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Compatibility of *Trichoderma viride* for Selected Fungicides and Botanicals

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ABSTRACT

Trichoderma viride can thrive in diverse environmental conditions as aggressive colonizers of soil and the roots of plants and act as natural bioagent to protect plants from infection by soil-borne fungal pathogens. Laboratory experiments were conducted to test the possibility of combining fungicides and botanicals with *Trichoderma viride* to work out their compatibility to devise a suitable integrated management of soil borne plant diseases. Five fungicides viz., dithane M-45, ridomil, captaf, blue copper, bavistin and five botanicals viz., *Parthenium hysterophorus, Urtica dioeca, Cannabis sativa, Polystichum squarrosum* and *Adiantum venustum* were evaluated at different concentration. Among fungicides only captaf and blue copper had recorded compatiblility to some extent with *T. viride*. While the water extracts of the tested botanicals were quite compatible with *Trichoderma* except for *C. sativa,* which have some inhibitory effect on the growth of pathogens. Present investigation suggests that compatible fungicides and botanicals can be used with *Trichoderma* in an IDM package to control soil borne plant pathogens.

Key words: *Trichoderma viride*, botanicals, fungicides, biocontrol, IDM

INTRODUCTION

Soil-borne diseases are consequence from the reduction of biodiversity of soil antagonistic organisms. Fungicide applications to soil, kills important beneficial fungi and also weakens the natural antagonistic activity (Lenteren and Woets, 1988). Inspite of well known side effects of chemicals on environment, they are continuously used to control soil borne plant pathogens. To reduce the use of pesticides, biological control method has been considered as more natural and environmentally acceptable approach (Bagwan, 2010). Several species of *Trichoderma* are well documented mycoparasites and have been used successfully against certain pathogenic fungi. *Trichoderma* strains are the key antagonists for the eco-friendly management of plant diseases. Significant growth inhibition by *Trichoderma* has been reported for *Armillaria mellea* (Tapwal *et al.*, 2004), *Dematophora necatrix* (Tapwal *et al.*, 2005), *Phytophthora cinnamomi* (Singh *et al.*, 2010), *Fusarium oxysporum* and *Rhizoctonia solani* (Dar *et al.*, 2011), *Sclerotium rolfsii* (Jegathambigai *et al.*, 2010) and *Fusarium oxysporum* f.sp. *psidii* (Jegathambigai *et al.*, 2009; Srivastava *et al.*, 2011). Many other workers (Salehpour *et al.*, 2005; Abdollahzadeh *et al.*, 2006; Mir *et al.*, 2011; Osman *et al.*, 2011) utilised *Trichoderma* species as a potential biological control agent. In an IDM package, incorporation of natural products provides a viable solution to the environmental problems caused by synthetic pesticides. Identification of these compounds and their further testing may be an effective approach to minimise the use of hazardous chemicals (Duke, 1990).

To develop an effective disease management programme, the compatibility of potential bioagents with fungicides and botanicals is essential. Combination of chemicals and compatible bioagents in an IDM strategy protects the seeds and seedlings from soil-borne and seed-borne inoculum (Dubey and Patil, 2001). Integration of compatible bioagent with pesticides, may enhance the effectiveness of disease control and provide better management of soil borne diseases (Papavizas and Lewis, 1981). The combination of biological control agents with fungicides would provide similar disease suppression as achieved with higher fungicide use (Monte, 2001). Combining antagonists with synthetic and non synthetic chemicals eliminates the chance of resistance development and reduces the fungicide application. In view of this, laboratory experiments were conducted to test the possibility of combining *Trichoderma viride* with fungicides and botanicals. The long term goal is to develop an effective IDM package for managing soil borne plant diseases as well as to prevent the resistance development in pathogens to chemicals. Integrating chemical resistant *Trichoderma* strains has an importance in the framework of integrated disease management. Disease prevention can be increased by using such tolerant strains that keeps pathogens under sufficient pressure so that they cannot thrive.

MATERIALS AND METHODS

Pure culture of *T. viride* was collected from the Department of Botany, Shoolini Institute of Life Sciences and Business Management, Solan, Himachal Pradesh. Compatibility tests were conducted under *in vitro* condition to find out safer fungicides and botanicals against *Trichoderma*. Five fungicides viz., Dithane M-45, Ridomil, Captaf, Blue Copper, Bavistin and were evaluated against *Trichoderma* by food poisoning technique. Fungicides were added to molten PDA just before pouring from the common stock solution to get final concentrations of 50, 100, 200, 300 ppm, respectively. *Parthenium hysterophorus, Urtica dioeca, Cannabis sativa, Polystichum squarrosum* and *Adiantum venustum* were collected from the undisturbed habitats of Solan district, Himachal Pradesh (India). Fresh leaves of healthy plant species were washed thoroughly with tap water and air dried. One hundred grams of plant tissue was ground using pestle and mortar by adding equal amount (100 mL) of sterilized distilled water (1:1 w/v). The pulverized mass was squeezed through cheese cloth and the extracts were centrifuged at 10000 rpm for 5-10 min. The supernatant was filtered through millipore filters (45 μm) using vacuum pump assembly under aseptic conditions to avoid contamination. A requisite amount of the filtrate was mixed in PDA just before pouring to get desired concentrations of 5, 10, 15 and 20% and gently shaken for thorough mixing of the extract.

The PDA plates amended with fungicides and plant extracts were inoculated aseptically with *Trichoderma* by transferring five mm diameter agar disc from fresh cultures. Three replications were maintained for each treatment. Unamended PDA served as the control. Inoculated petri plates were incubated at 25±1°C. The radial growth of *T. viride* was measured in all treatments after three days and compared with control. The percent growth inhibition of pathogen was estimated by using the formula following Vincent (1947) and converted into percent compatibility:

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

Where:
I = Percent growth inhibition
C = Colony diameter in control
T = Colony diameter in treatment

The data was recorded in triplicates and subjected to statistical analysis and conclusions were drawn on the basis of analysis of variance. The calculated value of F was compared with the tabulated values at 5% level of significance for an appropriate degree of freedom.

RESULTS AND DISCUSSIONS

Laboratory experiments were conducted to observe the compatibility of *T. viride* with fungicides and botanicals. The results revealed that at the selected concentrations of fungicides, only blue copper and Captaf were compatible to some extent (Table 1). The compatibility index of Blue copper with *T. viride* at different concentrations ranged in between 34.9-97.9%, followed by Captaf (16.7-25.0%). The percent compatibility decreased with an increase in the concentration of fungicide. *Trichoderma viride* was not compatible with Dithane, Bavistin and Ridomil in any level of selected concentration. The statistical analysis revealed that only blue copper has recorded significant differences in comparison to control and Captaf (SEM± = 3.10, CD (p = 0.05) = 9.31). Bagwan (2010) reported that thiram (0.2%), copper oxychloride (0.2%) and mancozeb (0.2%) are compatible with *Trichoderma harzianum* and *Trichoderma viride*. *Trichoderma* was most sensitive to captan, tebuconazole, vitavax, propiconazole and chlorothalonil. In a similar study, *T. harzianum* was found highly sensitive to mancozeb, tebuconazole and thiram, less sensitive to benomyl, triadimenol and dichlofluanid are relatively insensitive to procymidone and captan (Mclean *et al*., 2001). In the Present study, *Trichoderma* was found insensitive to blue copper and captaf and highly sensitive to dithane, bavistin and ridomil.

Botanicals are an important component of IPM. The aqueous extracts of tested plant species were quite compatible with *T. viride* (Table 2). The results revealed that extracts of *Parthenium, Adiantum* and *Urtica* recorded absolute compatibility at tested concentrations. The results revealed that extracts of *Parthenium, Adiantum* and *Urtica* recorded absolute compatibility at tested concentrations. This is followed by *Polystichum* recorded 100% compatibility at 5% concentration of aqueous extract and 90-95% compatibility at 10-20 concentration. The minimum compatibility was observed by *Cannabis* in the range of 40-77.5% at different concentration of phytoextract. The percent compatibility decreased with increase in the concentration of phytoextract. The statistical analysis revealed that the only *Cannabis* had recorded significant differences (Sem± = 0.96, CD (p = 0.05) = 2.89). Leaf extract of *Parthenium, Urtica* and *Adiantum*, were found effective against

Table 1: *In vitro* compatibility of selected fungicides with *T. viride*

	Compatibility (%) of *T. viride* with fungicides at different concentrations			
Fungicides	50 ppm	100 ppm	200 ppm	300 ppm
Dithane	0.0	0.0	0.0	0.0
Captaf	25.0	22.2	16.7	16.7
Bavistein	0.0	0.0	0.0	0.0
Ridomil	0.0	0.0	0.0	0.0
Blue copper	97.9	58.1	50.9	34.9

SEM±: 3.10, CD (p = 0.05): 9.31

Table 2: *In vitro* compatibility of selected botanicals with *T. viride*

	Compatibility (%) of *T. viride* with botanicals (leaf extract) at different concentrations			
Botanicals	5%	10%	15%	20%
C. sativa	77.5	57.5	45.0	40.0
P. hysterophorus	100.0	100.0	100.0	100.0
A. venustum	100.0	100.0	100.0	100.0
P. squarrosum	100.0	95.0	90.0	90.0
U. dioeca	100.0	100.0	100.0	100.0

SEM±: 0.96, CD (p = 0.05): 2.89

A. solani, A. zinnia, R. solani, F. oxysporum and *C. lunata* (Tapwal *et al*., 2011). Leaf Extracts of *Parthenium, Adiantum* and *Urtica* also showed absolute compatibility with *Trichoderma* in the present study. Similarly, Vanitha (2010) reported that wintergreen oil, lemongrass oil and their combination under *in vitro* conditions did not inhibit the growth of *Trichoderma*.

Antagonistic activity of biocontrol agents might be effective if it is integrated with other control practice and may result in acceptable levels of disease control with reduced level of chemicals use (Latorre *et al*., 1997). The present investigations provide evidence for the compatibility of *Trichoderma* with synthetic and natural chemicals. Curl *et al*. (1976) were of opinion that combined application of PCNB with *T. harzianum* effectively controlled *Rhizoctonia solani* in cotton seedlings than *T. harzianum* alone in greenhouse studies. Similar report of integration of biological agent and chemicals was reported by Henis *et al*. (1978).

Besides having great antagonistic potential, *Trichoderma* has the capability of degradading xenobiotic compounds and can survive in environments with remnants of fungicide molecules (Chaparro *et al*., 2011).

CONCLUSION

Present finding indicates that seed treatment or soil application of *Trichoderma* would be compatible with blue copper fungicide and plant extracts viz., *Parthenium*, *Adiantum* and *Urtica* for the integrated management of soil borne diseases. *T. viride* can be combined with seed treatment fungicides like blue copper and captaf at lower concentrations. Our future studies are directed to determine the compatibility of *Trichoderma* and chemicals in managing soil borne diseases of various crops under greenhouse and field conditions. Long term goal is to develop an integrated disease management strategy by combing *Trichoderma* and chemicals so as to prevent pathogen from gaining resistance as well as in building up of *Trichoderma* population levels in the soil that will be effective on a long term basis.

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