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Devendra Nishad
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Meghchand Dewangan
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Arvind Kumar Kurre
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Shweta Mishra
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Devendra Nishad
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Studies on the compatibility of *Trichoderma viride* and its interaction with different fungicides

Devendra Nishad, Meghchand Dewangan, Arvind Kumar Kurre and Shweta Mishra

Abstract
Trichoderma viride 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 Triehoderma viride to work out their compatibility to devise a suitable integrated management of soil borne plant diseases. Four fungicides Mancozeb, Thiram, Carbendazm and Captan were evaluated at different concentration. Present investigation suggests that compatible fungicides can be used with Triehoderma in an IDM package to control soil borne plant pathogen.The effect of compatibility of fungicides with *Trichoderma viride* experiment was conducted *in vitro* condition under poisoned food technique. Among the tested fungicides Mancozeb (0.1 and 0.2%) is fully compatible, Thiram and Captan (0.1%) is moderately compatible, Thiram (0.2%) and Captan (0.2%) is less compatible and Carbendazim (0.05 and 0.1%) is incompatible with *Trichoderma viride*.

Keywords: *Trichoderma viride*, compatibility, bio-agent and fungicides

Introduction
Biocontrol agents are safe and environmental friendly alternatives for pesticides in agriculture application. Trichoderma viride performed a high level of antagonistic activity toward a broad spectrum of phytopathogens and was determined as a biocontrol agent. Trichoderma viride is a filamentous fungus that is widely distributed in the soil, plant material, decaying vegetation, and wood that can be used as a bio fungicide. It is used for seed and soil treatment for suppression of various diseases caused by fungal pathogens. Colonies of Trichoderma viride grow rapidly and mature in 5 days. At 25 °C and on potato dextrose agar, the colonies are wooly and become compact in time. From the front, the color is white. As the conidia formed, scattered blue-green or yellow-green patches become visible. *Trichoderma spp*. from rhizosphere and non- rhizosphere soils of different geographical regions of Chhattisgarh in Central India and identified on the basis of their cultural characteristics structure of conidiophores and conidia (Agrawal and Kotasthane, 2009) [1]. The possibility of combining fungicides with *Trichoderma viride* to work out their compatibility to devise a suitable integrated management of soil borne plant diseases. 6 fungicides *viz.*, Blitox, Thiophenate methyl, Roxiltabucanazole, Ridomil, Bavistin and Captan were evaluated at different concentration (Singh *et al*. 2015) [3]. To check the compatibility of two insecticides, three fungicides and their combinations on *Trichoderma viride*. It shows variable responses against the tested pesticides (fungicides and insecticides) and their combinations at recommended concentrations for field studies (Vasundhara *et al*. 2015) [4]

Material and Method
The present studies were carried out at the Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, (C.G.).

Culture media
Common laboratory medium *i.e.* potato dextrose agar (PDA) medium was used for the isolation of the pathogen associated with the wilt of chickpea.

Chemicals
The chemicals were used for the preparation of media were obtained from Department of Plant Pathology, IGKV, Raipur.

Biological agent
The soil sample of different districts such as Raipur, Mahasamund, Gariyaband and Surguja were collected and *T. viride* was isolated by serial dilution method. The isolated *T. viride* isolates were designated as Tri-1, Tri-2, Tri-3 and Tri-4 respectively.

General procedure followed
Unless and otherwise mentioned for each set of experiment, four replications were kept for all *in vitro* studies. In general, sterilized and melted potato dextrose agar medium (15-20 ml) was poured in sterilized petriplates and allowed for solidification. The streptomycin was supplement in melted PDA medium in order to check the bacterial contamination prior to the pouring.
Wherever growth studies were conducted, 7 mm disc (always kept in inverted position) of the actively growing fungi was used for inoculating the medium in petriplates. Four replications were maintained and Complete Randomized Block Design (CRD) was used as per the requirement. The inoculated plates were incubated at 27±2 °C for a period of 3 to 7 days for growth of *Trichoderma viride*.

Isolation
Chickpea plant showing typical wilt symptoms were collected from research farm, IGKV, Raipur, Mahasamund, Gariyaband and Surguja districts. Isolation was made to isolate associated pathogen from wilted plants collected from different locations. The roots and stem of plant infected plant were washed in running tap water to remove soil adheres to roots. Root bark of the wilted plants were removed before isolation to remove contamination. The roots and stem were split open and small bits of the size 2.5 mm were cut with sterilized blade. These bits were disinfected with 0.1% of aqueous solution of mercuric chloride (HgCl2) for 30 second and washed with three subsequent changes sterilized water to remove traces of mercuric chloride. Each bit was picked up and placed on the solidified potato dextrose agar (PDA) plates. These plates were incubated at 27±2 ºC for 72 hrs. The fungal growth was observed on the bit and transferred to the slants of PDA media. The isolated fungi was identified as *Fusarium oxysporum* f. sp. *ciceri*.

Purification and maintenance of pure culture
All the isolates of pathogen obtained were purified from single micro-conidia on two per cent water agar and multiplied on potato dextrose agar slants starved at 4 °C for further studies.

Sub culturing of *T. viride*
Sub culturing was done on potato dextrose agar medium in petriplates and incubated at 27±2 ºC for 7 days with periodic observation for the development of colonies of *T. viride*. The green colour colonies were identified by key based on branching conidiophores, shape of phialides, emergence of phialides and spore characters.

In vitro* assay of fungicidal compatibility with *Trichoderma viride
In vitro four fungicides were evaluated to see their compatibility with *Trichoderma viride* by employing "Poisson food technique". The requisite amount of each fungicide based on active ingredient was added in potato dextrose agar medium at the time of pouring to obtain the desired concentrations, *i.e.* 0.1 and 0.2% in all fungicides except Carbendazim (0.05 and 0.1%). The same medium without fungicides served as the check. The medium was poured into 90 mm. Petriplates after solidification of medium each plate was inoculated with 7 mm mycelial disc of *Trichoderma viride*. The inoculated petriplates were incubated for 7 days at 27±2 °C. After incubation, radial growth was measured and per cent inhibition of growth was calculated from the mean colony diameter when petriplates fully covered by mycelial growth of the *Trichoderma viride* as per following formula.

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,
C = Growth of test fungus in control in mm.
T = Growth of test fungus in treatment in mm.

Result and Discussion
In vitro* assay of fungicidal compatibility with *Trichoderma viride
In vitro 4 fungicides were tested to see their compatibility with *T. viride*. The result revealed that the percent compatibility decreased with an increase in the concentration of fungicides.
The following fungicides were studied for interaction studies.

- **Carbendazim 50WP:** Observation with regard to the effect of various concentrations (0.05 and 0.01%) of Carbendazim on mycelial growth of *T. viride* was presented in Table 1, Plate1 and Fig.1. Both the concentrations of chemical showed complete inhibition of the mycelial growth for all the isolates of *T. viride*.
- **Mancozeb 75WP:** Table 2, Plate 2 and Fig 1. It is a clear from the data that Mancozeb did not show inhibitory effect on the growth and Sporulation *T. viride* at both concentration (0.1 and 0.2%).
- **Captan 75WP:** It was reveal from the data that at 0.1% concentration all the isolates grew with range of 25.25 mm in Tri-4 and 32.00 mm in Tri-1. At 0.2% isolates Tri-1 and Tri-2 show inhibitory effect while Tri-3 and Tri-4 showed less inhibitory effect against Captan. Table 3, Plate 3 and Fig 1.
- **Thiram 75WP:** Table 4, Plate 4 and Fig 1. It was reveal from the data that at 0.1% concentration all the isolates grew with range of 25.75 mm to 29.75 mm. At 0.2% gave 29.25 mm in Tri-4 and 24.75 mm Tri-3. Whereas very less growth was recorded in case of remaining two isolate *i.e.* Tri-1 (19.50 mm) and Tri-2 (11.00 mm).

The present findings are in accordance with studies by Bhattiprolu (2007) [2] Growth of *T. viride* in Mancozeb amended PDA fully compatible with *T. viride*. Singh (2015) [3] observed Captan was the only fungicide which showed with *T. viride* full compatibility up to 500 ppm dose only. Singh *et al*. (2015) [3] found that, possibility of combining fungicides with *Trichoderma viride* to work out their compatibility to devise a suitable integrated management of soil borne plant diseases. 6 fungicides *viz.*, Blitox, Thiophenate methyl, Roxiltabucanazole, Ridomil, Bavistin and Captan were evaluated at different concentration.

Table 1: Effect of various concentrations of Carbendazim 50WP on mycelial growth of *T. viride*

Concentration	Mycelial growth in diameter (mm)				
	Tri-1	Tri-2	Tri-3	Tri-4	Mean
0.05%	0.00	0.00	0.00	0.00	0.00
0.10%	0.00	0.00	0.00	0.00	0.00
Control	90.00	90.00	90.00	90.00	90.00

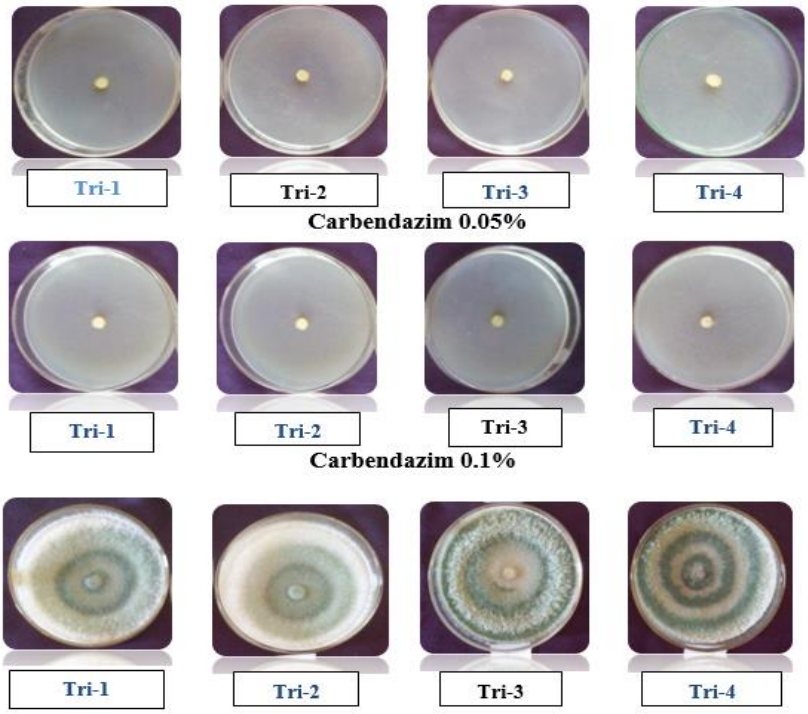

Plate 1: Radial growth of *T. viride* isolates in different concentration of Carbendazim

Table 2: Effect of various concentrations of Mancozeb 75WP on mycelial growth of *T. viride*

Concentration	Mycelial growth in diameter (mm)				
	Tri-1	Tri-2	Tri-3	Tri-4	Mean
0.10%	90.00	90.00	90.00	90.00	90.00
0.20%	90.00	90.00	90.00	90.00	90.00
Control	90.00	90.00	90.00	90.00	90.00

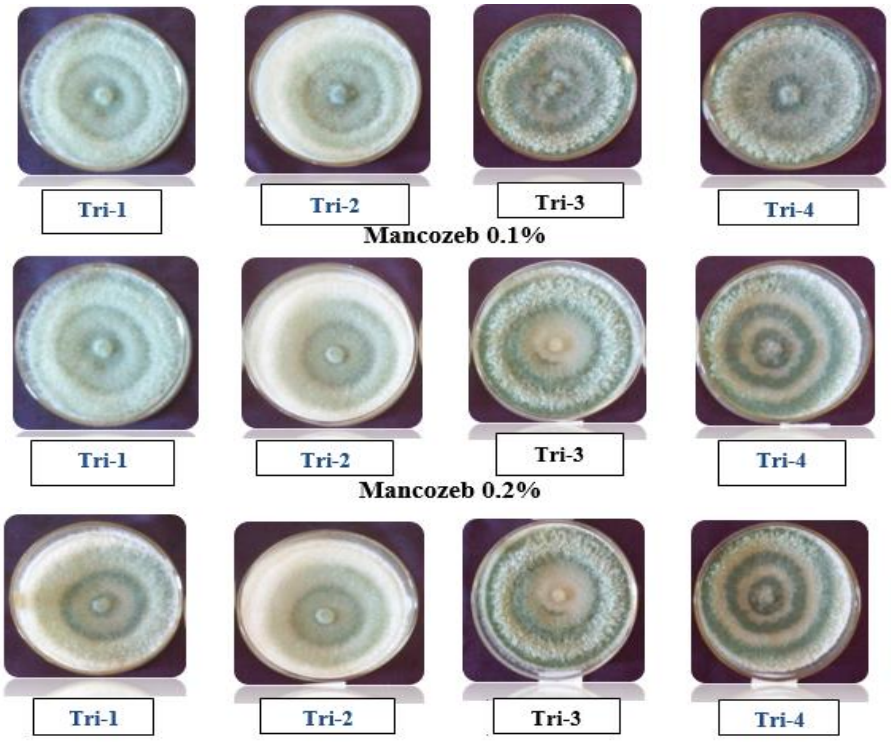

Plate 2: Radial growth of *T. viride* isolates in different concentration of Mancozeb

Table 3: Effect of various concentrations of Captan 75WP on mycelial growth of *T. viride*

Concentration	Mycelial growth in diameter (mm)				
	Tri-1	Tri-2	Tri-3	Tri-4	Mean
0.10%	32.00	30.50	33.75	25.25	30.37
0.20%	0.00	0.00	25.00	26.75	12.94
Control	90.00	90.00	90.00	90.00	90.00

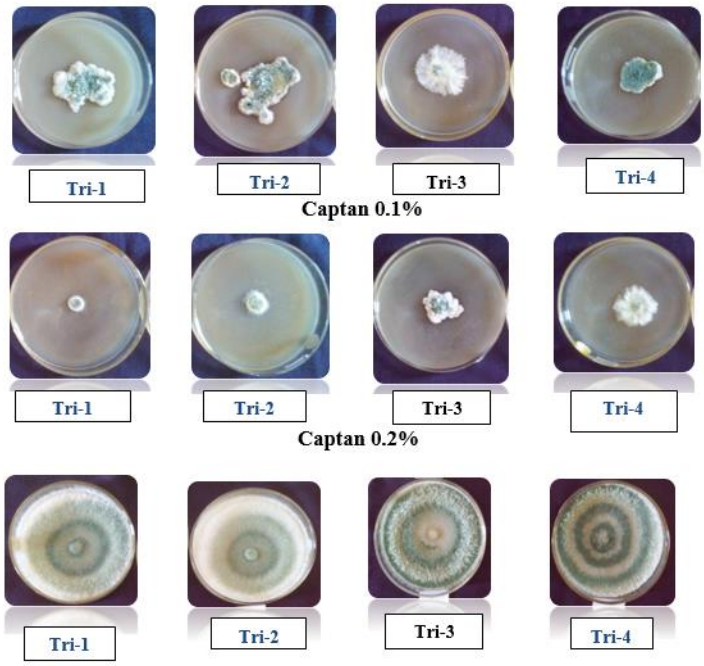

Plate 3: Radial growth of *T. viride* isolates in different concentration of Captan

Table 4: Effect of various concentrations of Thiram 75WP on mycelial growth of *T. viride*

Concentration	Mycelial growth in diameter (mm)				
	Tri-1	Tri-2	Tri-3	Tri-4	Mean
0.10%	28.75	25.75	28.25	29.75	28.13
0.20%	19.50	11.00	24.75	29.25	21.13
Control	90.00	90.00	90.00	90.00	90.00

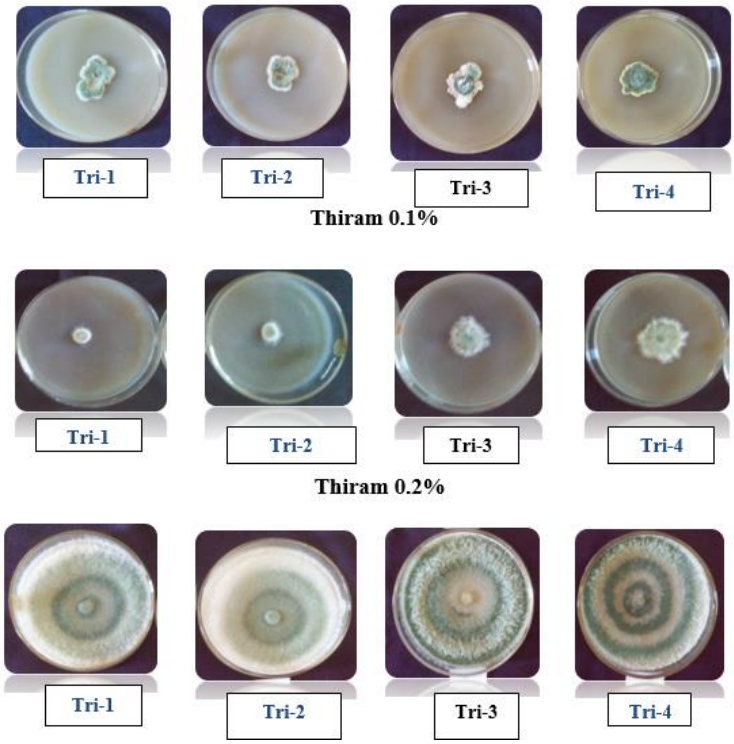

Plate 4: Radial growth of *T. viride* isolates in different concentration of Thiram

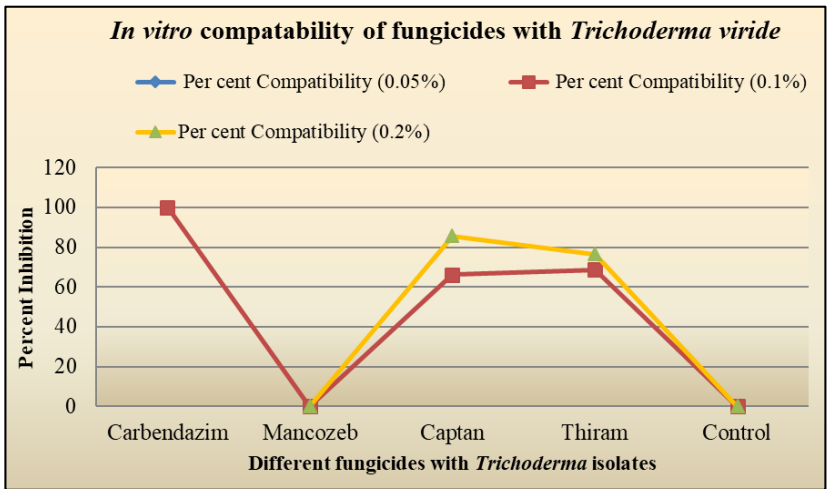

Fig 1: *In vitro* assay of compatibility of fungicides with *Trichoderma viride*

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