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Efficacy of *Trichoderma* spp. against *Phytophthora parasitica* and *Pythium* spp. causing foot rot and leaf rot of betelvine (*Piper betle* L.)

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ABSTRACT

Seventeen isolates of Trichoderma spp. isolated in Trichoderma Specific Medium (TSM) form soils of different betel vine plantations of West Bengal and two isolates form soils of Andhra Pradesh and Madhya Pradesh. Cultural, morphometric and antagonistic potential against Phytophthora parasitica (4 isolates) and Pythium spp. (6 isolates) of each Trichoderma spp. isolates were studied. The morphometric studies revealed that the highest phialides, phialospores, chlamydospore were recorded in isolate T_{13} and length of conidiophore was reordered highest in T_9, T_4, T_{13} and T_{12} isolates respectively. The chlamydospores recorded were in twins, chains or in intercalary in positions. The antagonistic potential of all the isolates of Trichoderma spp. were tested by Dual Plate Technique against all the isolates of Phytophthora parasitica and Pythium spp. and one isolate of Phytophthora parasitica were tested by inverted plate technique to find out the volatile properties against all the isolates of Trichoderma spp. From the cultural and morphometric characters, it was revealed that among the 17 isolates of Trichoderma spp., T_6, T_7, T_9, T_{10}, T_{11}, T_{12}, T_{13} and T_{JN} are Trichoderma viride, T_4 is T.virens and rest isolates are T. harzianum. The results of antagonistic property revealed that T_4, T_7, T_{12}, T_{14}, T_B and T_{JN} showed highest promise under in vitro conditions (Dual Plate Technique) by fully overgrowing the pathogens as reordered by Bell's scale within 7-8 days. Isolates T_7, T_{13}, T_{15} and T_4 of Trichoderma spp. showed some promise against isolate of Phytophthora parasitica by inverted plate technique within 4-5 days by releasing some volatiles. From the results, it could be concluded that isolates T_4 and T_7 were highly effective against the above pathogen by both the techniques tested.

Key words: Biocontrol, *Piper betle*, *Trichoderma viride*, *T. harzianum*, *Phytophthora parasitica* and *Pythium*

Betelvine is cultivated mainly under artificially erected structure, known as Boroj, Bareja or Bheet, which is a kind of hut whose sides and roof is made of jute slaths or straw on a light frame work of bamboo. In spite of the tremendous potentiality of the crop, cultivation of betel vine is highly risky and returns are uncertain because of its proneness to several diseases, aggravated by the moist and humid conditions of the plantation, that in turn are prerequisites for good harvest. Obviously the major constraint to cultivation of betelvine is its diseases that severally damage foot, stem, root and foliage. The serious diseases reported include a foot rot syndrome produced by a number of pathogens including *Phytophthora parasitica* var. *piperina*, *P. nicotianae* var *parasitica*, species of *Rhizoctonia*, *Pythium* and *Sclerotium rolfsii* Sace, and foliage diseases like leaf rot by *P. parasitica*, *P. palmivora*, leaf spot and stem anthracnose caused by *Colletorichum capsici*, bacterial leaf spot and stem rot caused by *Xanthomonas campestries* pv. *betlicola*. Among the pathogens, *Phytophthora* sp. and *Pythium* spp. perhaps ranks first in its destructiveness under both field and storage conditions. The extent of losses may vary from 30 – 100% in case of foot rot and 20 – 40% in case of leaf rot, leading to almost total crop failure (Maiti and Sen, 1982; Dasgupta *et al.*, 2000).

In last three decades, a lot of researches have been carried out on the antagonistic nature of several species of genus *Trichoderma* (Papavizas, 1985; Chet, 1987) which had shown highest potential against soil-borne fungal pathogens. Both *T. harzianum and T. viride* showed highest potential against many soil borne fungal pathogens. Researches on *T. harzianum* and *T. viride* as a biocontrol agent also showed differential antagonistic potential among isolates (D'Souza *et al.*, 2001; Mohanty, 2003). Our emphasis in the present studies was on the need for screening specific isolates of antagonists against various isolates of *P. parasitica* and *Pythium* spp.. Several antagonistic isolates of *Trichoderma sp.* were collected from different betelvine gardens of West Bengal and they were then tested under *in vitro* conditions against the pathogen *P. parasitica* and *Pythium* spp. which causes foot rot and leaf rot of betelvine (*Piper betle* L.).

MATERIALS AND METHODS

Isolates of *Trichoderma sp.* from soil

Seventeen different isolates of *Trichoderma* sp. were randomly isolated from soils which were collected from different barejas of West Bengal and two isolates form soils of Andhra Pradesh and Madhya Pradesh by dilution plate technique using TSM (*Trichoderma* Specific Medium) (Elad and Chet, 1983) modified by Saha and Pan (1997) (Table 1). All the isolates were maintained on PDA slants at 5°C.

Isolates of *Phytophthora parasitica* and *Pythium* spp. from soil

Four different isolates of *Phytophthora parasitica* and six different isolates of *Pythium* spp. were isolated from infected leaf and stem which were collected from different barejas of West Bengal using V8 juice agar medium (Table 2). All the isolates were maintained on Oat meal agar slants at 5°C.

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General Characteristic of *Trichoderma* isolates

Micrometric measurement of phialospores and phialides was done by mounting 4 day old young culture in lactophenol stained with cotton blue and observed under high power research microscope. Micrometric measurement of chlamydospores was made from one month old culture following the method described earlier. The length breadth ratios of phialospores, phialides and chlamydospores were recorded.

Antagonistic potential of *Trichoderma* Isolates

The antagonistic properties of 17 isolates of *Trichoderma* were tested on PDA medium by dual culture plate technique. Five days old cultures of *Phytophthora parasitica* and *Pythium* spp. were plated aseptically at the edge of petri plates 2 days before the placement of *Trichoderma* sp. Paired cultures were observed for a total of 9 days before being discarded. All the ratings were done after contacts between pathogen and antagonist using a modified Bell's (Bell *et a.l.*, 1982) scale (1-5) developed as follows:

Class I -The antagonist completely overgrew the pathogen (100% overgrowth).

Class II – The antagonist overgrew at least 2/3 rd of pathogen surface (75% overgrowth).

Class III – The antagonist colonized on half the growth of the pathogen (50% overgrowth).

Class IV- The pathogen & antagonist locked at the point of contact; and

Class V- The pathogen overgrew the mycoparasite.

Table 1: Source of isolates of *Trichoderma* spp.

Isolates	Place of collection	Name of bioagent	Growth after 96 hrs.
T_1	Plant Virus Research Farm, BCKV,Kalyani, Nadia (Baroj -1)	*Trichoderma harzianum*	Light green coloured full plate growth.
T_2	Plant Virus Research Farm, BCKV,Kalyani, Nadia (Baroj -2)	*T. harzianum*	Light green coloured full plate growth.
T_3	Plant Virus Research Farm, BCKV,Kalyani, Nadia (Baroj -3)	*T. harzianum*	Deep green sporulation all over the plate .
T_4	Mondauri Farm, BCKV, Mondauri, North 24 Parganas (Baroj -1)	*T. virens*	Dark green full plate growth.
T_5	Mondauri Farm, BCKV, Mondauri, North 24 Parganas (Baroj -2)	*T. harzianum*	Deep greenish sporulation, full plate mycelia growth.
T_6	Rautari, Nadia	*T. viride*	Dirty green coloured full plate growth.
T_7	Simurali, Nadia	*T. viride*	Light green coloured sporulation, full plate growth.
T_8	Simurali, Nadia	*T. harzianum*	Light green coloured full plate growth.
T_9	Rautari, Nadia	*T. viride*	Light greenish compact full plate growth.
T_{10}	Simurali, Nadia	*T. viride*	Compact yellowish green growth.
T_{11}	Rautari, Nadia	*T. viride*	Deep green sporulation all over the medium.
T_{12}	Simurali, Nadia	*T. viride*	Dirty green sporulation all over the medium.
T_{13}	Rautari, Nadia	*T. viride*	Deep green sporulation.
T_{14}	Rautari, Nadia	*T. harzianum*	Deep green sporulation.
T_{15}	Simurali, Nadia	*T. harzianum*	Deep green, compact sporulation
T_B	Bapatla, Andhra Pradesh	*T.viride*	Compact deep greenish full plate growth
T_{ZN}	Jabalpur, Madhya Pradesh	*T. viride*	Greenish appearance throughout the Petri plate.

Table 2: Source of isolates of *Phytophthora parasitica* and *Pythium* spp.

Isolates	Source	Name of the fungus	Place of collection
P_1	Leaf	*Phytophthora parasitica*	Gene Bank, BCKV,Kalyani
P_2	Leaf	*Pythium* spp.	Gene Bank, BCKV,Kalyani
P_3	Leaf	*Pythium* spp.	Gene Bank, BCKV,Kalyani
P_4	Stem	*Pythium* spp.	Mondauri Farm, BCKV, Mondauri
P_5	Leaf	*Pythium* spp.	Mondauri Farm, BCKV, Mondauri
P_6	Leaf	*Phytophthora parasitica*	Baroj of Aizwal Mondal, Simurali, Nadia
P_7	Stem	*Pythium* spp.	Baroj of Saidul Mondal, Simurali, Nadia
P_8	Stem	*Pythium* spp.	Baroj of Selim Mondal, Simurali, Nadia
P_9	Leaf	*Phytophthora parasitica*	Baroj of Aswini Mondal, Simurali, Nadia
P_{10}	Leaf	*Phytophthora parasitica*	Baroj of Uttam Das, Simurali, Nadia

RESULTS AND DISCUSSION

Identity of the isolates of *Trichoderma*

The identity of test isolates of antagonist was attempted to identify through a study of cultural and morphometric characters. The colony characters of seventeen isolates on PDA as observed visually at different time intervals (24-96 hrs) were recorded (Table 3). In general, colony morphology of all the isolates was more or less similar showing sparse to thin cottony mycelial mass with whitish border. Sporulation started after 48 hrs of incubation at 28±1° C for all the isolates. These observations on colony characters showed no difference from those made earlier by Rifai (1969), Domsch *et al.* (1980), Martha (1992) and D'Souza *et al.* (2001).

The micrometric measurements (Table 4) showed that the largest phialospore produced by isolate T_4 and it ranges from 3.75-7.50(5.62) µm and smallest ones were produced by isolates T_2, T_5, T_{10}, T_{11}, T_{13}, T_{14}, length rages from 2.5-3.50 µm and breadth ranges from 2.26-2.59 µm. The length breadth ratio was found to be highest in T_{13}. The length of Phialides ranged between 10-13.75 µm and width ranged between 2.66-3.10 µm. The longest phialides was produced by T_9 [12.5-15 µm (13.75)] and largest by also T_9 (12.5-15 µm × 2.51X-3 µm) and smallest Phialides was produced by T_{12} (6.25-10.25×2.71-2.99 µm). The length breadth ratio was highest in T_9 (5:1) where as smallest in T_{ZN} (1.16:1) (Table 4).

The morphometric characters and micrometric measurements of 17 isolates of *Trichoderma* spp revealed that T_6, T_7, T_9, T_{10}, T_{11}, T_{12}, T_{13} and T_{ZN} isolates are *T. viride*, T_4 isolate is *T. virens* and rest isolates are *T. harzianum* (Table 1).

Antagonistic potential of antagonist isolates against *P. parasiticaa*

The result (Table 5) showed that 3 isolates of*T.viride*., T_7, T_{12} and T_{ZN}, one isolate of *T. virens*, T_4 and two isolates of *T. harzianum*, T_{14} and T_B were highly antagonistic to *P. parasitica*, totally overgrew over the pathogenic organism within 7-8 days. Those isolates were categorized in class-I according to Bell's scale. The other *Trichoderma* spp. isolates gave an altogether different picture. *T. viride* isolate T_{11} and *T. harzianum* isolates T_1, T_2 and T_{15} were rated as R_2. Where as, *T. viride* isolates T_6, T_9, T_{10} and T_{13} and *T. harzianum* isolates T_3 and T_5 were rated as R_3. T_8 isolate of *T. harzianum* were antagonistic in the scale of R_2 and R_3 to isolates P_6 and P_{10} and P_1 and P_9 of *P. parasitica* respectively.

Trichoderma sp. specifically *T. viride* isolates T_7 and T_{12} reached in Class-I stage within 6 days of inoculation against most of the isolates of *Pythium* spp. However, based on this information the antagonistic *T. viride,* did not allow an easy selection of isolates as the variability in the antagonistic characteristic within isolate and isolate-pathogen was very high. But the antagonistic isolate T_7 and T_{12} appeared to be a nearly assured choice due to their effectivity against *P. parasitica*.

Antagonistic potential of antagonist isolates against *Pythium* spp.

Three isolates of *T. viride*., T_7, T_{12} and T_{ZN}, one isolate of *T. virens*, T_4 and two isolates of *T. harzianum*, T_{14} and T_B were highly antagonistic to *Pythium* spp., totally overgrew over the pathogenic organism within 7-8 days. Those isolates were categorized in class-I according to Bell's scale. The other *Trichoderma* spp. isolates gave an altogether different picture. *T. viride* isolate T_{11} and *T. harzianum* isolates T_1, T_2, T_8 and T_{15} were rated as R_2. Where as, *T. viride* isolates T_6, T_9, T_{10} and T_{13} and *T. harzianum* isolates T_3 and T_5 were rated as R_3.

Table 3: Colony characters of seventeen isolates of *Trichoderma* spp.

Isolate	Growth after 48 hrs.	Growth after 72 hrs.	Growth after 72 hrs.	Growth after 96 hrs.
T_1	White sparse growth.	White cottony mycelial growth.	Greenish white mycelial growth.	Light green coloured full plate growth.
T_2	White sparse growth.	White fluffy mycelial growth.	Full plate growth, whitish, non-sporulation.	Light green coloured full plate growth.
T_3	White cottony growth.	2/3plate white cottony growth.	Full mycelial growth, greenish coloured, sporulation at the older region .	Deep green sporulation all over the plate .
T_4	White sparce growth.	Same as 24hours, growth, white.	Full plate growth, greenish appearance to the periphery of the disc.	Dark green full plate growth.
T_5	White cottony appearance .	A raised growth pattern having whitish cottony mycelia growth.	Cottony, compact , light greenish growth.	Deep greenish sporulation, full plate mycelia growth.
T_6	Off-white mycelial growth.	White sparce growth, 2/3 of plate.	Full plate growth , whitish green growth at the periphery of the plate.	Dirty green coloured full plate growth.
T_7	White mycelial growth around the disc.	White sparce growth, no sporulation.	Full plate growth , greenish white mycelial growth.	Light green coloured sporulation, full plate growth.
T_8	White thin growth over the medium.	Round, white growth over the medium.	Light greenish sporulation surrounding the inoculated disc.	Light green coloured full plate growth.
T_9	White cottony appearance	Cottony mycelial growth, Light yellowish tinge.	Greenish white low sporulation, light yellowish tinge.	Light greenish compact full plate growth.
T_{10}	Fluffy cottony appearance	Same as 24has growth, myclia cover 2/3 of plate.	Full plate growth, light greenish appearance in entire plate.	Compact yellowish green growth.
T_{11}	White cottony myclial growth.	White cottony growth on the surface of medium.	Whitish green sporlation at 2/3 of the plate.	Deep green sporulation all over the medium.
T_{12}	White sparse growth.	Cottony growth over the medium.	Whitish green appearance nearly entire medium.	Dirty green sporulation all over the medium.
T_{13}	White fluffy growth.	White slow growth rate.	Very light greenish growth, myclia cover 2/3 of the plate.	Deep green sporlation.
T_{14}	White cottony appearance.	Sparse growth, cover 2/3 of the medium.	Light greenish appearance from centre to the periphery of the plate.	Deep green sporulation.
T_{15}	Bright white mycelia growth.	Cottony, white mycelia growth. Covers2/3 of the plate.	Full plate compact, cottony growth, sporulation. More or less in entire plate.	Deep green, compact sporulation
T_B	White sparse growth	Whitish mycelial growth	Whitish green sporulation covers 2/3rd of the petri plate	Compact deep greenish full plate growth
T_{ZN}	White cottony growth	Whitish mycelial growth covers 2/3rd of the petriplate	Full plate growth with whitish green appearance at the center.	Greenish appearance through out the Petri plate.

Table 4: Micrometric measurement of phialospores, phialides, chlamydospores of *Trichoderma* spp.

Isolate	Phialospore conidia^{-1}(µm)*			Phialide (µm)*			Chlamydospore (µm)*		
	L	B	L:B	L	B	L:B	L	B	L:B
T_1	2.50-3.75 (3.12)**	2.12-3.32 (2.72)	1.14:1	6.1-12.5 (9.3)	2.47-3.10 (2.78)	3.34:1	6.53-9.05 (7.79)	5.44-8.59 (7.01)	1.11:1
T_2	2.75-3.25 (3.0)	2.37-2.82 (2.59)	1.15:1	7.5-15.0 (11.25)	2.97-3.16 (3.06)	3.67:1	8.96-16.28 (12.62)	6.44-15.47 (10.95)	1.15:1
T_3	3.0-4.0 (3.5)	2.62-3.5 (3.09)	1.13:1	7.5-12.5 (10.00)	2.92-4.0 (3.46)	2.89:1	7.62-12.20 (9.91)	6.31-11.99 (9.15)	1.08:1
T_4	3.75-7.50 (5.62)	3.39-7.06 (5.22)	1.07:1	7.5-15 (11.25)	2.16-3.10 (2.63)	4.27:1	11.21-22.23 (16.72)	7.02-17.48 (12.25)	1.36:1
T_5	25-2.75 (2.62)	2.14-2.38 (2.26)	1.15:1	10-15 (12.5)	2.66-2.79 (2.72)	4.53:1	9.25-16.78 (13.01)	8.46-16.32 (12.39)	1.05:1
T_6	2.5-5.0 (3.75)	2.14-4.61 (3.15)	1.06:1	5-12.5 (8.75)	2.46-3.66 (3.06)	2.85:1	6.72-9.88 (8.3)	6.72-9.7 (8.21)	1.01:1
T_7	2.5-4.25 (3.37)	2.12-3.88 (3.0)	1.12:1	6.25-10 (8.12)	2.51-3.39 (2.95)	1.75:1	5.26-10.55 (7.90)	4.48-1023 (7.35)	1.07:1
T_8	2.75-4.25 (3.50)	2.39-3.88 (3.13)	1.11:1	7.5-10.25 (8.87)	2.20-2.99 (2.59)	3.42:1	12.62-32.16 (22.39)	11.23-28.55 (19.89)	1.12:1
T_9	2.5-4.25 (3.37)	2.07-2.88 (2.97)	1.13:1	12.5-15 (13.75)	2.51-3 (2.75)	5.0:1	12.61-17.02 (14.81)	10.49-13.03 (11.76)	1.25:1
T_{10}	2.5-3.25 (2.87)	2.07-2.86 (2.46)	1.16:1	5-7.5 (6.25)	2.92-3.9 (3.41)	1.83:1	6.14-15.15 (10.64)	5.72-11.36 (8.54)	1.24:1
T_{11}	2.5-3.5 (3.0)	2.12-3.06 (2.58)	1.16:1	7.5-12.5 (1.00)	2.16-2.66 (2.41)	4.41:1	15.11-24.97 (20.04)	11.19-16.0 (13.59)	1.47:1
T_{12}	3.5-5.25 (4.37)	3.12 -4.81 (3.96)	1.10:1	6.25-10.25 (8.25)	2.71-2.99 (2.85)	2.89:1	5.11-11.18 (8.14)	4.25-9.42 (6.83)	1.19:1
T_{13}	2.5-3.0 (2.75)	2.12-2.57 (2.34)	1.17:1	5-12.5 (8.75)	2.11-2.77 (2.44)	3.58:1	18.51-26.48 (22.49)	11.33-14.98 (13.15)	1.71:1
T_{14}	2.5-3.0 (2.75)	2.14-2.88 (2.51)	1.14:1	7.5-18.75 (13.12)	2.78-2.96 (2.87)	4.57:1	13.46-15.68 (14.57)	10.90-13.20 (12.05)	1.20:1
T_{15}	2.5-4.5 (3.5)	2.12-4.13 (3.12)	1.12:1	5-13.75 (9.37)	2.51-3.12 (2.81)	3.33:1	12.42-16.80 (14.61)	10.40-16.39 (13.41)	1.08:1
T_B	10-2.5 (10.5)	2.5-4.0 (3.3)	3.18:1	3.75-5.0 (4.5)	3.0-4.5 (3.80)	1.18:1	9.65-15.72 (12.59)	8.12-12.65 (11.14)	1.13:1
T_{JN}	15-20.0 (17.0)	5-9.5 (6.4)	2.65:1	5.0-6.5 (5.55)	3.75-5.75 (4.75)	1.16:1	7.21-13.10 (10.38)	6.10-12.10 (9.28)	1.11:1
SEm(±)	**0.37**	**0.36**		**1.79**	**0.27**		**2.41**	**1.37**	
LSD(0.05)	**1.02**	**1.00**		**NS**	**0.76**		**6.67**	**3.80**	

*Note: * Average of three replications ** Figures in parentheses are average of ten observations*

Table 6: Screening of *Trichodrma* sp. isolates against *Pythium* spp. (Average of three replications)

Isolates	Point of Contact (days)	Bell's scale after (days)* 4th						5th						6th						7th						8th					
		P_2	P_3	P_4	P_5	P_7	P_8	P_2	P_3	P_4	P_5	P_7	P_8	P_2	P_3	P_4	P_5	P_7	P_8	P_2	P_3	P_4	P_5	P_7	P_8	P_2	P_3	P_4	P_5	P_7	P_8
T_1	3	R_3	R_3	R_3	R_3	R_3	R_3	R_2R_2	R_3	R_3R_2	R_3R_3	R_2R_2	R_2R_2	R_2	R_2R_2	R_2R_2	R_2	R_2R_2	R_2R_2	R_2R_2	R_2R_2	R_1R_2	R_2R_2	R_2R_2	R_2R_2	R_2	R_2R_2	R_2R_2	R_2R_2	R_2R_2	R_2R_2
T_2	3	R_3	R_3	R_3	R_4	R_2	R_2	R_3	R_2	R_3	R_3	R_4	R_3	R_2	R_3	R_3	R_2	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_2	R_3	R_3	R_3	R_3	R_3
T_3	3	R_4	R_4	R_4	R_4	R_4	R_4	R_1	R_3	R_2	R_3	R_2	R_2	R_3	R_2	R_2	R_3	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_3	R_1	R_1	R_1	R_1	R_1
T_4	3	R_3	R_3	R_3	R_3	R_3	R_2	R_3	R_2	R_3	R_3	R_3	R_3	R_1	R_3	R_3	R_1	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_1	R_3	R_3	R_3	R_3	R_3
T_5	3	R_4	R_4	R_4	R_4	R_4	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_6	3	R_4	R_4	R_3	R_3	R_4	R_4	R_2	R_3	R_2	R_2	R_2	R_2	R_3	R_2	R_1	R_3	R_1	R_2	R_1	R_1	R_1	R_1	R_1	R_1	R_3	R_1	R_1	R_1	R_1	R_1
T_7	3	R_2	R_3	R_3	R_3	R_3	R_3	R_3	R_2	R_3	R_3	R_3	R_2	R_1	R_2	R_2	R_1	R_3	R_2	R_2	R_2	R_2	R_2	R_3	R_2	R_1	R_2	R_2	R_2	R_2	R_2
T_8	3	R_3	R_3	R_4	R_4	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_2	R_3	R_3	R_2	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_2	R_3	R_3	R_3	R_3	R_3
T_9	3	R_4	R_4	R_4	R_4	R_4	R_4	R_3	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_{10}	3	R_4	R_4	R_3	R_3	R_4	R_4	R_2	R_3	R_2	R_3	R_3	R_2	R_3	R_2	R_2	R_3	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_3	R_2	R_2	R_2	R_2	R_2
T_{11}	3	R_3	R_3	R_3	R_2	R_3	R_3	R_1	R_2	R_2	R_2	R_2	R_3	R_2	R_2	R_2	R_2	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_2	R_1	R_1	R_1	R_1	R_1
T_{12}	3	R_3	R_4	R_3	R_4	R_2	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_2	R_3	R_3	R_1	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_1	R_3	R_3	R_3	R_3	R_3
T_{13}	3	R_4	R_4	R_4	R_2	R_3	R_4	R_2	R_3	R_2	R_1	R_3	R_3	R_3	R_1	R_1	R_3	R_2	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_3	R_1	R_1	R_1	R_1	R_1
T_{14}	3	R_3	R_3	R_3	R_3	R_4	R_3	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_1	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_1	R_2	R_2	R_2	R_2	R_2
T_{15}	3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_2	R_2	R_2	R_3	R_3	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_1	R_1	R_1	R_1	R_2R_1	R_2	R_1	R_1	R_1	R_1	R_1
T_B	3	R_4	R_3	R_3	R_4	R_4	R_3	R_2	R_2	R_3	R_3	R_2	R_3	R_2	R_1	R_2	R_1	R_2	R_2	R_1	R_1	R_1	R_1	R_1		R_1	R_1	R_1	R_1	R_1	R_1
T_{JN}	3	R_3	R_3	R_3	R_4	R_3	R_4		R_2					R_2			R_2									R_1					

Table 5: Screening of *Trichoderma* spp. against *P. Parasitica* (Average of three replications)

Isolates	Point of Contact (days)	Bell's scale after (days)*																			
		4^{th}				5^{th}				6^{th}				7^{th}				8^{th}			
		P_1	P_6	P_9	P_{10}	P_1	P_6	P_9	P_{10}	P_1	P_6	P_9	P_{10}	P_1	P_6	P_9	P_{10}	P_1	P_6	P_9	P_{10}
T_1	3	R_3	R_3	R_3	R_3	R_2	R_3	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2
T_2	3	R_3	R_3	R_3	R_3	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2
T_3	3	R_4	R_4	R_4	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_4	3	R_3	R_4	R_4	R_3	R_1	R_3	R_3	R_2	R_1	R_2	R_2	R_2	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1
T_5	3	R_4	R_4	R_4	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_6	3	R_4	R_4	R_4	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_7	3	R_3	R_3	R_4	R_3	R_2	R_2	R_3	R_2	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1
T_8	3	R_3	R_3	R_3	R_4	R_3	R_3	R_2	R_3	R_3	R_2	R_2	R_2	R_3	R_2	R_2	R_2	R_3	R_2	R_3	R_2
T_9	3	R_4	R_4	R_4	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_{10}	3	R_4	R_3	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_{11}	3	R_3	R_3	R_3	R_3	R_2	R_2	R_3	R_3	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2
T_{12}	3	R_2	R_4	R_2	R_4	R_1	R_3	R_2	R_3	R_1	R_2	R_1	R_2	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1
T_{13}	3	R_4	R_4	R_3	R_4	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3	R_3
T_{14}	3	R_2	R_3	R_4	R_4	R_2	R_2	R_3	R_2	R_2	R_1	R_2	R_2	R_1	R_1	R_1	R_1	R_1	R_1	R_1	R_1
T_{15}	3	R_3	R_4	R_3	R_3	R_2	R_3	R_2	R_2	R_2	R_3	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2	R_2
T_B	3	R_4	R_4	R_3	R_3	R_3	R_3	R_2	R_2	R_3	R_3	R_1	R_2	R_2	R_2	R_1	R_1	R_1	R_1	R_1	R_1
T_{JN}	3	R_3	R_4	R_3	R_3	R_2	R_4	R_3	R_2	R_2	R_3	R_2	R_2	R_1	R_2	R_1	R_1	R_1	R_1	R_1	R_1

Trichoderma sp. specifically *T. viride* isolates T_7 and T_{12} reached in Class-I stage within 6 days of inoculation against most of the isolates of *P. parasiticaa* and *Pythium* spp. However, based on this information the antagonistic *T. viride,* did not allow an easy selection of isolates as the variability in the antagonistic characteristic within isolate and isolate-pathogen was very high. But the antagonistic isolate T_7 and T_{12} appeared to be a nearly assured choice due to their effectively against *P. parasitica* and *Pythium* spp.

It is well known that there is sufficient selectivity of isolates of *T. viride* in their antagonistic efficiency towards a particular pathogen (Papavizas and Lumsden, 1980; Cook and Baker, 1983). Efforts to use these microbes to control such pathogen gained momentary during last 2 decades (Papavizas, 1981). Bell *et al.* (1982) tested antagonistic activities of *Trichoderma* sp. isolates against different plant pathogen and recorded pathogen-antagonistic interactions. Reports (Elad *et al.*, 1980) showed that while some isolates were highly antagonistic to some pathogen yet there was a clean isolate to isolate variability in the degrees of parasitism. It could be concluded that there is ample scope to control foot rot and leaf rot of betelvine disease through the use of biocontrol agents under field conditions as few antagonistic obtained from the results showed high activity against *P. parasitica* and *Pythium* spp under *in-vitro* conditions. The overgrowth by the antagonist under *in-vitro* conditions may be a good criterion of selecting an antagonist provided the isolate showed uniform performance under *in-vitro* conditions.

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