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Effect of different organic matter sources and *Trichoderma viride* Pers.: Fr. on damping-off of tomato (*Lycopercicum esculentum* L.) var. CO-1 seedlings caused by *Pythium indicum* Bal.

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ABSTRACT: Four different organic matter sources (dried leaf materials of *Azadiracta indica, Madhuca indica, Morinda tinctoria, Peltophorum pterocarpum* and *Thespesia populnea*), tested against *Pythium indicum*, either alone or in combination with *Trichoderma viride*. Application of *T. viride* and *Thespesia* leaf recorded maximum seed germination (100 %), low disease incidence (5.0 %), increased seedling growth (15.6 cm/pl), seedling vigour index (1560), and seedling biomass (23.8 mg/pl) in *P. indicum* inoculated unsteilized soil.

KEY WORDS: Damping-off, integrated control, organic matter source, *Pythium indicum*, *Trichoderma viride*, tomato

INTRODUCTION

Chemical control of plant diseases caused by soil pathogens is difficult, expensive and creates soil pollution. Biocontrol using antagonistic organisms offers a reliable approach either alone or in integration with other disease management practices (Patibanda and Prasad, 2004). There are several reports about the positive (Alice and Venkta Rao, 1986) and negative effects of plant materials on damping-off disease (Sawada *et al.*, 1965; Mitchell, 1979; Martin and Hancock, 1986). The control of plant diseases is more effective, when fungal antagonists and organic matter is integrated than using them separately. *Trichoderma* species are the most frequently studied fungal antagonists in relation to biocontrol of pathogenic fungi. Damping-off disease, caused by several species of *Pythium*, is one of the most wide spread and serious diseases of crops found in different regions of India. In the present study, an attempt was made to control the damping-off of tomato (*Lycopercicum esculentum* L.) var. Co-1 seedlings caused by *Pythium indicum* by integrating *Trichoderma viride* and organic matters of certain common plants under pot culture condition.

MATERIALS AND METHODS

The fungal antagonist, *Trichoderma viride*, obtained for this study from Tamil Nadu Agricultural University, Coimbatore was used as an entagonist. Leaves of *Azadiracta indica* A. Juss., *Madhuca indica* J. Gmelin., *Morinda tinctoria* Roxb., *Peltophorum pterocarpum* (DC) Backer ex K. Ileyne and *Thespesia populnea* (L) Sol. Ex. Serr., were used as organic amendments either alone or in combination with the antagonist. The fungal pathogen (*Pythium indicum*) was isolated from diseased tomato seedlings and identified based on the characters described by Balakrishnan (1948). Its pathogenicity was established on tomato varieties. Liquid inoculum of *P. indicum* was prepared following the procedure of Kauraw and Singh (1982) and added to sterilized and unsterilized soil (10% v/w). *Trichoderma viride* was multiplied in wheat bran/ peat mixture (Sivan *et al.*, 1984) and mixed with sterilized and unsterilized soil (0.5 % w/w).

The green leaves, collected from Neem, *Madhuca*, *Morinda*, *Peltophorum* and *Thespesia* plants, were dried for 24 hours at 80°C, powdered and used. These organic matter sources (OMS) were mixed with soil (at 0.5% w/w level). The experiments were carried out in both sterilized and unsterlized sandy loam soil. Greenhouse studies were carried out (Botanical garden, Department of Botany at Annamalai University) in plastic cups (7 x 5 cm), which were filled with 150 g of sieved (2-mm sieve) sandy loam soil. Both sterilized and unsterilized soil was mixed well with the inoculum of *P. indicum* (10 % v/w) either alone or in combination with various OMS (0.5% w/w). This soil was incubated for 3 days. Then the antagonist (*T. viride*) was mixed thoroughly with pathogen/ OMS treated soil (0.5% w/w). Twenty-five seeds of tomato (variety Co-1) were sown immediately after the application of antagonist (0.5%) and OMS (0.5%) in pathogen inoculated/ uninoculated sterilized and unsterilized soil. Control was maintained parallel to the other treatments at similar conditions. The seedling growth was observed continuously for 20 days. The seed germination and disease incidence was calculated based on the data collected at 5-day interval for 20 days. The seedling length (root + shoot) and biomass were estimated in 20 days old tomato seedling. The seedling vigour indices were calculated by following the procedure suggested by Abdul-Baki and Anderson (1973). Four replications were maintained for each treatment and the experimental data were analyzed statistically (ANOVA).

RESULTS AND DISCUSSION

The seed germination, seedling height, seedling vigour index, biomass production and incidence of damping-off disease were observed and the data were presented in Tables (1-3).

Effect of organic matter sources on tomato seed germination

The results revealed that the OMS tested in unsterilized soil favour the seed germination of tomato at lower concentrations, while it was inhibited at higher concentration as compared to control (Table 1). The inhibitory effect on seed germination may be due to the increased activity of soil saprophytes favoured by excess of organic matter, which leads to accumulation of toxic substances produced by them. However, *Thespesia* leaf amended unsterilized soil shows increasing trend with increasing concentration. On the other hand, the OMS amended in sterilized soil has no significant effect on seed germination with increasing concentration, while it was reduced as compared to control. This could be due to lack of native microbes and the release and accumulation of toxic metabolites from decomposing OMS used. The seed germination was noticed higher in sterilized soil than in unsterilized soil. Further none of the organic matter caused post emergence death of tomato seedling in both soils. Hence, they were used to incorporate with fungal antagonist, *T. viride*, to control damping-off disease in tomato seedlings.

Effect of organic matter sources and *T. viride* on tomato seed germination

Addition of *T. viride* in *P. indicum* inoculated soil promoted the seed germination (Table 2) in both unsterilized (62%) and sterilized (67%) soil as compared to soil inoculated with pathogen alone (46% and 39%, respectively). The increased seed germination in sterilized soil as compared to unsterilized soil indicate that the introduced fungal antagonist was less effective in unsterilized soil as suggested by Liu and Vaughan (1965) due to the presence of native soil microbes. The absence of

Table 1. Effect of organic matter sources on seed germination of tomato seedlings

Sl. no.	Treatment	Soil type	Seed germination (%) Concentration of plant leaf (%)			CD (P = 0.05)	F- value
			0.1	0.5	1.0		
1	Neem leaf	US SS	93 (77.03) 97 (82.84)	84 (66.72) 95 (78.82)	77 (61.45) 92 (73.83)	9.8 14.31	** **
2	*Madhuca* leaf	US SS	89 (71.30) 93 (75.06)	84 (67.25) 94 (77.86)	66 (54.55) 96 (81.88)	9.09 5.19	* NS
3	*Morinda* leaf	US SS	86 (68.29) 92 (74.23)	67 (54.97) 89 (71.09)	45 (42.12) 85 (68.08)	9.9 86.72	* *
4	*Peltophorum* leaf	US SS	100 (89.67) 95 (81.06)	100 (89.67) 100 (89.67)	91 (72.87) 100 (89.67)	7.44 4.91	* NS
5	*Thespesia* leaf	US SS	89 (71.09) 94 (79.70)	91 (72.87) 96 (80.04)	98 (84.07) 97 (82.84)	8.27 5.52	* NS
6	Control (without leaf material)	US SS	82 (65.07) 100 (89.67)			– –	– –

US = Unsterilized Soil SS = Sterilized Soil NS = Non-significant.
*/** = Significance at (P = 0.01) and (P = 0.05) level, respectively;
Figures in parentheses represent arcsine percentage transformation values.

such soil microbes in sterilized soil favoured better growth of introduced fungal antagonist and ultimately promoted the seed germination.

Among the OMS amended in *P. indicum* inoculated soils, neem and *Madhuca* leaf materials increased the seed germination, while others suppressed the same in unsterilized soil. However, in sterilized soil all OMS tested favoured the seed germination as compared to *P. indicum* inoculated soil. Maximum seed germination of 79 per cent (unsterilized soil) and 73 per cent (sterilized soil) was recorded in *P. indicum* inoculated soil amended with neem leaf and *Peltophorum* leaf, respectively (Table 2). Application of OMS and *T. viride* in pathogen inoculated soil, generally, favours higher seed germination (except in sterilized soil amended with *Morinda* leaf) as compared to other treatments (Table 2). Maximum seed germination (100%) was observed in unsterilized soil amended with neem leaf and *Thespesia* leaf material, whereas in sterilized soil, *T. viride* added with *Peltophorum* leaf favours maximum seed germination (79%). The seed germination was generally more in unsterilized soil than in sterilized soil, which may due to the additive role of native saprophytic and antagonistic organism in unsterilized soil on the control of inoculated and native soil pathogenic organisms.

Effect on the incidence of damping-off disease

In *P. indicum* inoculated soil, application of fungal antagonist reduced the damping-off disease incidence in tomato seedlings to 44 per cent in unsterilized soil and 56 per cent in sterilized soil (Table 2) as compared to *P. indicum* inoculated control soil (84% and 89%, respectively).

Organic matter sources (except neem) amended in pathogen inoculated soil favours the incidence of damping-off of tomato in unsterilized soil, while in sterilized soil they shows inhibitory effect (except *Morinda* and *Thespesia*) as compared to control. This might be due to the proliferation of natural soil pathogenic microbes, which was absent in sterilized soil. The reverse condition in both soils

Table 2. Effect of organic matter sources (0.5%) and *T. viride* (0.5%) on seed germination and damping-off of tomato seedlings in *P. indicum* (10 %) inoculated soil

Sl. no.	Treatment	Seed germination (%)		Damping-off incidence (%) #	
		US	SS	US	SS
1	Control (*P. indicum*)	46 (42.70)	39 (33.60)	84 (66.77)	89 (71.09)
2	*T. viride* only	62 (51.98) +34.78	67 (55.03) +71.79	44 (41.53) -47.62	56 (48.46) -37.08
3	Neem leaf	72 (58.13) +56.52	66 (54.55) +69.23	76 (60.80) -09.52	86 (68..29) -03.37
4	*T. viride* + Neem leaf	100 (89.67) +117.39	76 (60.80) +94.87	12 (20.14) -85.71	38 (38.03) -57.30
5	*Madhuca* leaf	79 (62.97) +71.74	63 (53.56) +61.54	86 (68.29) +02.38	83 (65.89) -06.74
6	*T. viride* + *Madhuca* leaf	89 (71.30) +93.48	69 (56.26) +76.92	24 (29.23) -71.43	53 (46.73) -40.45
7	*Morinda* leaf	20 (26.51) -56.52	41 (39.77) +05.13	88 (73.06) +04.76	93 (75.06) +04.49
8	*T. viride* + *Morinda* leaf	96 (80.04) +108.70	54 (47.35) +38.46	69 (56.26) -17.86	76 (61.00) -14.61
9	*Peltophorum* leaf	34 (35.63) -26.09	73 (58.74) +87.18	92 (75.81) +09.52	74 (59.67) -16.85
10	*T. viride* + *Peltophorum* leaf	86 (68.29) +86.96	79 (63.40) +102.56	26 (30.49) -69.05	47 (43.27) -47.19
11	*Thespesia* leaf	36 (36.78) -21.74	64 (53.78) +64.10	85 (68.29) +01.19	89 (71.30) ±00.00
12	*T. viride* + *Thespesia* leaf	100 (89.67) +117.39	73 (58.74) +87.18	05 (11.18) -94.05	32 (34.43) -64.04
	CD (P=0.05)	6.98	10.77	10.09	5.73

Pre- and Post- Emergence Damping-off disease. US= Unsterilized Soil; SS= Sterilized Soil.
* Significance at (P = 0.01) level. (+) OR (-) = per cent change over control.
Figures in parentheses represent arcsine percentage transformation values.

amended with certain OMS might be due to the additive role of toxic metabolites produced by saprophytic microbes in unsterilized soil and by OMS in sterilized soil. On the other hand, integrated application of OMS and *T. viride* in pathogen inoculated soil effectively control damping-off disease incidence as compared to soil treated with *P. indicum* either alone or in combination with *T. viride* or OMS. This might be due to the increased population of antagonistic organisms in response

Table 3. Effect of organic matter sources (0.5 %) and *T. viride* (0.5%) on early growth of tomato seedlings in *P. indicum* (10 %) inoculated soil

Sl. no.	Treatment	Seedling growth (cm / pl)@		Seedling vigour Index		Seedling biomass (mg / pl)	
		US	SS	US	SS	US	SS
1	Control (*P. indicum*)	12.0 (0.0)	10.9 (0.0)	555.5 (0.0)	430.2 (0.0)	12.8 (0.0)	9.3 (0.0)
2	*T. viride*	14.5 (+20.8)	11.7 (+7.3)	896.0 (+61.3)	778.7 (+81.0)	15.3 (+19.5)	13.0 (+39.8)
3	Neem leaf	15.0 (+25.0)	12.3 (+12.8)	1075.8 (+93.7)	810.3 (+88.35)	16.0 (+25.0)	14.3 (+53.8)
4	*T. viride* + Neem leaf	12.9 (+7.5)	14.1 (+29.4)	1290.0 (+132.2)	1070.0 (+148.7)	18.8 (+46.9)	15.3 (+64.5)
5	*Madhuca* leaf	10.8 (-10.0)	12.3 (+12.8)	857.3 (+54.3)	775.8 (+80.3)	7.8 (-39.1)	10.3 (+10.8)
6	*T. viride* + *Madhuca* leaf	14.3 (+19.2)	15.0 (+37.6)	1270.5 (+128.7)	1034.6 (+140.5)	12.5 (-2.3)	11.3 (+21.5)
7	Morinda leaf	11.1 (-7.5)	12.7 (+16.5)	218.9 (-60.6)	518.8 (+20.6)	7.3 (-42.97)	9.5 (+2.2)
8	*T. viride* + *Morinda* leaf	13.1 (+9.2)	16.0 (+46.8)	1257.0 (+126.3)	866.4 (+101.4)	20.0 (+56.3)	10.3 (+10.8)
9	*Peltophorum* leaf	11.3 (-5.8)	13.1 (+20.2)	382.6 (-31.1)	961.1 (+123.4)	8.0 (-37.5)	5.0 (-46.2)
10	*T. viride* + *Peltophorum* leaf	16.7 (+39.2)	13.6 (+24.8)	1438.6 (+158.9)	1071.9 (+149.2)	15.3 (+19.5)	9.0 (-3.2)
11	*Thespesia* leaf	13.9 (+15.8)	13.7 (+25.7)	499.8 (-10.0)	873.9 (+103.1)	13.3 (+3.9)	14.0 (+50.5)
12	*T. viride* + *Thespesia* leaf	15.6 (+30.0)	15.0 (+37.6)	1560.0 (+180.8)	1098.8 (+155.4)	23.8 (+85.9)	19.0 (+104.3)
	CD (P = 0.05)	1.64	1.59	127.4	170.9	1.49	0.99

US = Unsterilized Soil; SS = Sterilized Soil.
@ Root and shoot length of tomato seedlings.
Figures in parentheses (+ or -) represent per cent change over control.

to the addition of organic matter sources (Lumsden *et al.*, 1983; Chung *et al.*, 1988). Among the OMS tested along with *T. viride*, *Thespesia* leaf effectively reduced the damping-off disease incidence in tomato (up to 94%) and is followed by nem leaf (up to 85.71%) in unsterilized soil (Table 2). Further, the control of damping-off disease was observed higher in unsterilized soil than in sterilized soil.

Effect on seedling growth

Treatment with *T. viride*, in pathogen inoculated soil, promote the seedling growth in both unsterilized soil (20.83%) and sterilized soil (7.34%) as compared to *P. indicum* inoculated soils. The seedling growth of tomato was increased more in unsterilized soil than in sterilized soil. All OMS amended in pathogen inoculated sterilized soil promote the seedling growth, while in unsterilized soil the trend was reversed (except in neem and *Thespesia*). Among the OMS, neem and *Thespesia* leaf material favoured maximum seedling growth of tomato in unsterilized soil (15cm/pl) and sterilized (13.7 cm/pl) soil, respectively.

Application of different OMS with fungal antagonist in pathogen inoculated soil increased the seedling growth of tomato as compared to all other treatments. Increased length of tomato seedling by *Trichoderma* species has been reported (Chung *et al.*, 1986) and ascribed to the control of minor pathogens and/or production of growth regulatory factors (Baker, 1988). Further most of the OMS amended in *P. indicum* inoculated soil, either alone or in combination with *T. viride*, favoured the seedling growth more in sterilized soil than in unsterilized soil.

Effect on seedling vigour index (SVI)

The SVI of tomato was increased considerably due to the addition of fungal antagonist in pathogen inoculated unsterilized soil (61.3%) and sterilized soil (81.01%) as compared to *P. indicum* inoculated control soil. Incorporation of OMS with pathogen promoted the SVI in sterilized soil, whereas in unsterilized soil it was inhibited, except in neem leaf and *Madhuca* leaf. However, OMS applied with *T. viride* in pathogen inoculated soil, increased the SVI of tomato many folds (Table 3). It was observed that the SVI of tomato was higher in unsterilized soil than in sterilized soil. Maximum increase of SVI (1560 and 1098.8) was recorded in *P. indicum* inoculated unsterilized and sterilized soil, respectively, amended with *T. viride* and *Thespesia* leaf as compared to control (Table 3).

Effect on biomass production

Biomass production in tomato seedling was promoted by the addition of *T. viride* (Table 3) in pathogen inoculated unsterilized soil (15.3mg/pl) and sterilized soil (13mg/pl) as compared to control (12.8 and 9.3mg/pl, respectively). Incorporation of neem and *Thespesia* leaf material in *P. indicum* inoculated soil increased the seedling biomass of tomato in unsterilized soil, while other OMS decreased the same. On the other hand, all organic matter sources (except *Peltophorum*) tested, favour the biomass production in sterilized soil as compared to control. Application of OMS and *T. viride* in pathogen inoculated soil, increased the biomass production in both soil as compared to control. Maximum seedling biomass (19mg/pl and 23.8 mg/pl) of tomato was recorded in *Thespesia* leaf and fungal antagonist applied pathogen inoculated sterilized and unsterilized soil, respectively (Table 3).

In general, the results of present study revealed that the application of organic matter sources with the fungal antagonist, *T. viride*, could serve as a food base to improve the proliferation of antagonist introduced and quicken the antagonistic activity. This may lead to effective control of damping-off of tomato caused by *P. indicum* and ultimately it promotes the maximum seed germination, seedling growth and biomass production (Chung *et al.*, 1986).

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