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Biocontrol potential of *Trichoderma* spp. against important soilborne diseases of vegetable crops

A.S. KAPOOR*
Department of Plant Pathology, CSK HPKV, Palampur 176 062

ABSTRACT: *In vitro* studies on the efficacy of *Trichoderma* spp. against soilborne pathogens revealed maximum inhibition of mycelial growth by *T. harzianum* against *Rhizoctonia solani*, *Pythium debaryanum, Sclerotinia minor and Fusarium oxysporum* f. sp. *pisi.* Bioagent, *T. viride* showed maximum inhibition of mycelial growth of *S. rolfsii*. Similarly, the culture filtrate of *T. harzianum* showed maximum inhibition of mycelial growth *of R. solani*, *Pythium debaryanum, S. minor, F. oxysporum* f.sp. *pisi* and *S. rolfsii. T. harzianum* inhibited significantly the conidial germination of *F. oxysporum* f.sp. *pisi* and sclerotial germination of *S. rolfsii* as compared to other bioagents. Wheat bran alone or in combination with FYM in the ratio of 1:1 and 1:2 respectively supported maximum multiplication of *T. harzianum* followed by shelled maize cob powder, maize flour and FYM. Wheat bran based formulation as soil application @ 2 g/kg soil was found the best delivery system followed by seed treatment with same formulation @ 2g /kg seed or talc based formulation as seed treatment @ 2g/kg seed in controlling the root rot of pea and collar rot of tomato in pot experiments.

Key words: Biocontrol, *Trichoderma* spp., soilborne diseases, vegetable crops

Soilborne pathogens, *viz., Rhizoctonia solani, Sclerotium rolfsii, Pythium debaryanum*, *Fusarium oxysporum* and *Fusarium solani* are the most destructive pathogens of many vegetables. The extent of damage could be judged from fact that under favourable conditions it would cause total loss of the crop. The management of soilborne pathogens is difficult as compared to foliar diseases. The ability of resting structures of some of these soil borne pathogens i.e. sclerotia in case of *R. solani* and *S. rolfsii* withstand adverse conditions and their wide host range makes them highly successful pathogens. Management of soilborne pathogens with fungicides has been attempted for long time. However, it is difficult to manage these diseases economically with fungicides alone because of their soilborne nature and wide host range.

Management through host resistance is also unsatisfactory in many cases, because of non-availability of resistant sources against these pathogens. Biological control of these pathogens therefore, is an alternative possibility. *Trichoderma* species as a potential biocontrol agent was recognized in the early 1930's yet, after sixty seven years Jutsum (1988) reported that only five per cent biocontrol agents had actually achieved control of the organisms. Most of these failures have been attributed to inappropriate formulations and lack of understanding of the mode of action and ecology of the biological control agents (Baker, 1986).

Mass multiplication, efficacy of the bioproduct and delivery system of bioagents have been considered to be the major limitations in the success of *Trichoderma* spp., in the management of soilborne pathogens of vegetables. The present paper deals with these aspects of biological control.

MATERIALS AND METHODS

Diseased samples were collected from different vegetable growing areas of Himachal Pradesh and isolated *S. rolfsii* and *S. minor* from diseased samples of tomato plants. *F. oxysporum* f.sp. *pisi, P. debaryanum, R. solani* were isolated from infected

*Corresponding author: askapoor@hillagric.ernet.in

pea seedlings. The pure cultures of the test pathogens were made by hyphal tip/single spore methods. Pathogenicity tests of all the isolated pathogens were made on their respective major hosts in pots under polyhouse conditions. Then these were preserved in potato dextrose agar (PDA) slants at 5^0C and revived on PDA as and when required by sub culturing.

Antagonistic activity of bioagents *viz., Trichoderma viride, T. harzianum, T. koningii, T. pseudokoningii* and *T. viride* – strain E against various soilborne pathogens, *viz., F. oxysporum* f. sp. *pisi, S. minor, S. rolfsii, R. solani* and *P. debaryanum* was tested on PDA using dual culture technique described by Huang and Hoes (1976). The cultures were incubated 23 ± 1^0C. Each treatment was replicated thrice. The linear growth of the bioagents and the soilborne pathogens from the centre of the plate was recorded after the control plates were completely covered by the pathogen. Growth inhibition of pathogen by bioagent over control was calculated by using the formula (Vincent, 1947).

Effect of non-volatile metabolites of *Trichoderma* spp. on mycelial growth and Sclerotial germination

Culture broth of bioagents was filtered through a 50 mm Whatman filter paper and this culture filtrate of bioagent thus obtained was evaluated for mycelial growth, spore germination and sclerotial germination inhibition studies.

For mycelial growth studies, culture filtrate of bioagents obtained through filtration was sterilized by autoclaving at 1.1 kg cm^{-2} pressure for 15 minutes and then tested for their antagonistic activity against soilborne pathogens. Five ml of heat sterilized culture filtrate of the bioagent was added to 20 ml of PDA in Petriplates and 3 mm diameter culture discs of 5-7 days old 5 soilborne pathogens were placed at the centre and incubated at 25 +1^0C. The colony size was compared with the control where only PDA was used. Colony diameter and percent inhibition was recorded when the control plates were completely covered with the pathogen.

Spore germination test of *F. oxysporum* f.sp. *pisi* obtained from 10 days old culture was studied by hanging drop method. Spore germination was tested by mixing spores in culture filtrate of bioagents with proper control where spores were mixed in sterilized distilled water. Each treatment was replicated thrice and 10 microscopic fields per replication were used for germination studies. Spore germination was recorded under microscope (10x) after 8h and per cent germination of the spore was calculated.

Fifty sclerotia of *S. rolfsii* were dipped in the culture filtrates of bioagents for 30 minutes and then evaluated for sclerotial germination in sterilized sand in plastic Petriplates. These Petriplates were incubated at 25 ± 1^0C. Sclerotia dipped in sterilized water served as control.

Evaluation of organic substrates for mass multiplication and delivery system of biocontrol agents

Six organic substrates *viz.,* rice husk, wheat bran, FYM, chicken manure, maize flour, shelled maize cobs were evaluated for mass multiplication of *T. harzianum*. The powdered form of these organic substrates were passed through a 2 mm sieve, mixed with water (1:1 ratio w/v) and autoclaved in large graduated (22x2.5cm) test tubes for one hour at 1.1 kg cm^{-2} pressure for two consecutive days. After sterilization and cooling each test tube was inoculated by placing 3 mm diameter mycelial disc of 7 days old culture of *T. harzianum* on top of the substrate under aseptic conditions. The linear growth of the bioagent was recorded on alternate day until the whole tube was covered with the bioagent. These tubes were incubated at 25 ± 1^0C. Each treatment was replicated thrice. The best organic substrate (wheat bran) was then evaluated in different combinations for mass multiplication and standardization of organic based bioagent formulation. Different organic substrates were mixed in 1:1 and 1:2 ratios by weight and filled in graduated test tubes.

Preparation of bioagent formulations and their evaluation

Ten days old culture of *T. harzianum,* multiplied in potato broth was sieved through cheese cloth and spore suspension which has 10^9 cfu / ml was prepared. Talc powder was sterilized at 1.1 kg cm^{-2} pressure for 30 minutes for 2 successive days

and then transferred 1kg of sterilized substrate (talc powder) into a sterilized polythene bag under aseptic conditions and added 400-500 ml of bioagent suspension per bag. Nutritive additive Carboxyl methyl cellulose (CMC) @ 5g/kg of talc substrate was added in it and mixed thoroughly. The produce contained minimum of 2 x 10^8 cfu g^{-1} substrate.

T. harzianum was grown at 25 ± 1^0C for 1 week in potato dextrose broth in 250 ml flasks on a rotary shaker, with 12 hours light and 12 hours darkness/day. The hyphal biomass was strained, rinsed with sterilized water and added 1% aqueous sodium alginate solution. Two gram wheat bran was also added and mixed in mixture. This mixture was then poured drop wise to 0.25 M $CaCl_2$ solution. Pellets formed in calcium chloride solution were removed within 10 minutes, rinsed with distilled water, allowed to air dry on filter paper and stored in glass beaker at 22 ± 1^0C. The pellets were used as one of the delivery systems of bioagents in evaluation of delivery systems of bioagent (s).

The formulations were evaluated in pot experiments against the pathogens *viz., R. solani* and *S. rolfsii*. Field soil was sterilized and filled in plastic pots of 15 cm dia. The pots were inoculated with the *R. solani/S. rolfsii* @ 2g /kg soil and covered with transparent polythene sheets. After 4-5 days, the bioagent was added as wheat bran based formulation as seed treatment @ 2g/kg seed, as soil application @ 2g/kg soil, talc based formulation as seed treatment @2g/kg seed, as soil application @2g/kg soil; Sodium alginate pellet as soil application (400/m^2) and control. As soil application bioagent formulations were added in each pot by mixing top 2-3 cm soil of pots and thereafter added seeds of peas for *R. solani* and tomato for *S. rolfsii.* Data on disease incidence was recorded periodically till the termination of experiment. The treatments were replicated 4 times in RBD.

RESULTS AND DISCUSSION

Mycelial growth inhibition : Maximum mycelial growth inhibition (92.60%) of *R. solani* was caused by the *T. harzianum* followed by *T. pseudokoningii* (68.16%) which was statistically at par with *T. koningii* (65.86%) and *T. viride* (54.06%). *T.viride* – strain E. was least effective (Table 1). *S. rolfsii* inhibition was maximum (54.66%) by *T. viride* followed by *T.viride* – strain E (41.13%) and *T. pseudokoningii* (41.10%) which were statistically at par with each other. *T. koningii* was comparatively less effective (23.60%), whereas, *T. harzianum* (18.03%) was least effective. Maximum inhibition of *P. debaryanum* was found by *T. harzianum* and *T. koningii* (58.53%) followed by *T.viride* – strain E (55.70%) which was statistically at par with both of them. Maximum inhibition of *S. minor* was found by *T. harzianum* (95.20%) followed by *T. viride* (64.10%), *T.viride* – strain E (53.30%) and *T.*

Table 1. Antagonistic activity of *Trichoderma* spp. on soilborne pathogens of vegetable crops

Bioagent	Per cent mycelial inhibition*				
	R. solani	*S. rolfsii*	*P. debaryanum*	*S. minor*	*F. oxysporum* f.sp. *pisi*
Trichoderma harzianum	92.60 (74.62)	18.03 (25.04)	58.53 (49.89)	95.20 (77.37)	71.80 (57.91)
T. viride – strain E	49.96 (44.96)	41.13 (39.86)	55.70 (48.26)	53.30 (46.88)	29.90 (33.03)
T. viride	54.06 (47.32)	54.66 (47.66)	39.50 (38.91)	64.10 (53.28)	28.665 (32.10)
T. koningii	55.86 (48.40)	23.60 (29.05)	58.53 (49.89)	52.66 (46.51)	62.03 (51.08)
T. pseudokoningii	68.16 (56.44)	41.10 (39.84)	47.60 (43.60)	40.80 (39.63)	36.50 (37.10)
CD (P< 0.05)	(15.48)	(3.93)	93.90)	(7.94)	(7.20)

*Average of three replications

Figures in parentheses are arc sine transformed values

koningii (52.66%) which were statistically at par with each other. Maximum mycelial growth inhibition (71.80%) of *F. oxysporum* f. sp. *pisi* was also found by *T. harzianum* followed by *T. koningii* (62.03%). It is evident from these studies that except against *S. rolfsii, T. harzianum* was most effective against all the test pathogens.

All the five biocontrol agents used in the present study showed high antagonistic activity as indicated by inhibition of mycelial growth of pathogens in dual culture experiments, which was further confirmed by inhibiting the mycelial growth, spore germination and sclerotial germination in culture filtrates of these bioagents. Arya and Kaushik (2001) reported maximum inhibition of *F. oxysporum* and *R. solani* (74.7-75.2%) with *G. virens* followed by *T. harzianum* (65.9 – 72.4%). In the present study also *T. harzianum* showed maximum inhibition of *S. minor*, *R. solani* (92.60%) and *F. oxysporum* f. sp. *pisi* (71.80%).

Effect of non-volatile metabolites of *Trichoderma* spp.

Though all the non-volatile metabolites of *Trichoderma* spp. inhibited the mycelial growth significantly, but the maximum inhibition (64.40%) of *R. solani* was achieved by *T. harzianum* and *T. viride* (Table 2). Culture filtrate of *T. viride* caused maximum inhibition of *S. rolfsii* followed by *T. harzianum* (66.63%), *T. pseudokoningii* (51.66%) and *T. viride*-strain E (46.63%). Similarly culture filtrate of *T. harzianum* showed maximum inhibition (52.40%) of *P. debaryanum* followed by *T.viride* – strain E (51.90%) and were statistically at par with each other. Second best was *T. viride* followed by *T. koningii* and *T. pseudokoningii* causing 46.9, 40.9 and 38.9 percent inhibition, respectively. Culture filtrate of *T. harzianum* also inhibited 77.50 per cent mycelial growth of *S. minor* followed by *T. pseudokoningii* (62.80%). *T. viride* – strain E. and *T. viride* both inhibited 57.90 per cent whereas, *T. koningii* was least effective (52.30%).Cultural filtrate of *T. harzianum* was also best in inhibiting mycelial growth of *F. oxysporum* f. sp. *pisi* (69.40%) which was followed by *T. viride* (64.43%), *T. pseudokoningii* (54.43%), *T. koningii* (50.00%) and *T. viride* – strain E (48.86%).

Maximum inhibition of spore germination was shown by *T. harzianum* (44.96%) followed by *T.viride* – strain E (38.70%) and were statistically at par with each other. *T. viride* (17.73%) and *T. koningii* (15.93%) were comparatively less effective whereas, *T. pseudokoningii* (5.03%) was least effective. Similarly though all the bioagents significantly reduced the myceliogenic germination of sclerotia over control but maximum germination inhibition was observed with *T. harzianum* (47.33%) and *T. viride* (42.06%) which were statistically at par with each other.

Kucuk and Kivanc (2003) reported that cultural filtrate of *T. harzianum* T19 had an inhibitory effect against several plant pathogenic fungi. In the present

Table 2. Effect of non-volatile metabolites of *Trichoderma* spp. on soilborne pathogens of vegetable crops

Bioagent	Per cent mycelial inhibition*				
	R. solani	*S. rolfsii*	*P. debaryanum.*	*S. minor*	*F. oxysporum*
Trichoderma harzianum	64.40 (53.36)	66.63 (54.71)	52.40 (46.35)	77.50 (61.67)	69.40 (56.44)
T.viride – strain E	51.06 (45.59)	46.63 (43.04)	51.90 (46.07)	57.90 (49.52)	48.86 (44.33)
T. viride	64.40 (53.36)	71.63 (57.81)	46.90 (43.20)	57.90 (49.53)	64.43 (53.41)
T. koningii	51.96 (46.11)	40.40 (39.44)	40.90) (39.73	52.30 (46.30)	50.00 (44.98)
T. pseudokoningii	56.40 (48.68)	52.66 (45.93)	38.90 (38.53)	62.80 (52.42)	54.43 (47.53)
CD (P< 0.05)	(5.60)	(3.46)	(4.82)	(4.28)	(4.36)

*Average of three replications

Figures in parentheses are arc sine transformed values

studies culture filtrate of *T. harzianum* exhibited 64.40 per cent inhibition against *R. solani* and (66.63 per cent) against *S. rolfsii*. The antifungal effect of the culture filtrate is attributed because of the viridin as a fungistatic metabolite produced in the culture by *Trichoderma* (Chet *et al.,* 1979). Many isolates of *Trichoderma* spp. produce volatile and non-volatile metabolites, which are active against a wide range of fungi (Dennis and Webster, 1971).

Evaluation of different organic substrates for mass multiplication of *T.harzianum*

Evaluation of six different organic substrates for mass multiplication of most effective bioagent *T. harzianum* was done by measuring the mycelial growth. On 18th day relative mycelial growth was observed maximum on wheat bran (93.30%) followed by on shelled maize cob powder (90.63%), maize flour (72.86 %) and FYM (59.10%). Whereas, there was no growth in rice husk and chicken manure (Table 3). The rate of growth of mycelium of *T. harzianum* was maximum in wheat bran (5.18%/ day) followed by a shelled maize cob powder (5.04 % / day) .

The best substrate (wheat bran) for mass multiplication of *T. harzianum* was also evaluated in combination with different organic substrates *viz.,* maize flour, and FYM and rice husk in the ratios 1: 1 and 1: 2. The observations were taken up to 14 days. On 14th day the combination of wheat bran: FYM (1: 1) showed 100% mycelial growth followed by wheat bran: FYM (1: 2) (65.40%), wheat bran: rice husk (1: 1) (59.36%), wheat bran: maize flour (1: 2) (52.06%) and wheat bran: maize flour (1: 1) (51.46%) as compared to wheat bran (62.96%)(Table.4). Rate of mycelial growth was also maximum (7.14 and 4.67%/day) in mixture of wheat bran : FYM (1:1and 1:2). This indicated the enhancement of mycelial growth by supplementing wheat bran with FYM which will increase its economic viability.

Wheat bran substrate has been used for multiplication of antagonists by several workers (Henis *et al.,* 1978; Sivan *et al.,* 1984; Singh, 1991). Panicker and Jeyaranjan (1993) reported FYM as the best medium for mass multiplication of *Trichoderma* spp. followed by wheat bran and rice bran.

Evaluation of delivery systems of *Trichoderma harzianum vis-à-vis* management of root rot of peas and collar rot of tomato in pots under poly house condition

Root rot of peas: Application of wheat bran formulation *of T. harzianum* as soil application @ 2g/kg of soil has led to the hundred per cent

Table 3. Evaluation of organic substrates for the mass multiplication of *Trichoderma harzianum*

Treatment	Relative per cent growth of bioagent*						
	Days after inoculation						
	6	8	10	12	15	18	r/day (%)
FYM	20.00 (4.58)	28.40 (5.40)	31.96 (5.73)	38.63 (6.28)	44.40 (6.72)	59.10 (7.74)	3.8
Wheat bran	23.06 (4.89)	39.96 (6.38)	47.53 (6.95)	54.16 (7.41)	68.86 (8.32)	93.93 (9.70)	5.18
Maize bran	14.63 (3.92)	23.96 (4.87)	35.50 (6.00)	38.16 (6.21)	47.10 (6.89)	72.86 (8.58)	4.05
Shelled maize cob powder	22.63 (4.85)	33.30 (5.85)	41.26 (6.48)	51.96 (7.26)	69.30 (8.36)	90.63 (9.56)	5.04
Chicken manure	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00
Rice husk	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00
CD. (P £ 0.05)	(0.56)	(1.10)	(0.87)	(0.90)	(1.07)	(0.58)	-

*Average of three replications r: Rate of mycelial growth

Figures in parentheses are square root transformed values

Table 4. Evaluation of wheat bran in combination with different organic substrates for the mass multiplication of *Trichoderma harzianum*

Treatment	Relative per cent growth of bioagent*					
	Days after inoculation					
	6	8	10	12	14	r/day (%)
Wheat bran (WB)	18.73 (25.53)	23.56 (28.94)	30.26 (33.32)	52.09 (46.20)	62.96 (52.68)	4.50
WB: maize bran (MB) (1:1)	10.23 (17.85)	17.53 (24.44)	27.20 (31.20)	42.40 (40.54)	61.46 (45.82)	3.68
WB:FYM (1:1)	39.96 (39.17)	48.43 (44.08)	60.56 (51.11)	92.06 (73.96)	100.00 (89.96)	7.14
WB: Rice husk (RH) (1:1)	21.13 (26.55)	31.46 (34.02)	41.16 (39.88)	59.33 (50.40)	59.36 (50.42)	4.24
WB:MB (1:2)	15.10 (22.54)	22.96 (28.55)	32.66 (34.82)	41.16 (39.88)	52.06 (46.16)	3.72
WB:FYM (1:2)	30.86 (33.65)	39.96 (39.13)	53.30 (46.89)	73.90 (59.61)	100.00 (89.96)	7.14
WB:RH (1:2)	33.26 (35.16)	41.16 (39.88)	50.26 (45.13)	61.76 (51.82)	65.40 (53.95)	4.67
CD. (P< 0.05)	(9.90)	(6.96)	(6.65)	((9.12)	(7.99)	-

*Average of three replications r: Rate of mycelial growth

Figures in parentheses are arc sine transformed values

Table 5. Evaluation of delivery system of *Trichoderma harzianum* on the incidence (%) of root rot of pea and collar rot of tomato under polyhouse condition

Delivery system	Pea root rot*		Tomato root rot*	
	Disease incidence	Per cent control	Disease incidence	Per cent control
Wheat bran as seed treatment @ 2g/kg seed	4.15 (1.79)	94.21	29.15 (4.86)	57.58
Wheat bran as soil application @ 2g/kg soil	0.00 (1.00)	100	7.47 (2.49)	89.12
Talc based formulation as seed treatment @ 2g/kg seed	13.82 (3.07)	80.74	20.27 (4.05)	70.50
Talc based formulation as soil application @ 2g/kg soil	44.00 (6.68)	38.69	19.02 (4.43)	72.32
Sodium alginate pellets (400/m^2)	54.92 (7.44)	23.47	30.00 (5.27)	56.34
Control	71.77 (8.49)	-	68.72 (8.21)	-
C.D. (P £ 0.05)	2.07		3.07	

*Average of four replications

Figures in parentheses are square root transformed values

control of disease followed by the wheat bran formulation as seed treatment @ 2g/kg seed with 94.21% (Table 5). Talc based formulation of *T.harzianum* as seed treatment @ 2g/kg seed controlled disease by 80.74 per cent, and as soil application @ 2g/kg soil by 38.69 per cent, whereas sodium alginate pellets (400/m^2) controlled disease by only 23.47 per cent.

Collar rot of tomato: Minimum collar rot disease incidence (7.47%) was observed in wheat bran based formulation applied as soil application @ 2g/kg of soil followed by talc based formulation as soil application @ 2g/kg soil (19.02%) and talc based formulation as seed treatment @ 2g/kg seed (20.27%). Wheat bran formulation as seed treatment @ 2g/kg seed (29.15%) and sodium alginate pellets (400/m^2) formulations were comparatively less effective.

Hadar *et al*. (1979) observed that *T. harzianum* in the form of wheat bran culture reduced the damping off of bean, tomato and brinjal due to *R. solani.* Application of wheat bran preparation of *T. harzianum* brought out an excellent control of damping-off of tomato and egg plant and wilt and root rot of lentil (Elad *et al.,* 1980). *Trichoderma* spp. have been found effective against *S. rolfsii* under *in vitro* and *in vivo* conditions (Pal and Saraswathi, 1986; Das *et al.* 2003; Dutta and Das 2002). .

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