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Interaction of fungicides on the growth of *Trichoderma viride*

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Abstract
Trichoderma viride is a potential biocontrol agent for plant disease management especially seed borne and soil borne diseases. *Trichoderma* is a free living saprophytic fungus with high Competitive Saprophytic Ability (CAS), soil inhabitant and also survived on aerial parts of the plant. Biological control alone will not be feasible to combat the disease in case of severe incidence. Based on high cost and environmental concerns it is not advisable to protect the crops for the entire period by conventional fungicides. Biological control, in integration with fungicidal treatment was more reliable approach to manage soil borne and seed borne pathogens. In the present study the native isolate of *T.viride* was used for assessing their growth on fungicide amended medium (poisoned food technique). The fungicides *viz.*, carbendazim (0.1, 0.2 and 0.3 per cent), copper oxy chloride (0.2, 0.3 and 0.4 per cent), thiophanate methyl (0.05, 0.1 and 0.2 per cent), benomyl (0.05, 0.1 and 0.2 per cent) and sodium hypochlorite (5, 10 and 15 per cent) were used. Mycelial growth in fungicide amended solid medium and mycelial dry weight in fungicide amended liquid medium was studied to assess the compatibility. The radial mycelial growth of *T.viride* was significantly reduced by the fungicides at all concentrations tested. Among them, copper oxy chloride showed minimum inhibition of 45.55 per cent in mycelial growth and maximum weight of 77.00 mg of mycelial dry weight at 0.2 per cent concentration.

Keywords: *Trichoderma viride*, carbendazim, copper oxy chloride, thiophanate methyl, benomyl, sodium hypochlorite, mycelial growth and mycelial dry weight

Introduction
Nowadays pesticides and organic compounds are widely used to control various plant pathogens in many countries. Fungicide applications kill important beneficial fungi and also weakens the natural antagonistic activity. In spite of well known side effects of chemicals on environment they are continuously used to control plant pathogens. Biological control of soil borne plant pathogens is a potential alternative to the use of chemical pesticides, which already proved to be harmful to the environment. Several strains of the fungus *Trichoderma* was isolated and become effective bio control agents of various soil borne plant pathogenic fungi under green house and field conditions (Duke, 1990) [3]. Species of *Trichoderma* are well documented mycoparasites and have been used successfully against pathogenic fungi due to their high efficacy ad broad spectrum activity (Ramesh Babu, 2006) [6]. Biological control method has been considered as more natural and environmentally acceptance approach against the use of pesticides (Bagwan, 2010) [2]. Considering this background, the present study was carried out to access the compatibility of native isolate of *Trichoderma viride* with common fungicides at their lower, actual and higher quantity of recommended doses.

Materials and Methods
The culture of native antagonist *Trichoderma viride* collected from nearby village of Adhiparasakthi Agricultural College, G.B. Nagar, Kalavai was used in this study.

Fungicides used in this experiment

Table 1.

S. No.	Common name	Formulations	Chemical name
1.	Bavistin	50 % WP	Carbendazim (Methyl -2- Benzimidazole Carbamate)
2.	Fytolan	50 % WP	Copper oxychloride
3.	Hexamar	70 % WP	Thiophanate methyl
4.	Benofit	50 % WP	Benomyl
5.	Bleaching powder	3 – 8 %	Sodium hypochlorite

Fungicides on radial mycelial growth of *T. viride*

The native antagonist *T. viride* was grown on fungicides amended poisoned Czapek's medium by the method of poisoned food technique. Fungicides with various concentrations used for this study were carbendazim (0.1, 0.2 and 0.3%), copper oxy chloride (0.2, 0.3 and 0.4 %), thiophanate methyl (0.05, 0.1 and 0.2 %), benomyl (0.05, 0.1 and 0.2 %) and sodium hypochlorite (5, 10 and 15 %). Concentrations of various fungicides were determined as actual, lower and higher than the dose recommended for seed treatment and soil application.

The poisoned medium was poured in to the sterilized Petri plates with three replications. Control plates were maintained without fungicides. From three days old culture of *T. viride* grown on PDA medium, 9 mm disc was taken and inoculated at the centre of the Petri plates. Plates were incubated at room temperature (30 ± 2ºC). From third day onwards the radial mycelial growth was observed. Finally radial growth was measured by comparing the full growth of control plates with *T. viride*.

The per cent growth inhibition of antagonist *T. viride* was estimated by using the formula given by Vincent (1947) [8] and converted in to per cent compatibility.

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

Where,

I - Per cent growth inhibition

C – Colony diameter in control

T – Colony diameter in treatment

Fungicides on mycelial dry weight of *T. viride*

The mycelial dry weight *T. viride* was obtained by using Czapek's broth. In 100 ml of sterilized Czapek's broth in 250 ml conical flasks, different concentrations of various fungicides were added *viz*., carbendazim (0.1, 0.2 and 0.3%), copper oxy chloride (0.2, 0.3 and 0.4 %), thiophanate methyl (0.05, 0.1 and 0.2 %), benomyl (0.05, 0.1 and 0.2 %) and sodium hypochlorite (5, 10 and 15 %). Control flasks were maintained without fungicides. Three replications were used for each treatment. The 9 mm disc of three days old culture of *T. viride* was inoculated in all flasks and incubated at room temperature for 15 days. At the end of incubation period, the contents were filtered through previously dried and weighed Whatman No.1 filter paper. The mycelial mats were dried in an oven at 80ºC and weighed. Again it was dried and weighed to get the constant weight.

Statistical analysis

Data of the experiments were analysed by Completely Randomized Block Design (CRD) using data entry module for Ag Res Statistical Software© 1994 Pascal International Software Solutions, version 3.01 for data entry and version 7.01 for analysis.

Results and Discussion

The native isolate of *T. viride* was tested against various fungicides with different concentrations under *in vitro* condition. Along with actual dose of fungicides, lower and higher concentrations were also tried to study the tolerance of *T. viride* as radial mycelial growth and mycelial dry weight.

Radial mycelial growth of *T. viride* on fungicides amended medium

In vitro experiment was conducted to find out the effect of various fungicides on the radial mycelial growth of *T. viride* and the results are summarized in Table 1. The radial mycelial growth of *T. viride* was significantly reduced by the fungicides at all concentrations tested. Among the fungicides, sodium hypochlorite (disinfectant) showed better radial mycelial growth of 40.00, 38.00 and 23.00 mm at 5, 10 and 15 per cent concentrations. Copper oxy chloride was effective in all concentrations and recorded 49.00, 28.00 and 17.00 mm of radial mycelial growth. At lower concentration, copper oxy chloride (0.20%) showed more than 50 per cent of growth (49.00 mm) compared to control (90.00 mm). Benomyl was less effective as 10.00, 9.00 and 8.00 mm of growth at 0.05, 0.10 and 0.20 per cent concentrations.

Table 1: Effect of different fungicides on the radial mycelial growth of *Trichoderma viride*

S. No.	Fungicides	Concentration (%)	Growth of Mycelium (mm)	Per cent decrease over control	Per cent compatibility
1.	Carbendazim	0.10	35.00 d	61.11	38.89
		0.20	23.00 f	74.44	25.56
		0.30	14.00 g	84.44	15.56
2.	Copper oxy chloride	0.20	49.00 b	45.55	54.45
		0.30	28.00 e	68.88	31.12
		0.40	17.00 g	81.11	18.89
3.	Thiophanate methyl	0.05	16.00 g	82.22	17.78
		0.10	15.00 g	83.33	16.67
		0.20	14.00 g	84.44	15.56
4.	Benomyl	0.05	10.00 h	88.88	11.12
		0.10	9.00 h	90.00	10.00
		0.20	8.00 h	91.11	8.89
5.	Sodium hypochlorite	5.00	40.00 c	55.55	44.45
		10.00	38.00 cd	57.77	42.23
		15.00	23.00 f	74.44	25.56
6.	Control	-	90.00 a	-	-

S.E. = 1.52 C.D.(0.05) = 3.11

The results of this study coincided with the report of Shukla (2011) [7] as fytolan showed least inhibitory effect on growth and bavistin completely inhibited the growth of *T. viride*. Rahulkumar *et al*. (2018) [5] also reported that carbendazim showed almost complete suppression of *T. viride* followed by carbendazim + mancozeb. Whereas thiophanate methyl at lower concentrations would be compatible for the integrated management of soil borne diseases (Rahulkumar *et al*., 2018) [5] it was contradictory to this result. Results of Kumar *et al.* (2019) [4] was coincided with this findings as *T. viride* is completely incompatible with carbendazim, propiconazole and hexaconazole at 50 ppm concentrations and growth of *T.*

viride was also inhibited greatly by tridemorph and thiophanate methyl at 50 ppm concentration. In this experiment also lower concentration of copper oxy chloride showed better growth. Carbendazim was next to sodium hypochlorite on growth. Sodium hypochlorite was recommended for the management of soil-borne pathogens especially *Sclerotium rolfsii*. Benomyl completely inhibited the growth at al concentrations.

Mycelial dry weight of *T. viride* on fungicides amended medium

Experiment was conducted to assess the efficacy of different fungicides on myelial dry weight of antagonist *T. viride* and the results are summarized in Table 2. All fungicides inhibited the growth of antagonistic fungi *T. virde* at all concentrations in the liquid medium compared to control which recorded 86.00 mg mycelial dry weight. Among the fungicides tried, copper oxy chloride recorded the lowest inhibition (10.46 per cent) followed by sodium hypochlorite at 5 per cent concentration (26.74 per cent). Highest inhibition was obtained in benomyl as 83.72, 86.04 and 87.20 per cent followed by thiophanate methyl and carbendazim.

Table 2: Effect of different fungicides on the mycelial dry weight of *Trichoderma viride*

S. No.	Fungicides	Concentration (%)	Mycelial dry weight (mg)	Per cent decrease over control	Per cent compatibility
1.	Carbendazim	0.10	57.00 fg	33.72	66.28
		0.20	54.00 g	37.20	62.80
		0.30	47.00 h	45.34	54.66
2.	Copper oxy chloride	0.20	77.00 b	10.46	89.54
		0.30	65.00 c	24.41	75.59
		0.40	58.00 e	32.55	67.45
3.	Thiophanate methyl	0.05	61.00 de	29.06	70.94
		0.10	48.00 h	44.18	55.82
		0.20	46.00 h	46.51	53.49
4.	Benomyl	0.05	14.00 k	83.72	16.28
		0.10	12.00 k	86.04	13.96
		0.20	11.00 k	87.20	12.80
5.	Sodium hypochlorite	5.00	63.00cd	26.74	73.26
		10.00	40.00 i	53.48	46.52
		15.00	31.00 j	63.95	36.05
6.	Control	-	86.00 a	-	-

S.E. = 1.79 C.D.(0.05) = 3.66

Findings of this experiment coincided with the report of Shukla (2011) [7] and Ashwani Tapwal *et al*. (2012) [1] as fytolan or blue copper (copper oxy chloride) and captaf were compatible with *T. viride* and bavistin completely inhibited the growth. In this results benomyl and thiophanate methyl were highly inhibitory followed by carbendazim (bavistin). Copper oxy chloride was compatible.

Conclusion

The radial mycelial growth and mycelial dry weight of *T.viride* were significantly reduced by fungicides at all concentrations tested. Among fungicides, copper oxy chloride exhibited the lowest inhibition of mycelial growth and dry weight followed by sodium hypochlorite. Radial mycelial growth may be spares or dense but mycelial dry weight represented the actual growth.

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