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Mass screening of *Trichoderma* spp. for their antagonism against some plant pathogenic oomycetes fungi

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Abstract
In vitro efficacy of twenty five *Trichoderma* isolates (twenty were TCMS series viz., TCMS 2, 4, 5, 12, 14a, 14b, 15, 16, 24, 32, 34, 36, 43, 60, 62, 64, 65, 72, 85 and 93, and five Th series; Th 1, 3, 14, 19 and 32) were ascertained for their antagonistic activity against few major plant pathogenic oomycetes namely, *Phytophthora infestans, P. parasitica* and *Pythium aphenidermatum* using dual culture technique. *P. infestans* was isolated from infected potato leaves and *Pythium aphenidermatum* from infected brinjal. *P. parasitica* culture was collected from Central Potato Research Institute (CPRI), Simla. The present study was conducted at Biological Control Laboratory, Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar. All the 25 *Trichodrma* isolates were found significantly effective against the test pathogens. TCMS-36 and TCMS-72 were found highly effective against *P. aphinidermatum* with 59.57 per cent inhibition of radial growth of the fungus. Maximum reduction in mycelial growth of *P. infestans* was recorded with isolate TCMS-64 (60.40%) followed by TCMS-65 (59.41%), TCMS-34 (58.42%), TCMS-24, TCMS-43 and TCMS-93 with 57.43 per cent inhibition. While, maximum inhibition of *P. parasitica* was recorded with TCMS-4 (92.75%) followed by TCMS-36 (92.23%), TCMS-2 (91.71%), TCMS-14a (91.17%) and TCMS-32 (90.67%). The selected potential isolates may be applied to sustainable and eco-friendly management of many major crop diseases caused by the oomycetes and other fungi.

Keywords: Antagonism, Bioagent, Dual culture, Management, Oomycetes

INTRODUCTION

Several phyto-pathogenic oomycetes fungi, such as *Phytopthora*, *Pythium*, *Peronospora,* etc. have been causing enormous losses since many decades due to changes introduced in agriculture system which resulted in detrimental effects on economically important crops. Agrochemicals have been used to manage plant diseases in generally, but chemical control has so many drawbacks like resistance development, effects on non target organisms, environmental pollution, food poisoning etc. While, biological control is risk-free, eco-friendly, sustainable and self perpetuating. In general biocontrol agents interfere with the life cycle of pathogens.

Among the micro-organisms fungi are most commonly used as biological control agents to manage soil-borne plant pathogens. Biocontrol fungi are mostly saprophytic and can selfperpetuate abundantly in various kinds of soil (Mukhopadhyay and Mukjerjee, 1998; Singh *et al*., 2001a; Chaube *et al*., 2002). *Trichoderma*, *Gliocladium*, *Penicillium*, *Aspergillus*, *Neurospora*, *Chaetomium*, *Arthobotrys* etc. are among the important genera of fungi used to control plant diseases. For the first time role of *Trichoderma lignorum* was used as biological control agent against citrus seedling disease caused by *Rhizoctonia solani* was implicated (Weindling, 1932). Mycoparasitism is a complex phenomenon, at first bioagent fungus detect host fungus and tropically grow towards it (Chet *et al*., 1981). For establishing contact, *Trichoderma* spp. produces appressorium and then coil around the host fungus (Inbar *et al*., 1996). A hole is formed at the

site of the Appressorium attachment on the target fungus and directs the entry of *Trichoderma* spp. into the lumen of the pathogenicfungus (Rey *et al*., 2001; Freeman *et al*., 2002). *Trichoderma* spp. has different infection patterns including extracellular enzyme production. Diffusion of enzymes in-turn stimulates the target fungus to release cell-wall oligomers, and such oligomers enhance the production of endochitinases from the *Trichoderma* spp. which are toxic to the target fungus (Viterbo *et al*., 2002; Brunner *et al*., 2003). The cell wall degrading enzymes from *Trichoderma* includes β-1, 3-glucanase, β-1, 6-glucanases, proteases, hydrolases, cellulases etc. (Benitez *et al*., 1998). The combined effect of different enzyme, antibiosis and mycoparasitism results in dissolution of the cell wall of target fungus (Mendoza-Mendoza *et al*., 2003). Competition is another general mechanism of biological control. Competition exhibits between bioagent and plant pathogen for water, nutrients, vitamins and space for their growth (Cook and Baker, 1983; Muslim *et al*., 2003). *Trichoderma* spp. are irradiated with ultraviolet light to produce mutants which lacks antibiotic production and mycoparsitism ability (Howell, 2002). Competition at different rhizosphere varies depending on the availability of carbon, nitrogen, sulphur, phosphorus and other nutrient sources (Benitez *et al*., 2004).

Hence, the present experiment was ascertained on mass screening of *Trichoderma* spp. for their antagonism against some plant pathogenic oomycetes fungi in order to screen out the efficient strains of *Trichoderma* as a sustainable alternative method to manage crop diseases caused by the oomycetes.

MATERIALS AND METHODS

Phytophthora infestans and *Pythium aphenidermatum* were isolated from infected potato and brinjal, respectively. *Phytophthora parasitica* isolate was collected from Central Potato Research Institute (CPRI), Simla. *P. aphenidermatum* and *P. parasitica* cultures were grown on potato dextrose agar (PDA) medium at 26±1^{0}C while *P. infestans* was cultured at 16±1^{0}C. The cultures were preserved at 4^{0}C in a refrigerator. For isolation of *Trichoderma* spp. Soil samples were collected from copper mining sites/areas of Uttarakhand (Hence, the isolates were named as TCMS series) and the fungus was isolated on Trichoderma Specific Medium (TSM) using serial dilution and pour plate techniques.

Twenty five *Trichoderma* isolates (twenty TCMS series; TCMS 2, 4, 5, 12, 14a, 14b, 15, 16, 24, 32, 34, 36, 43, 60, 62, 64, 65, 72, 85, 93, and five Th series; Th 1, 3, 14, 19; 32) were ascertained for their antagonistic potential against *P. infestans, P. aphenidermatum* and *P. parasitica* by using dual culture plate technique. The studies were performed in Petri dishes poured with 20 ml PDA. After solidification of medium, a 5.0 mm disc of the pathogen was cut from the edge of an actively growing culture, using a sterile cork borer, and inoculated in the Petri dish about 1.5 cm apart from the edge. Another 5.0 mm disc of a *Trichoderma* spp. was inoculated at the opposite end in the Petri dish at 1.5cm from the edge. The experiment was carried out in three replications. The Petri dishes were incubated at 26±1^{0}C while for *P. infestans* it was incubated at 16±1^{0}C. Observations on the growth of bio-control agent and test fungi were recorded after 72 hours of inoculation. Per cent inhibition of radial growth of test pathogen was calculated by applying the formula:

$$I\ (\%) = \frac{(C - T)}{C} \times 100$$

Where,

I = Per cent inhibition of radial growth of test fungus (pathogen)

C = Radial growth of test fungus in control plate (without *Trichoderma* spp.)

T = Radial growth of test fungus in dual culture plate

RESULTS AND DISCUSSION

Antagonistic potential of 25 *Trichoderma* isolates was carried out against three oomycetes fungi under *in votro* conditions. Most of the *Trichoderma* spp. tested ware found significantly effective on inhibiting the radial growth of target pathogen/s.

Treatments were significantly differed from check and one another in inhibiting the radial growth of *P. aphinidermatum*. TCMS-36 and TCMS-72 were found highly effective against *P. aphinidermatum*. While, Th-14 was found least effective with 31.9% inhibition of radial growth followed by TCMS-62 with 34.04% inhibition (Table 1, Fig 1 and Plate 1). Efficacy of *Trichoderma* spp. against *P. infestans* was in the range of 36.67 to 60.40 per cent. Maximum reduction in hyphal growth was recorded with isolate TCMS-64 (60.40%) followed by TCMS -65 (59.41%), TCMS-34 (58.42%), TCMS-24 (57.43%), TCMS-43 (57.43%) and TCMS-93 (57.43%) (Table 1, Fig 1 and Plate 2). Per cent inhibition of hyphal growth of *P. parasitica* by *Trichoderma* spp. after 72 hr was ranged from 54.92 to 92.75 per cent. Maximum inhibition was recorded with TCMS-4 (92.75%) followed by TCMS-36 (92.23%), TCMS-2 (91.71%), TCMS-14a (19.17%) and TCMS-32 (90.67%) while minimum inhibition in hyphal growth of *P. parasitica* was noticed with TCMS-34 (54.92%), TCMS-72 (60.62%), Th-32 (61.66%) and Th-3 (62.18%) (Table 1, Fig 1 and Plate 3). However the efficacy of tested *Trichoderma* spp. against *P. parasitica* under laboratory conditions was above 50 per

Table 1. Antagonism of *Trichoderma* spp. against some oomycetes.

Sl. No.	*Trichoderma* Isolate	*Pythium spp.*		*P. infestans*		*P.parasitica*	
		Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)
1	TCMS 2	0.77	51.06	2.03	39.60	0.53	91.71
2	TCMS 4	0.93	40.43	1.57	53.47	0.47	92.75
3	TCMS 5	0.80	48.94	2.27	32.67	2.20	65.80
4	TCMS 12	0.73	53.19	2.00	40.59	2.00	68.91
5	TCMS 14a	0.70	55.32	1.70	49.50	0.57	91.19
6	TCMS 14b	0.70	55.32	1.73	48.51	1.47	77.20
7	TCMS 15	0.73	53.19	2.13	36.63	1.07	83.42
8	TCMS 16	0.70	55.32	1.63	51.49	0.87	86.53
9	TCMS 24	0.77	51.06	1.43	57.43	2.27	64.77
10	TCMS 32	0.80	48.94	1.77	47.52	0.60	90.67
11	TCMS 34	0.77	51.06	1.40	58.42	2.90	54.92
12	TCMS 36	0.63	59.57	1.47	56.44	0.50	92.23
13	TCMS 43	1.03	34.04	1.43	57.43	1.83	71.50
14	TCMS 60	0.67	57.45	1.60	52.48	1.00	84.46
15	TCMS 62	1.03	34.04	2.17	35.64	0.70	89.12
16	TCMS 64	0.67	57.45	1.33	60.40	1.47	77.20
17	TCMS 65	0.70	55.32	1.37	59.41	0.93	85.49
18	TCMS 72	0.63	59.57	1.73	48.51	2.53	60.62
19	TCMS 85	0.87	44.68	1.67	50.50	2.30	64.25
20	TCMS 93	0.73	53.19	1.43	57.43	0.83	87.05
21	Th 1	0.80	48.94	1.67	50.50	1.43	77.72
22	Th 3	0.78	50.43	1.97	41.58	2.43	62.18
23	Th 14	1.07	31.91	1.53	54.46	1.13	82.38
24	Th 19	0.87	44.68	1.80	46.53	1.77	72.54
25	Th 32	0.73	53.19	1.53	54.46	2.47	61.66
26	Control	1.57	0.00	3.37	0.00	6.43	0.00
SEm ±		0.32		0.44		0.36	
CD (0.01)		0.12		0.17		0.14	

cent. Isolates TCMS-16, 24, 34, 36, 60, 64, 65, 93 and Th-32 were found significantly effective against all the test fungi (*P. parasitica, P.infestans* and *P. aphinidermatum)* with inhibition of mare than fifty per cent of mycelial growth of the pathogen.

Similar results were reported by the previous workers on the efficacy of *Trichoderma* spp. on many soil borne fungi. *T. harzianum,* and *T. polysporum* reduced the mycelial growth of *R. solani* under laboratory conditions varied from 59.6 to 78.4 % (El-Kafrawy, 2002). *Trichoderma* species were found highly effective on *Sclerotinia sclerotiorum*, with *T. atroviride* the best in reducing fungal growth by 85-93% (Matroudi *et al*., 2009). Sixty two *Trichoderma* isolates were evaluated for their antagonism against soil-borne plant pathogens. Most of the tested *Trichoderma* spp. found highly

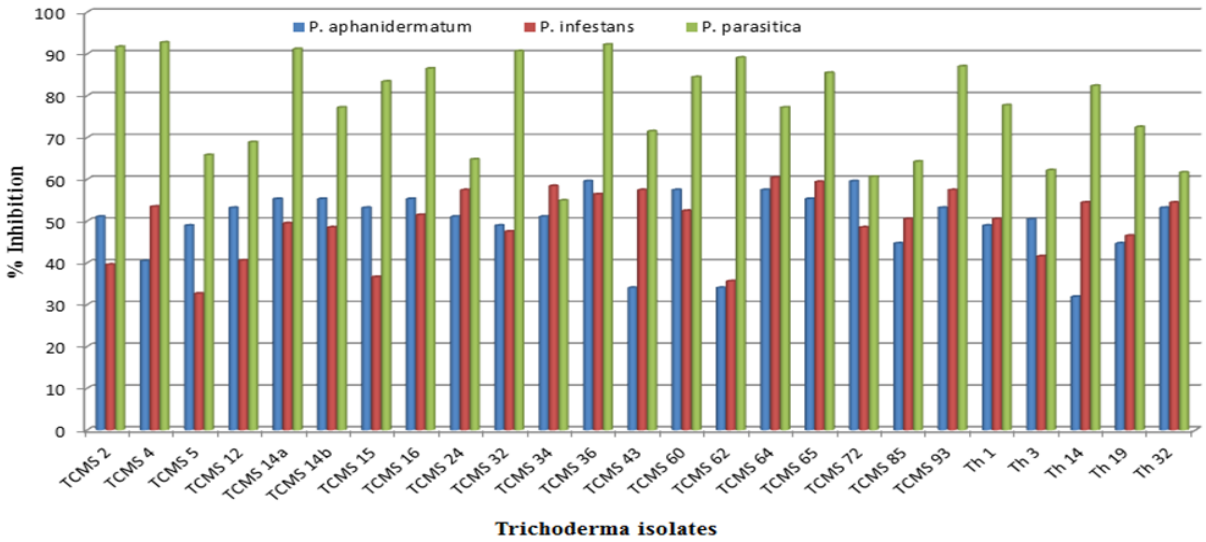

Fig. 1. *Efficacy of Trichoderma isolates against some Oomycetes fungi.*

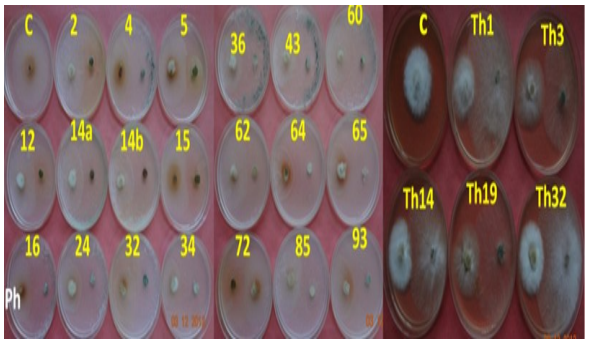

Plate 1. *Antagonism of Trichoderma isolates on Pythium aphanidermatum.*

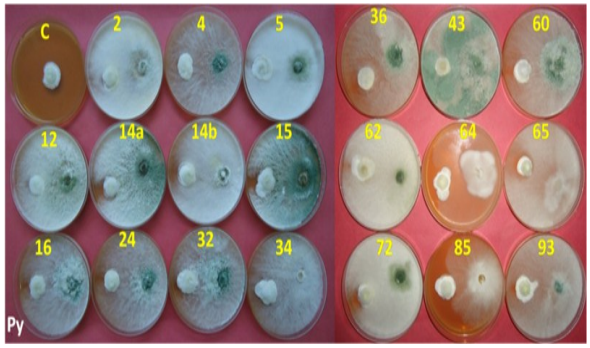

Plate 2. *Antagonism of Trichoderma isolates on Phytophthora infestans.*

significant in inhibiting the growth of the test pathogens (Joshi *et al*., 2010). Five per cent isolates were found efficient against *S. rolfsii* and 13% against *R. solani*, showed above 80% inhibition of mycelial growth. The antagonistic potential of five biocontrol fungi viz., *T. harzianum, B. subtilis, S. noursei, G. roseum,* and *S. natalensis*, was evaluated under *in vitro* against *C. Gloeosporioides* and *C. acutatum* (Svetlana *et al*., 2010). The antagonistic fungi inhibited vegetative growth and germination of conidia of *Colletotrichum* spp. Eighteen *Trichoderma* isolates showed considerable biocontrol potential while, *T. atroviride* was found best (Poornima Sharma, 2011). Antagonist of *Trichoderma harzianum* on *P. aphanidermatum* was more aggressive than *T. viride*. Per cent inhibition among *T. harzianum* isolates was varied only between 80 to 86% (Muthu Kumar and Pratibha Sharma, 2011). Many isolates of *Trichoderma viride*, were screened for their antagonism against several fungal plant pathogens. Among the tested isolates, Tr 8 showed 70, 68.2, 70, 73.3, 69.3 and 70.1 per cent inhibition of *R. solani, S. rolfsii, M. phaseolina, A. alternata, F. solani and C. Capsici,* respectively (Mishra *et al*., 2011). The inhibitory effect of *Trichoderma* spp. may mainly due to cellulolytic activity, antibiosis, mycoparasitism and competition for nutrients and space.

Conclusion

There was a significant difference in antagonism among the tested *Trichoderama* spp. against the

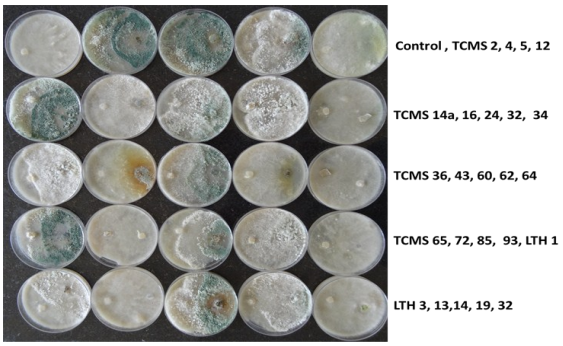

Plate 3. *Antagonism of Trichoderma isolates on Phytophthora parasitica.*

different oomycetes pathogens. Some isolates were found good against all the test pathogens. The range of inhibition by the same set of isolates also varied against *Pythium* and *Phytophthora* species. However, few isolates found most affective against all the test pathogens viz., *P. aphanidermatum, P. infestens* and *P. parasitica.* The selected potential candidates could be further screened in field conditions and can be effectively utilized to manage many plant diseases caused by oomycetes and other fungi (ascomycetes and basidiomycetes) in eco-friendly and sustainable manner.

REFERENCES

1. Benitez, T., Limon, J., Delgado Jarana, J. and Rey, M. (1998). Glucanolytic and other enzymes and their control. *In*: *Trichoderma* and *Gliocladium*, Vol. 2. G.E. Harman and C.P. Kubicek, eds. Taylor and Francis, London. pp. 101-127.
2. Benitez, T., Rincón, A.M., Limón, M.C. and Codón A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains, *Int. Microbiol*. 7: 249-260.
3. Brunner, K., Peterbauer, C.K., Mach, R.L., Lorito, M., Zeilinger, S. and Kubicek, C.P. (2003). The Nag1 *N*-acetylglucosaminidase of *Trichoderma atroviride* is essential for chitnase induction by chitin and of major relevance to biocontrol. *Curr.Genet.* 43: 289-295.
4. Chaube, H.S., Mishra, D.S., Varshney, S. and Singh, U.S. (2002). Biological control of plant pathogens by fungal antagonists: Historical background, present status and future prospects. *Annu. Rev. Plant Pathol*. 2: 1-42.
5. Chet, I., Harman, G.E. and Baker, R. (1981). *Trichoderma harzianum.* its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microb. Ecol.* 7: 29-38.
6. Cook, R.J. and Baker, K.F. (1983). The Nature and Practice of Biological Control of Plant Pathogens. The American Phyto-pathological Society. St. Paul. Minnesota. pp. 539.
7. El-Kafrawy, A. A. (2002). Biological control of bean damping-off caused by *Rhizoctonia solani*. *Egyptian Journal Agricultural Research* 80 (1): 57-70.
8. Freeman, S., Maymon, M., Kirshner, B., Rav, D. and Elad, Y. (2002). Use of GUS transformants of *Trichoderma harzianum* isolate T39 (TRICHODEX) for studying interactions on leaf surfaces. *Biocontrol Sci. Tech.* 12 (3): 401-407.
9. Howell, C.R. (2002). Cotton seedling pre-emergence

damping-off incited by *Rhizopus oryzae* and *Pythium* spp. and its biological control with *Trichoderma* spp. *Phytopathology* 92: 177-180.

10. Inbar, J., Menendez, A. and Chet, I. (1996). Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. *Soil Bio.Biochem.* 28 (6): 757-763.
11. Joshi, B.B., Bhatt, R.P. and Bahukhandi, D. (2010). Antagonistic and plant growth activity of Trichoderma isolates of Western Himalayas Journal of Environmental Biology 31(6): 921-928.
12. Matroudi, S., Zamani, M.R and Motallebi, M. (2009). Antagonistic effects of three species of *Trichoderma* sp. on *Sclerotinia sclerotiorum,* the causal agent of canola stem rot. *Egyptian Journal of Biology,* 11:37-44
13. Mendoza-Mendoza, A., Pozo, M.J., Grzegorski, D., Martinez, P., Garcia, J.M., Olmedo-Monfil, V., Cortés, C., Kenerley, C., and Herrera-Estrella, A. (2003). Enhanced biocontrol activity of *Trichoderma* through inactivation of a mitogen-activated protein kinase. *Proceedings of the National Acacemy of Sciences of the United States of America* 100: 15965-15970.
14. Mishra, B.K., Rohit, Kumar Mishra.. Mishra, R.C. Amit Kumar, Tiwari, Ramesh Singh, Yadav and Anupam Dikshit. (2011). Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiata* L. *Archives of Applied Science Research* 3 (2): 361-369.
15. Mukhopadhyay, A.N. and Mukjerjee, P.K. (1998). Biological control of plant diseases: Status in India. *In*: Biological suppression of phytopathogens, Nematodes and Weeds (Singh, S. P. and Husain, S. S., eds.). Project Directorate of Biological Control, Bangalore.
16. Muslim, A. Horinouchi, H. and Hyakumachi, M. (2003). Biological control of Fusarium wilt of tomato with hypovirulent binucleate *Rhizoctonia* in greenhouse conditions. *Mycosci.* 44:77-84.
17. Muthu Kumar, A. and Pratibha Sharma. (2011). Molecular and morphological characters: Anappurtenance for antagonism in *Trichoderma* spp. *African Journal of Biotechnology* 10(22): 4532-4543.
18. Poornima Sharma (2011). Complexity of *Trichoderma-Fusarium* interaction and manifestation of biological control. *AJCS.* 5(8): 1027-1038.
19. Rey, M., Delgado-Jarana, J. and Benitez, T. (2001). Improved antifungal activity of a mutant of *Trichoderma harzianum* CECT 2413 which produces more extracellular proteins. *Appl. Microbio.Biotech.* 55 (5): 604-608.
20. Singh, U.S., Mishra, D.S., Prasad, R.D., and Chaube, H.S. (2001a). Biological Control of Plant Pathogens in India: Historical perspectives, present status and future prospects. *In*: *Hundred Years of Biological in India* (ed., S. P. Singh), ICAR, New Delhi
21. Svetlana, Z., Stojanović1, S., Ivanović, Ž., Gavrilović, V., Tatjana, P. and jelica, B. (2010). Screening of antagonistic activity of microorganisms against C*olletotrichum acutatum* and C*olletotrichum gloeosporioides*.*Arch. Biol. Sci*., Belgrade 62 (3): 611-623.
22. Viterbo, A., Ramot, O., Chernin, L. and Chet, I. (2002). Significance of lytic enzymes from *Trichoderma* spp. in the biocontrol of fungal plant pathogens. *Anton. Leeuw.* 81:549-556.
23. Weindling, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology* 22: 834-845.