J. Biol. Control, 16(1): 65-69, 2002

Integrated application of *Trichoderma viride* Pers.: Fr. and farm yard manure to control damping-off of tomato [*Lycopersicum esculentum* Mill.]

R. NEELAMEGAM * and T. GOVINDARAJALU
Department of Botany, Annamalai University
Annamalai Nagar 608 002, Tamil Nadu, India

ABSTRACT: **In the present study, efforts were made to control the damping-off of tomato caused by *Pythium indicum* by using *Trichoderma viride* alone or in combination with farm yard manure (FYM). *T. viride* effectively controlled the disease in both sterilized and unsterilized soil than FYM when treated alone. But the combined treatment of *T. viride* and FYM showed better results in controlling the disease with concominent increase in seedling length and biomass of tomato.**

KEYWORDS: Damping off, farm yard manure, integrated control, *Pythium indicum*, tomato, *Trichoderma viride*

Tomato (*Lycopersicum esculentum* Mill.) is affected by various diseases and the most serious one is the damping off caused by several species of *Pythium*, which accounts for severe crop loss. Due to wide host range, soil borne nature and prolonged survival of propagules in soil by saprophytic growth and by resistant resting structures which persist for many years (Hendrix and Campbell, 1973), the direct control of *Pythium* spp. is difficult and expensive (Hendrix and Powell, 1970). The increasing awareness on possible deleterious effects of pesticides on ecosystem, growing interest in pesticides free agricultural products, the biological control of plant pathogenic fungi has received considerable attention (Mehrotra *et al.*, 1997). This helps to attain better plant growth and yield by the destruction of the resting propagules of many soil borne pathogens without any environmental hazard (Papavizas and Lumsden, 1980). The last two decades witnessed a flurry of sustained research activity on biocontrol using several species of *Trichoderma* (Lewis and Lumsden, 1995; Bagyaraj and Govindan, 1996; Aggarwal *et al.*, 1999). There is not much information on the biocontrol of *Pythium indicum* with organic matter amendment integrated with an antagonist. The objective of the present investigation is to control the damping off disease of tomato caused by *Pythium indicum* by using *Trichoderma viride* alone or in combination with farm yard manure under pot culture conditions.

MATERIALS AND METHODS

In the present study, *Trichoderma viride*, obtained from Tamil Nadu Agricultural University, Coimbatore was used as an antagonist in the exzperiment. Farm yard manure (FYM) was used as organic amendment alone or in combination with

* Department of Botany, S.T. Hindu College, Nagercoil 629 002, Tamil Nadu, India

the antagonist. The test pathogen, Pythium *indicum* was isolated from diseased tomato seedlings (Isolate P_3), and identified based on the characters described by Balakrishnan (1948) and its pathogenicity was established.

Preparation of *P. indicum* Inoculum

Liquid inoculum of *P. indicum* was prepared following the procedure of Kauraw and Singh (1982). A homogenous suspension of the fungal hyphae was obtained by macerating the mycelial mat with sterile distilled water in a warring blender (5 Petri-plates 90mm size, each having 15ml of Potato Dextrose Agar Medium and full growth of fungus mixed in one liter of sterile distilled water). This inoculum was added to sterilized and unsterilized soil at 100ml / kg.

Preparation of *T. viride* inoculum

Trichoderma viride was multiplied in Wheat bran / Peat mixture (Sivan *et al.*, 1984). The medium contains Wheat bran and Peat soil at 1:1 ratio (w/w). The moisture content of the medium was adjusted to 40 percent (w/w). This substrate mixture was sterilized in 500ml Ehrlenmeyer flasks for one hour at 20-lb. pressure on three successive days. Then the substrate mixture was inoculated with 8mm discs of *T. viride* and incubated in an illuminated chamber for 14 days. This preparation was mixed with sterilized and unsterilized soil at 5 g / kg (0.5 %) level.

Greenhouse studies were carried out in plastic cups (7x 5cm), which were filled with 150g of sieved (2mm sieve) sandy loam field soil. The soil was mixed well with the inoculum of pathogen (*P. indicum*) and/or FYM (100ml/Kg soil and 1g/Kg soil, respectively) either alone or in combination. This soil was incubated for 3 days. Then the antagonist (*T. viride*) was mixed thoroughly with pathogen/ FYM treated soil at the level of 50g/Kg soil. Control was maintained parallel to the other treatments at similar conditions. Water was sprayed regularly for maintaining proper moisture in the soil.

The tomato variety, Co-1 was selected for this work, since it is a susceptible to damping - off disease. Twenty-five seeds were sown immediately after the application of antagonist (0.05%w/w)/ organic matter (0.1%w/w) in soil. The seedlings were watered regularly as per requirement. The seedlings were observed continuously for 20 days to record the germination and disease incidence was calculated based on the data collected at 5day interval for 20 days. The seedling length and biomass were estimated in 20 days old tomato seedling. Four replications were taken for each treatment and various combinations of treatments were tried.

The above experiments were carried out in both sterilized and unsterlized sandy loam soil which was collected from agricultural experimental field, Faculty of Agriculture, Annamalai University, Annamalainagar. The soil was dried in shade, stored in bulk and used for raising the seedlings. Soil characteristics were as follows: sand (63.1%), silt (23.7%), clay (11.5%) and organic matter (1.3%); pH 7.3, water holding capacity (49.5%); Nitrogen (N) 14 µg/g, Phosphorus (P) 9µg/g and Potasium (K) 31µg/ g.

Experimental data were statistically analysed following Duncan's Multiple Range Test (DMRT) and one way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Effect on seed germination

The maximum germination was recorded in soil treated with *T. viride* and *T. viride* in combination with FYM (Table 1). The germination was significantly more in sterilized soil than in unsterilized soil treated with *T. viride* and FYM either alone or in combination with *P. indicum*. This might be due to the presence of native microbes which decreased the active growth of *P. indicum*, whereas in sterilized soil the activity of *P. indicum* increased unchecked and ultimately reduced seed germination. Singh and Reddy (1979) recorded more population of *Pythium* species in sterilized soil than in unsterilized soil. In *P. indicum* inoculated soil, the introduction of *T. viride* generally promoted the germination of tomato seeds, however the seed

germination was more in sterilized soil (67%) than in unsterilized soil (62%). This indicates that *T. viride* was less effective in unsterilized soil as suggested by Liu and Vaughan (1965) due to the presence of native soil microbes. The absence of such soil microbes in sterilized soil favoured better growth of antagonist and ultimately promoted seed germination.

Addition of FYM in *P. indicum* inoculated unsterilized soil resulted in low seed germination (41%) in comparison to sterilized soil (53%). However, the integrated application of *T. viride* and FYM still increase the seed germination in unsterilized soil to 100 percent as against 84 percent in sterilized soil (Table 1). This might be due to the additive role of native saprophytic and antagonistic organisms in unsterilized soil on the control of inoculated and native pathogenic organisms. Several workers reported that the organic matters are known to stimulate the resting structures of parasitic fungi (Chinn *et al.*, 1953; Mitchell and Alexander, 1961) and at the same time enhanced the activity of other soil microflora including antagonists. The increased population of *Trichoderma* by incorporation of organic matters was reported by Khan *et al.* (1974) and Mc Fadden and Sutton (1975).

Effect on damping off of tomato

As compared to sterilized soil (89%), the disease incidence was low in *P. indicum* inoculated unsterilized soil (84%). This increased activity of

Table 1. Effect of integrated application of *T. viride* and FYM on the control of damping-off incidence and seedling growth in tomato

Sl. No.	Seed treatment	Seed germination (%)		Damping- off (%)*		Seedling length (cm/plant)		Biomass (mg/ plant on dry weight basis)	
		US	SS	US	SS	US	SS	US	SS
1	Control	82 (65.07)	100 (89.67)	0.00 (00.00)	0.00(00.00)	15.2	12.1	13.5	11.3
2	*P. indicum*	46 (42.70)	39 (33.60)	84 (66.77)	89 (71.09)	12.0	10.9	12.8	09.3
3	*T. viride*	98 (84.07)	100 (89.67)	0.00 (00.00)	0.00 (00.00)	15.9	14.7	20.8	19.3
4	Farm Yard Manure	78 (62.15)	100 (89.67)	0.00 (00.00)	0.00 (00.00)	09.7	15.5	13.3	17.2
5	*T. viride* + FYM	84 (66.77)	91 (72.87)	0.00 (00.00)	0.00 (00.00)	15.4	13.5	20.3	17.5
6	*P. indicum* + *T. viride*	62 (51.98)	67 (55.03)	44 (41.53)	56 (48.46)	14.5	11.7	15.3	13.0
7	*P. indicum* + FYM	41 (39.80)	53 (46.73)	79 (62.89)	81 (64.24)	12.5	14.8	10.8	15.0
8	*P. indicum* + *T. viride* + FYM	100 (89.67)	84 (66.77)	14 (21.71)	41 (39.77)	15.5	16.8	21.5	16.8
	CD (P = 0.05)	9.09	6.80	7.23	4.35	0.93	0.93	1.13	0.93

US — Unsterilized Soil; SS —Sterilized Soil;

* Pre- and Post- emergence damping - off disease

Figures in parentheses represent arcsine transformed values.

native saprophytic soil microflora could be due to the presence of native antagonistic microbes. The increased disease incidence in sterilized soil might be due to the absence of competing soil microflora.

Addition of *T. viride* either alone or in combination with FYM in *P. indicum* inoculated soil effectively reduced the disease incidence in unsterilized soil (44% and 14%, respectively) as compared to sterilized soil (56% and 41%, respectively). This might be due to the increasing population of antagonistic organisms in response to the addition of organic matter (Lumsden *et al.*, 1983; Chung *et al.*, 1988).

Effect of different treatments on tomato seedling growth

Inoculation of *P. indicum* reduced the seedling length and biomass of tomato probably by the production of toxic metabolites while introduction of *T. viride* / with FYM promoted the length and biomass of seedling as compared to pathogen inoculated soil. This was more in unsterilized soil than in sterilized soil. Increased growth of plants by *Trichoderma* species has been reported as an added advantage (Chung *et al.*, 1988). However, introduction of FYM in pathogen inoculated and uninoculated unsterilized soil shows a reduction in the seedling length and biomass. The integrated application of *T. viride* with FYM, generally, promotes the seed germination, seedling length and biomass of tomato and ultimately minimizes the damping - off disease incidence.

The results, thus, indicate that the integration of FYM with *T. viride* can serve as an excellent food base to improve the proliferation and antagonistic activity of *T. viride*. This may lead to effective biological control of damping- off of tomato caused by *P. indicum*.

ACKNOWLEDGEMENTS

The senior author is grateful to University Grants Commission, New Delhi, for the financial support. The author also extend sincere thanks to Dr. T. Govindarajalu, the Principal Investigator of the Project, Annamalai University, Tamil Nadu, for his valuable guidance.

REFERENCES

Aggarwal, A., Kumar, J., Sharma, D. and Mehrotra, R. S. 1999. Biological control of root-rot of sunflower using *Trichoderma harzianum* and a mycorrhizal fungi. *Journal of Indian Botanical Society*, **78**: 87-90.

Bagyaraj, D. J. and Govindan, M. 1996. Microbial control of fungal root pathogens, pp 293-321. In: K.G. Mukerji (Ed.). Advances in Botany. APH Publishing Corporation, New Delhi.

Balakrishnan, K. A. 1948. South Indian Phycomycetes-1. *Pythium indicum* sp. nov. causing a fruit rot of *Hibiscus esculentus* L. *Proceedings of Indian Academy of Science*, **27**: 161-173.

Chinn, S. H. F., Ledingham, R. J., Sallans, B. J. and Simmonds, P. M. 1953. A mechanism for the control of common root-rot of wheat. *Phytopathology*, **43**: 701.

Chung, Y. R., Hoitink, H. A. H. and Lipps, P. E. 1988. Interactions between organic matter decomposition level and soil borne disease severity. *Agricultural Ecosystem Environment*, **24**: 183-193.

Hendrix, F. F. and Campbell, W. A. 1973. *Pythium* as plant pathogens. *Annual Review of Phytopathology*, **11**: 77-98.

Hendrix, F. F. and Powell, W. M. 1970. Control of root pathogens in peach decline sites. *Phytopathology*, **60**: 16-19.

Kauraw, L. P. and Singh, R. S. 1982. Effect of organic amendment of soil on the incidence of root rots of wheat. *Indian Journal of Mycology and Plant Pathology*, **12**: 271-277.

Khan, M. W., Khan, A. M. and Saxena, S. K. 1974. Rhizosphere fungi and nematodes of egg plant as influenced by oil cake amendments. *Indian Phytopathology*, **27**: 480-484.

Lewis, J. A. and Lumsden, R. D. 1995. Do pathogenic fungi have the potential to inhibit biocontrol fungi? *Journal of Phytopathology*, **143**: 585-588.

Liu, S. D. and Vaughan, E. K. 1965. Control of infection in table beet seedlings by antagonistic microorganisms. *Phytopathology*, **55**: 986-989.

Lumsden, R. D., Lewis, J. A. and Papavizas, G. C. 1983. Effect of organic amendments on soil borne plants diseases and pathogen antagonists, pp 51-70. In: W. Lockeretz (Ed.). *The environmentally sound agriculture.* Praeger Press, New York.

Mc Fadden, A. G. and Sutton, J. C. 1975. Relationships of populations of *Trichoderma* spp. in soil to disease in maize. *Canadian Journal of Science*, **55**: 579-586.

Mehrotra, R. S., Aneja, K. R. and Aggarwal, A. 1997. Fungal control agents, pp. 111-138. In: Nancy A. Recheigl and Jack E. Recheigh (Eds.). *Environmentally Safe Approaches to Crop Disease Control.* CRC Press Boca Raton Florida.

Mitchell, R. and Alexander, M. 1961. The mycolytic phenomenon and biological control of *Fusarium* in soil. *Nature*, **190**: 109-110.

Papavizas, G. C. and Lumsden, R. D. 1980. Biological control of soil borne fungal propagules. *Annual Review of Phytopathology*, **18**: 389-413.

Singh, R. S. and Reddy, C. S. 1979. Suppression of damping-off of tomato and seedling blight of chickpea and sugarbeet by strains of *Streptomyces diastalicus. Indian Phytopathology*, **32**: 374-377.

Sivan, A., Elad, Y. and Chet, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum. Phytopathology*, **74**: 498-501.