Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7333

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (3):** 44-51 (2019)

Research Article

Compatibility of *Trichoderma viride* with Fungicides for Plant Disease Management

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Received: 5.03.2019 | Revised: 13.04.2019 | Accepted: 24.04.2019

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 with T. viride to work out their compatibility to devise a suitable integrated management of soil borne plant diseases. A set of two isolates of T. viride namely T_1 and T_2 were used to evaluate compatibility with eight fungicides at five different concentrations. In-vitro compatibility study of Trichoderma spp with Mancozeb 75%WP revealed that this fungicide is compatible with Trichoderma viride upto 50 ppm. However, above 50 ppm concentration it started inhibiting the growth of both the isolates of T. viride. With thiram 75%WP, the maximum average per cent inhibition of 69.26 per cent was recorded at 1000 ppm which shows 30.74 per cent compatibility of Trichoderma with Thiram 75%WP at 1000 ppm concentration. However, Chlorothalonil 75%WP showed 75.29% inhibition in growth of T. viride at 1000 ppm concentration. Further, Tridemorph and thiophanate methyl showed only 100 % inhibition in growth of T. viride at 1000 ppm fungicide concentration. In-vitro compatibility study of Carbendazim 50%WP, propiconazole 25%EC and Hexaconazole 5%EC revealed that these fungicides are not compatible with T. viride even at 50 ppm concentration and 100 per cent inhibition in growth of T. viride was observed at 50 ppm and above concentrations.

Key words: *Trichoderma, Fungicides, ppm, Per cent inhibition*

INTRODUCTION

Trichoderma, a filamentous soil inhabiting mycoparasite, is used in commercial preparation for biological control of many fungal plant pathogens[7] and included the mechanisms like antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solublization and sequestration of inorganic nutrients and inactivation of pathogen enzymes[5].

Cite this article: Kumar, A., Bansal, R.D., Chelak, Y.K., Compatibility of *Trichoderma viride* with Fungicides for Plant Disease Management, *Int. J. Pure App. Biosci.* **7(3):** 44-51 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7333

However, with the increasing interest in biological control, owing to environmental and economic concerns, and with the rapid development of biotechnology, several *Trichoderma* species were formulated in a commercial production for protection and growth enhancement of a number of crops in several countries[12].

The combined use of bio-control agents and chemical pesticides has attracted much attention in order to obtain synergistic or additive effects in the control of plant diseases[9]. Even reduced amounts of the fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist[10]. Chemical protectants are effective under climatic conditions in which biological antagonists are less effective, while an active biological control agent can prophylactically colonize wounds or senescing plant tissue[6]. Usually fungicide resistant or tolerant isolates of bioagents for use in integrated control are readily obtained by selection on pesticide containing media[1]. *Trichoderma* strains differ in their sensitivity to different pesticides[8]. There are reports where insecticides used at recommended concentrations are inhibitory to *Trichoderma* spp. than fungicides[20]. However, the compatibility of *Trichoderma* to pesticides needs confirmation before its use in integrated management system. Recently, biological control combined with chemical fungicide at lower concentration is applicable. It is being a part of IPM (Integrated Plant disease Management) strategy which was applied since chemical control was used individually cause environmental pollution. Integrating fungicide resistant antagonists with suitable fungicidal treatment has importance in the framework of integrated disease management. Species of *Trichoderma* have been extensively tested by the Plant Pathologists due to their high efficacy, broad spectrum activity and ease in mass multiplication. Integration of chemicals and bio-agents has been the subject of research during recent years. Integration of biological seed protectant with fungidical treatment that eliminate competitors, may enhance the establishment of desired biocontrol agents and provide better control of seed and seedling diseases that either used separately[16].

MATERIAL AND METHODS

Trichoderma harzianum isolates

A set of two isolates of *T. harzianum* were procured from Department of Plant Pathology, College of Agriculture, Rewa and used in the present investigation. Both the isolates were isolated from soil of Satna and Rewa (Kuthulia) and coded as T_1 and T_2 respectively. Standard serial dilution method was followed for isolation of *Trichoderma* on selective media (Hi media). Plates were incubated at 25 ±2ºC for 5days. Different *Trichoderma* colonies appearing on the plates were purified in the Potato Dextrose Agar (PDA). The procured isolates of *T. harzianum* were maintained throughout the study by periodical transfers on Potato dextrose agar (PDA) medium.

Fungicide

Eight fungicides namely Mancozeb 75% WP, Carbendazim 50% WP, Thiram 75% WP, Tridemorph 75% EC, Chlorothalonil 75% WP, Hexaconazole 5% EC, Propiconlazole 25% EC and Thiophanate methyl 70% WP were screened against two isolates of *Trichoderma viride* by Poisoned Food Technique[15]. The fungicides were evaluated at five different concentrations of 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm.

Poison food technique

The poisoned food technique[15] was followed to evaluate the efficacy of different systemic, non-systemic and combiproducts fungicides for radial mycelial growth inhibiting of the *Trichoderma*. Stock solutions of fungicides were prepared by dissolving the required quantities of each fungicide separately in sterile distilled water. The fungicidal suspension was added to the PDA melted medium to obtain the required concentrations on commercial formulation basis of the fungicide. Twenty ml of poisoned medium was poured in each sterilized Petriplates under aseptic condition. Suitable check was

maintained without addition of fungicide. Mycelial disc of 5 mm was taken from the periphery of seven days old colony of *Trichoderma* and was placed in the centre of Petriplates and incubated at 27 ±1^{o} C for 12 days and three replications were maintained for each treatment. Overall experimental design followed was factorial Completely Randomized Design where in fungicides formed the factors and concentration formed the levels. The diameter of the colony was measured in two directions and average growth was recorded. Per cent inhibition of mycelial growth of the fungus was calculated by using the formula C-T/ C x100 (where C= growth in control and T =growth in treatment) given by Vincent[21].

Statistical analysis

The data obtained in these experiments were statistically analyzed by using completely randomized design (CRD). The data pertaining to percentages were angularly transformed. Results were analyzed by following appropriate statistical methods as per the procedure suggested by Gomez and Gomez[4].

Results and discussion

Compatibility of fungicides with *Trichoderma viride*

In total eight fungicides namely Mancozeb 75% WP, Carbendazim 50% WP, Thiram 75% WP, Propiconazole 25% EC, Chlorothalonil 75% WP, Hexaconazole 5% EC, Tridemorph 75% EC and Thiophanate 70% WP methyl were tested at five different concentrations of 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm along with control with two isolates of *T. viride* T_1 and T_2.

Mancozeb 75% WP

In-vitro compatibility study of *Trichoderma viride* with Mancozeb (Table 1) revealed that this fungicide is compatible with *Trichoderma viride* upto 50 ppm. However, above 50 ppm concentration it started inhibiting the growth of both the isolates of *Trichoderma viride.* The full Petriplate growth of 85 mm was recorded with both the isolates of *Trichoderma* at 50 ppm concentration which shows its 100 per cent compatibility at 50 ppm concentration. However, on gradually increasing the concentration of Mancozeb from 50 ppm to upto 1000 ppm, the compatibility kept on reducing. It was observed that, at 100 ppm concentration respectively 25.00 percent and 27.21 per cent inhibition in growth was recorded with *Trichoderma* isolate 1 and 2 in comparison to control. At 1000 ppm concentration of Mancozeb 70.88 per cent and 71.03 per cent inhibition in growth of *Trichoderma* isolate 1 and 2 was recorded.

Thiram 75% WP

The compatibility assay of *T. viride* along with different tested concentrations of thiram (Table 2), showed that inhibition in growth of both the isolates of *T. viride* started even at 50 ppm concentration. In comparision to control, where full Petriplate growth of 85.00 mm was recorded, 4.12 per cent and 4.85 per cent inhibition was calculated in growth of T_1 and T_2 isolate of *T. viride* respectively. However on increasing the concentration of Thiram from 50 ppm upto 1000 ppm the percent inhibition in growth of both the isolates of *Trichoderma* kept on increasing and upto 69.71 per cent inhibition was recorded in *T. viride* isolate T_2. The average percent inhibition ranged from 4.85 per cent to 69.26 per cent. The detailed data for colony diameter and per cent inhibition of both the isolates of *T. viride* at different concentrations of thiram has given in table 2.

Chlorothalonil 75% WP

Compatibility analysis of chlorothalonil 75% WP (Table 3) revealed that inhibition in growth of both the isolates of *T. viride* started from 50 ppm concentration of chlorothalonil showing 14.12 per cent and 15.88 per cent inhibition in comparison to control at 50 ppm concentration respectively in T_1 and T_2 isolate. On increasing the concentration of chlorothalonil the colony diameter kept on reducing. The average per cent inhibition in growth of *T. viride* ranged from 15.00 per cent to 75.29 per cent in comparison to control. This shows that chlorothalonil is 85 per cent compatible with chlorothalonil at 50 ppm concentration. However on increasing the concentration of chlorothalonil, the compatibility of *T. viride* kept on reducing and at 1000 ppm concentration, the compatibility reduced to 24.71 per cent only.

Carbendazim 50% WP

In-vitro compatibility study revealed that this fungicide is not compatible with *T. viride* even at 50 ppm concentration of carbendazim (Table 4). The colony of both the isolates of *T. viride* did not grow in all the tested concentrations of carbendazim.

Propiconzole 25 %EC

In-vitro compatibility study of *T. viride* with Propiconazole revealed that this fungicide completely inhibited the growth of both the isolates of *T. viride* at all the tested concentrations (Table 5). This shows that *T. viride* isolates are not compatible with Propiconazole even at 50 ppm concentration and above.

Tridemorph 75% EC

Compatibility analysis of tridemoph revealed that this fungicide started inhibiting the growth of *T. viride* at lower concentration of 50 ppm (Table 6) and 72.94 per cent and 74.41 per cent inhibition was calculated in growth of T_1 and T_2 isolate of *T. viride* respectively. However on increasing the concentration of Tridemorph from 50 ppm to upto 500 ppm the percent inhibition in growth of both the isolates of *Trichoderma* kept on increasing and upto 94.71 per cent and 96.18 per cent inhibiton was recorded in T_1 and T_2 respectively. On increasing the concentration of Tridemorph *viride* from 500 ppm to 1000 ppm, the colony growth of both the isolates of *Trichoderma* was completely checked and 100 percent inhibition was observed.

Thiophanate methyl 70% WP

The compatibility assay of *T. viride* along with different tested concentrations of Thiophanate methyl showed that thiophanate methyl significantly started inhibition in growth of both the tested isolates of *Trichoderma* at 50 ppm concentration and 69.71 per cent and 71.47 per cent inhibition was recorded in T_1 and T_2 respectively in comparison to control (Table 7). On increasing the concentration of thiophanate methyl, to 500 ppm, the growth of both the isolates of *Trichoderma* was completely checked and 100 per cent inhibition was recorded which showed the incompatibility of *Trichoderma* with thiophanate methyl at 500 ppm and above concentration.

Hexaconazole 5% EC

In-vitro compatibility study of *T. viride* with Hexaconazole revealed that this fungicide completely inhibited the growth of *Trichoderma* isolates at all the tested concentrations (Table 8). Hexaconazole started inhibiting the growth of *Trichoderma* from 50 ppm concentration itself and it was observed that there was no growth of both the isolates of *Trichoderma* at 50 ppm and above tested concentrations. This showed that *Trichoderma* is not compatible with hexaconazole at 50 ppm and above concentrations.

To develop an effective disease management programme, the compatibility of potential bioagents with fungicides is essential. Combination of chemicals and compatible bioagents in an IDM strategy protects the seeds and seedlings from soil-borne and seed-borne inoculums[3]. Integration of compatible bioagent with pesticides, may enhance the effectiveness of disease control and provide better management of soil borne diseases[16]. The combination of biological control agents with fungicides would provide similar disease suppression as achieved with higher fungicide use[13]. Combining antagonists with synthetic and non synthetic chemicals eliminates the chance of resistance development and reduces the fungicide application. Earlier fungicide compatibility studies with *T. viride* showed that mancozeb 75%WP was most compatible followed by pyrroclosterobin. Carbendazim 50%WP was found to be highly inhibitory which is in agreement with the findings of Somasekhara *et al*.[17].

The present investigation provides evidence for the compatibility and in-compatibility of *Trichoderma* with fungicides. Similar reports of compatibility of *Trichoderma* with fungicides and other agro-chemicals has been reported by Nallathambi *et al*.[14], Madhusudan *et al*.[11], Sunil and Kulkarni[18], Bagwan[2], Tewari *et al*.[19], and results pertaining to present investigations are matching with their findings. Consequently, more detailed studies are still needed among the various isolates of *Trichoderma* species in order to provide a better understanding of the

threshold limit of different chemicals compatible with *Trichoderma* to utilize them in integrated disease management.

Conclusion

Present findings indicate fungicide compatibility analysis of *Trichoderma* which revealed that *T. viride* is completely incompatible with carbendazim 50%WP, propiconazole 25%EC and hexaconazole 5%EC even at 50 ppm concentrations and found lethal. *T. viride* is compatible with Mancozeb 75%WP upto 50 ppm. With thiram 75%WP and chlorothalonil 75%WP *T. viride* is more than 70 % compatible upto 100 ppm. Growth of *Trichoderma* is inhibited greatly by Tridemorph and thiophanate methyl at 50 ppm concentration and showed its incompatibility with these fungicides.

Table 1: Compatibility of *T. viride* isolates with mancozeb 75 % WP

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	85.00	0.00	85.00	0.00	0.00
100 ppm	63.75	25.00	60.00	29.41	27.21
250 ppm	47.75	43.82	46.75	45.00	44.41
500 ppm	31.75	62.65	30.00	64.71	63.68
1000 ppm	24.75	70.88	24.50	71.18	71.03
Control	85.00	0.00	85.00	0.00	0.00
C.D. (5%)	2.62	-	2.23	-	-

Table 2: Compatibility of *T. viride* isolates with thiram 75% WP

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	81.50	4.12	80.25	5.59	4.85
100 ppm	79.50	6.47	78.50	7.65	7.06
250 ppm	63.00	25.88	61.75	27.35	26.62
500 ppm	32.50	61.76	31.75	62.65	62.21
1000 ppm	26.50	68.82	25.75	69.71	69.26
Control	85.00	0.00	85.00	0.00	0.00
C.D. (5%)	1.79	-	2.23	-	-

Table 3: Compatibility of *T. viride* isolates with Chlorothalonil 75% WP

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	73.00	14.12	71.50	15.88	15.00
100 ppm	62.50	26.47	60.50	28.82	27.65
250 ppm	48.25	43.24	47.00	44.71	43.97
500 ppm	24.75	70.88	23.75	72.06	71.47
1000 ppm	21.50	74.71	20.50	75.88	75.29
Control	85.00	0.00	85.00	0.00	0.00
C.D. (5%)	1.55	-	1.72	-	-

Table 4: Compatibility of *T. viride* isolates with carbendazim 50 % WP

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	0.00	100.00	0.00	100.00	100.00
100 ppm	0.00	100.00	0.00	100.00	100.00
250 ppm	0.00	100.00	0.00	100.00	100.00
500 ppm	0.00	100.00	0.00	100.00	100.00
1000 ppm	0.00	100.00	0.00	100.00	100.00
Control	85.00	0.00	85.00	0.00	0.00

Table 5: Compatibility of *T. viride* isolates with propiconzole 25% EC

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	0.00	100.00	0.00	100.00	100.00
100 ppm	0.00	100.00	0.00	100.00	100.00
250 ppm	0.00	100.00	0.00	100.00	100.00
500 ppm	0.00	100.00	0.00	100.00	100.00
1000 ppm	0.00	100.00	0.00	100.00	100.00
Control	85.00	0.00	85.00	0.00	0.00

Table 6: Compatibility of *T. viride* isolates with tridemorph 75% EC

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	23.00	72.94	21.75	74.41	73.68
100 ppm	14.25	83.24	12.75	85.00	84.12
250 ppm	10.25	87.94	9.25	89.12	88.53
500 ppm	4.50	94.71	3.25	96.18	95.44
1000 ppm	85.00	100.00	85.00	100.00	100.00
Control	85.00	0.00	85.00	0.00	0.00
C.D. (5%)	0.90	-	0.93	-	-

Table 7: Compatibility of *T. viride* isolates with Thiophanate methyl 70% WP

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	25.75	69.71	24.25	71.47	70.59
100 ppm	16.75	80.29	13.75	83.82	82.06
250 ppm	6.00	92.94	3.50	95.88	94.41
500 ppm	0.00	100.00	0.00	100.00	100.00
1000 ppm	0.00	100.00	0.00	100.00	100.00
Control	85.00	0.00	85.00	0.00	0.00
C.D. (5%)	1.08	-	0.90	-	-

Table 8: Compatibility of *T. viride* isolates with hexaconazole 5 % EC

Concentration	T_1		T_2		Average Per cent inhibition
	Colony diameter (mm)	Per cent inhibition	Colony diameter (mm)	Per cent inhibition	
50 ppm	0.00	100.00	0.00	100.00	100.00
100 ppm	0.00	100.00	0.00	100.00	100.00
250 ppm	0.00	100.00	0.00	100.00	100.00
500 ppm	0.00	100.00	0.00	100.00	100.00
1000 ppm	0.00	100.00	0.00	100.00	100.00
Control	85.00	0.00	85.00	0.00	0.00

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