates of *Trichoderma* against *R. solani* and *F. oxysporum*, these isolates were subjected to a further *in vitro* screening. For this, the pathogen was inoculated on sterilized PDA and grown for 7 days. After the establishment of the pathogen in the petriplates, a 5 mm culture disc of the antagonist was inoculated. The plates were kept at room temperature for 9 days. Each set of treatment was replicated thrice. Antagonism expressed as sporulating *Trichoderma* over the pathogen was noted at regular intervals from the fifth day onwards. Based on the observation, ratings were made using the modified Bell's (Bell et al., 1982) scale (Classes 1 to 5) as follows.

Class 1 = The antagonist completely overgrown the pathogen (100 % overgrowth).
Class 2 = The antagonist overgrown at least 3/4th of pathogen surface (75% overgrowth).
Class 3 = The antagonist colonized on half of the growth of the pathogen (50% overgrowth).
Class 4 = The pathogen and the antagonist locked at the point of contact.
Class 5 = The pathogen overgrown the mycoparasite.

Production of volatile and non-volatile/diffusible metabolites

All *Trichoderma* isolates selected from the primary screening were evaluated for the production of volatile inhibitory substances *in vitro* following the technique of Dennis and Webster (1971). Five mm disc of *Trichoderma* was inoculated centrally in petriplates containing PDA medium in triplicates. The petriplates

were sealed at the edges, kept in polythene bags, and incubated at room temperature. After 5 days, the test pathogen was inoculated on fresh PDA and the lids of the petriplates inoculated with antagonist were replaced by the culture of the pathogen on PDA. The plates were fixed with cello-tape and incubated for another 7 days. Control consisted of plates inoculated with pathogen inverted over dish containing PDA alone. Growth of the pathogen was measured after 5 days of incubation and the percent inhibition calculated as explained before.

The effect of non-volatile substances produced by the selected *Trichoderma* isolates against the pathogens was studied using the methods described by Anith (1997). The culture filtrate was prepared by growing *Trichoderma* spp. in potato dextrose broth (PDB) for 15 days at room temperature (28±2ºC). The mycelial mat of the fungus was removed from the broth and centrifuged at 10,000 rpm for 15 min. The supernatant collected and concentrated to $1/10^{th}$ of its original volume, was passed through bacteria proof filter (Millipore, 0.45 µ) and used for further study.

For the well assay, sterile molten PDA was poured into sterile petriplates. Wells of 10 mm diameter were made towards the edge in the PDA plates by removing agar discs from the medium using a cork borer. The wells were then partially sealed with molten soft agar. When the agar solidified, 100 µl concentrated culture filtrate was poured carefully into the well using a micropipette and allowed to percolate. Bioassay against the pathogen was performed by co-inoculating an agar disc of the pathogen at the centre of the plate. Pathogen growth was observed after two days of incubation.

Fluorescent pseudomonads

Fifty-six isolates of *P. fluorescens* obtained from the rhizosphere and phylloplane of healthy tomato plants were tested for their antagonistic activity against *R. solani* and *F. oxysporum* following the dual culture technique. Culture discs of 5 mm size of the pathogen were placed at the centre of the sterilized petriplates containing sterilized media. Both KMB and PDA were used in the screening process. The respective bacterial isolate was then streaked 2 cm away from the pathogen at the centre, in a triangular pattern. Inhibition zone was measured after 5 days of incubation.

Compatibility of the selected antagonistic isolates under in vitro *conditions*

Selected isolates of *Trichoderma* namely, TR17, TR20, TR19, and TR22 and *P. fluorescens* P28, were further evaluated for their compatibility following two methods under *in vitro* conditions. In the first method, spore suspension of *Trichoderma* spp. was prepared by dispensing a 5 mm fungal disc in 2 to 3 ml sterile distilled water. One ml of this was plated in KMB agar to get a uniform fungal growth over the media. The *Pseudomonas* isolate was inoculated at three points on the *Trichoderma* plated media. The culture plates were incubated and inhibition of *Trichoderma* growth monitored. In the second method, *Pseudomonas* cell suspension was prepared, mixed with KMB agar, poured into sterile petriplates and a 5 mm disc of *Trichoderma* culture was cut out, and inoculated on the media and observed for the inhibition of growth of *Pseudomonas.* The experiment was repeated on PDA also. All the laboratory experiments mentioned in this paper were laid out in completely randomized design with three replications and the data were subjected to analysis of variance.

Results and Discussion

Trichoderma spp.

A comparison of the data presented in Table 1 indicate that of the 26 *Trichoderma* isolates tested *in vitro*, 11 (TR2, TR13, TR15, TR17, TR18, TR19, TR20, TR21, TR22, TR24, and TR26) were effective in suppressing *R. solani.* These isolates inhibited pathogen growth by 59% within 6 days of inoculation. Eight isolates (TR2, TR13, TR17, TR18, TR19, TR20, TR22, and TR24) were promising against *F. oxysporum* and they reduced the growth of the pathogen by more than 60% within 6 days of inoculation. The remaining isolates, although inhibited the growth of *R. solani* and *F. oxysporum*, were inferior to the ones listed above in performance.

Table 1. In vitro screening of *Trichoderma* isolates against *Rhizoctonia solani* and *Fusarium oxysporum.*

Trichoderma isolates	Mean radial growth (cm) of three replications		% inhibition (6th day of inoculation)	
	R. solani	*F. oxysporum*	*R. solani*	*F. oxysporum*
TR1	2.30	2.10	49	53
TR2	1.85	1.75	59[a]	61[a]
TR3	2.40	2.80	47	38
TR4	2.70	2.70	40	40
TR5	2.40	2.50	47	44
TR6	2.35	2.60	48	42
TR7	2.30	2.10	49	53
TR8	2.20	2.0	51	56
TR9	3.25	2.50	28	44
TR10	2.40	2.50	47	44
TR11	3.15	2.40	30	47
TR12	2.80	2.70	38	40
TR13	1.85	1.75	59[a]	61[a]
TR14	2.20	2.30	51	49
TR15	1.85	2.0	59[a]	56
TR16	2.20	2.40	51	47
TR17	1.85	1.75	59[a]	61[a]
TR18	1.85	1.75	59[a]	61[a]
TR19	1.83	1.75	59[a]	61[a]
TR20	1.83	1.75	59[a]	61[a]
TR21	1.83	1.90	59[a]	58
TR22	1.85	1.70	59[a]	62[a]
TR23	3.2	2.80	29	38
TR24	1.83	1.75	59[a]	61[a]
TR25	2.20	2.60	51	42
TR26	1.85	1.90	59[a]	58
Control	4.50	4.50	–	–
CD (0.05)	0.04	0.028	–	–

Values with superscripts differ significantly.

On further screening it was observed that two isolates *viz*., *T. harzianum* TR20 and *T. pseudokoningii* TR17 were more effective against *R. solani* than others whereas *T. viride* TR19 and TR22 were the most promising against *F. oxysporum* (Table 2). These isolates completely overgrew the pathogens and suppressed it within 7 days of inoculation.

In vitro studies on the inhibitory mechanisms showed that *Trichoderma* cultures apparently produced volatile and non-volatile substances in the growth medium that suppressed the pathogen growth. The data presented in Table 3 clearly indicate that *T. pseudokoningii* TR17 derived volatile substances caused maximum inhibition of the mycelial growth of both pathogens. This isolate inhibited *R. solani* and *F. oxysporum* by 54% and 48% respectively. *T. longibrachiatum* TR2 and *T. viride* TR22 were ranked next in respect of growth inhibition of *R. solani* (41%) and *F. oxysporum* (43%).

Diffusible (non-volatile) metabolite activity of the antagonists was more potent against *R. solani* than *F. oxysporum.* Culture filtrates from both *T. harzianum* TR20 and *T. viride* TR22 produced an inhibition zone of 6 mm for *R. solani*. Among the eight *Trichoderma* isolates tested for non-volatile metabolite activity against *F. oxysporum*, *T. harzianum* TR20 was the best and it produced an inhibition zone of 4 mm. This was followed

Table 2. Ratings of selected isolates of *Trichoderma* spp. on the inhibition of *Rhizoctonia solani* and *Fusarium oxysporum*

Trichoderma spp. and isolates	Bell's scale (modified) (days)									
	R. solani					*F. oxysporum*				
	5	6	7	8	9	5	6	7	8	9
T. longibrachiatum TR2	C4	C4	C2	C1	C1	C4	C4	C4	C4	C2
T. virens TR13	C4 – C3	C3	C2	C1	C1	C4	C4	C3 – C2	C1	C1
T. viride TR15	C4	C4	C4	C3	C3	NA	NA	NA	NA	NA
T. pseudokoningii TR17	C4	C4	C1	C1	C1	C4	C4	C4	C4	C4
T. viride TR18	C4	C4	C2	C2	C1	C4	C4	C3 – C2	C1	C1
T. viride TR19	C4	C4	C3	C2	C2	C3 – C2	C2	C1	C1	C1
T. harzianum TR20	C3 – C2	C2 – C1	C1	C1	C1	C4	C4 – C3	C3	C3	C3
T. viride TR21	C4	C4	C4 – C3	C4 – C3	C4 – C3	NA	NA	NA	NA	NA
T. viride TR22	C4	C3	C2	C1	C1	C3 – C2	C2	C1	C1	C1
T. harzianum TR24	C4	C3	C2	C1	C1	C4	C4 – C3	C3	C3	C2
T. viride TR26	C4	C4	C4	C4 – C3	C4 – C3	NA	NA	NA	NA	NA

C1 = Class 1 (Antagonist completely overgrew the pathogen: 100% over-growth); C2 = Class 2 (75% over-growth); C3 = Class 3 (50% over-growth); C4 = Class 4 (pathogen and antagonist locked at the point of contact); C5 = Class 5 (Pathogen overgrew the mycoparasite); NA = Not applicable.

by *T. virens* TR13 and *T. viride* TR19 (Table 3). Overall, considerable variations in the inhibitory properties of *Trichoderma* isolates were discernible. Variations in the inhibitory potential may be due to the differences in the quantity and quality of the inhibitory substances (volatile and non-volatile) produced by the antagonists. This is consistent with the reports of Bell et al. (1982).

Fluorescent pseudomonads

The inhibitory potential of pseudomonad isolates also differed significantly. Against *R. solani*, *Pseudomonas*

Table 3. In vitro inhibitory effect of volatile and non-volatile metabolites of selected *Trichoderma* spp. against *Rhizoctonia solani* and *Fusarium oxysporum*

Trichoderma spp. and isolates	Volatile metabolites (% inhibition)		Non-volatile metabolites inhibition zone (mm)	
	R. solani	*F. oxysporum*	*R. solani*	*F. oxysporum*
T. longibrachiatum TR2	41^{b}	33^{d}	0^{f}	0^{e}
T. virens TR13	20^{j}	18^{g}	2^{e}	3^{b}
T. viride TR15	30^{f}	NA	5^{b}	NA
T. pseudokoningii TR17	54^{a}	48^{a}	4^{c}	1^{d}
T. viride TR18	35^{d}	28^{e}	5^{b}	1^{d}
T. viride TR19	28^{g}	35^{c}	4^{c}	3^{b}
T. harzianum TR20	38^{c}	24^{f}	6^{a}	4^{a}
T. viride TR21	27^{h}	NA	3^{d}	NA
T. viride TR22	33^{e}	43^{b}	6^{a}	2.5^{c}
T. harzianum TR24	30^{f}	24^{f}	5^{b}	0^{e}
T. viride TR26	21^{i}	NA	0^{f}	NA
Control	0	0	0	0
CD (0.05)	0.13	0.07	0.18	0.04

NA = not applicable; means with the same superscript do not differ significantly.

fluorescens isolates P28 and P51 exerted the maximum inhibitory effect as evidenced by the widest inhibition zone (14.25 and 14 mm respectively for P28 and P51 in KMB and 7.5 mm for both in PDA; Table 4). P20 and P28 were the most inhibitory isolates against *F. oxysporum* (11.25 and 10.75 mm respectively in KMB and 7.5 and 7.25 mm respectively in PDA; Table 5). The biocontrol potential of fluorescent pseudomonads against *R. solani* (Rini and Sulochana, 2006) and *F. oxysporum* f. sp. *lycopersici* (Hebbar et al., 1992) have been previously reported. Among the 56 pseudomonads tested, the isolates obtained from the phylloplane were generally ineffective in inhibiting the growth of *R. solani* and *F. oxysporum*.

Our results also indicate that the antagonistic activity of the bacterial strains depended on the media used. Among the 56 isolates tested against *R. solani*, 17 inhibited *R. solani* in both PDA and KMB media (Table 4). However, the inhibitory potential was more profound

Table 4. In vitro screening of fluorescent pseudomonads against *Rhizoctonia solani*.

Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)		Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)	
	KMB	PDA		KMB	PDA
P1[1]	12.0 (3.60)	5.0 (2.45)	P29[1]	13.0 (3.74)	0.5 (1.22)
P2[2]	8.5 (3.08)	OG	P30[2]	6.0 (2.65)	OG
P3[1]	11.5 (3.52)	4.3 (2.29)	P31[2]	3.0 (2.0)	OG
P4	OG	OG	P32[2]	4.0 (2.24)	OG
P5	OG	OG	P33	OG	OG
P6	OG	OG	P34[2]	1.0 (1.41)	0.0 (1.0)
P7	OG	OG	P35	OG	OG
P8[2]	4.5 (2.35)	OG	P36	OG	0.0 (1.0)
P9[1]	12.5 (3.65)	6.0 (2.65)	P37	OG	OG
P10	OG	OG	P38[1]	2.8 (1.94)	2.2 (1.79)
P11[1]	12.8 (3.71)	7.5 (2.92)	P39	OG	OG
P12[1]	5.3 (2.50)	2.3 (1.80)	P40	OG	OG
P13[2]	6.5 (2.74)	OG	P41	OG	OG
P14[2]	9.0 (3.16)	OG	P42	OG	1.0 (1.41)
P15[1]	5.5 (2.55)	0.8 (1.32)	P43[1]	3.0 (2.0)	1.0 (1.41)
P16[2]	11.3 (3.50)	OG	P44	0.0 (1.0)	0.0 (1.0)
P17[1]	11.8 (3.57)	2.0 (1.73)	P45[2]	5.3 (2.50)	OG
P18[2]	13.3 (3.77)	0.0 (1.0)	P46	OG	OG
P19[2]	4.0 (2.24)	OG	P47	OG	OG
P20[1]	9.5 (3.24)	1.0 (1.41)	P48	0.0 (1.0)	0.0 (1.0)
P21[2]	6.5 (2.74)	OG	P49	0.0 (1.0)	0.0 (1.0)
P22[2]	13.0 (3.74)	OG	P50	OG	OG
P23	OG	OG	P51[1]	14.0[a] (3.87)	7.5[a] (2.92)
P24[1]	12.3 (3.64)	2.8 (1.94)	P52[1]	8.0 (3.0)	1.0 (1.41)
P25[2]	13 (3.74)	OG	P53[2]	4.5 (2.35)	OG
P26	OG	3.3 (2.06)	P54[2]	8.0 (3.0)	OG
P27[1]	11.3 (3.50)	5.5 (2.55)	P55	OG	OG
P28[1]	14.3[a] (3.91)	7.5[a] (2.92)	P56[1]	5.8 (2.59)	1.0 (1.41)
			CD	0.36	0.34

Figure in parenthesis indicates $\sqrt{x+1}$ transformed value; OG – antagonist overgrown by the pathogen; [a] significantly superior; [1]antagonism shown in both the media used; [2] antagonism shown only in KMB agar and not in PDA.

in KMB rather than PDA. Another 17 isolates though inhibited the pathogen in KMB could not exhibit antagonism in PDA medium while two isolates viz., P26, and P42 inhibited *R. solani* in PDA but not in KMB. A similar trend was discernible for *F. oxysporum* also (Table 5). The nutrient source or its concentration in the medium might have affected the production of antifungal compounds that in turn are responsible for the antagonistic activity of fluorescent pseudomonads in different media, as reported by Hebbar et al. (1992).

Compatibility of the selected isolates

Data on compatibility show that both *Trichoderma* spp. and fluorescent pseudomonads were compatible to each other in dual cultures. Either of the organisms formed no inhibition zone in any of the plates. Similar observations have been reported previously also. For example, *T. viride*/*T. harzianum* and *P. fluorescens* were reported to be compatible and improved plant growth, as well as suppressed seedling disease of chilli and

Table 5. In vitro screening of fluorescent pseudomonads against *Fusarium oxysporum*.

Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)		Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)	
	KMB	PDA		KMB	PDA
P1[1]	9.5 (3.20)	6.0 (2.65)	P29[1]	9.0 (3.16)	6.5 (2.7)
P2[1]	8.5 (3.08)	5.5 (2.54)	P30[1]	6.0 (2.64)	5.0 (2.4)
P3[1]	10.0 (3.30)	4.8 (2.40)	P31[1]	3.0 (2.0)	2.0 (1.7)
P4	OG	OG	P32[1]	4.0 (2.24)	3.5 (2.1)
P5	OG	OG	P33[2]	3.0 (2.0)	OG
P6	OG	OG	P34[2]	1.5 (1.58)	OG
P7	OG	OG	P35	OG	OG
P8[1]	3.3 (2.06)	3.3 (2.06)	P36	OG	OG
P9[1]	6.5 (2.74)	4.5 (2.34)	P37	OG	OG
P10[1]	4.5 (2.35)	3.5 (2.15)	P38	OG	OG
P11[1]	9.5 (3.24)	5.0 (2.45)	P39	OG	OG
P12[1]	5.3 (2.50)	5.5 (2.54)	P40	OG	OG
P13[1]	5.0 (2.45)	5.0 (2.45)	P41	OG	OG
P14[1]	8.5 (3.08)	6.0 (2.64)	P42	OG	OG
P15[2]	5.5 (2.55)	0.0 (1.0)	P43	OG	OG
P16[1]	8.5 (3.08)	6.5 (2.74)	P44	OG	OG
P17[1]	8.5 (3.08)	5.0 (2.45)	P45	OG	OG
P18[1]	9.3 (3.20)	6.8 (2.78)	P46	OG	OG
P19[2]	3.0 (2.0)	OG	P47	OG	OG
P20[1]	11.3[a] (3.46)	7.50[a](2.90)	P48	0.0 (1.0)	OG
P21[1]	4.5 (2.35)	4.5 (2.34)	P49[2]	2.0 (1.73)	OG
P22[1]	9.8 (3.28)	6.3 (2.70)	P50	OG	OG
P23	OG	OG	P51[1]	9.5 (3.24)	6.0 (2.6)
P24[1]	7.5 (2.90)	5.5 (2.54)	P52[1]	8.0 (3.0)	5.3 (2.5)
P25[1]	7.0 (2.83)	3.3 (2.06)	P53[1]	5.5 (2.55)	5.5 (2.6)
P26[2]	2.5 (1.87)	OG	P54[1]	8.0 (3.0)	6.0 (2.6)
P27[1]	8.0 (3.0)	6.5 (2.74)	P55	0.0 (1.0)	OG
P28[1]	10.8[a] (3.35)	7.3[a] (2.87)	P56[1]	7.5 (2.90)	4.5 (2.3)
			CD	0.37	0.25

Figure in parenthesis indicates $\sqrt{x+1}$ transformed values; OG – antagonist overgrown by the pathogen;
[a] significantly superior; [1] antagonism shown in both the media used; [2] antagonism shown only in KMB agar and not in PDA.

tomato significantly when these were applied together (Rini and Sulochana, 2006; Chaube and Sharma, 2002).

On a final note, this study demonstrated the efficacy of native isolates of *Trichoderma* viz*.,* T. *harzianum* TR20, *T. pseudokoningii* TR17 and *T. viride* isolates TR19 and TR22 and *P. fluorescens* isolate P28 in controlling *R. solani* and *F. oxysporum*. It also showed the involvement of volatile and non-volatile antibiotics in biocontrol. Further, the bacterial and fungal antagonists are compatible with one another and hence a *consortium approach* could be used in controlling the pathogens effectively.

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