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Study on compatibility of *Trichoderma viride* with different fungicides

SANJEEV KUMAR[1], AMARENDRA KUMAR[1], RAKESH KUMAR[2]* and RAJESHA G[3]

Bihar Agricultural University, Sabour, Bhagalpur, Bihar 813 210, India

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ABSTRACT

Aggressive colonization of *T. viride* can occurs in diverse environmental condition as a biological control and prevents many soil borne diseases. In this context, laboratory experiment was conducted during 2018–19 at Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar to test the sensitivity of *T. viride* isolated with selected fungicides to devise the best management practice. In course of investigation, nine fungicides of systemic, non-systemic and their combination were evaluated at lower concentrations compared to recommended dose on sensitivity and sporulation of *T. viride* with food poisoned techniques. Growth and sporulation of *T. viride* was totally inhibited by carbendazim, hexoconazole, carbendazim+ mancozeb and iprodione + carbendazim at all the concentrations. Sporulation affected by all the fungicides, but in copper-oxychloride, inhibition of sporulation had minimum, i.e. 19.7, 37.1 and 53.9 at 200, 500 and 1000 µg/ml, respectively. Since, the minimum inhibitory effect on growth and spore production was recorded in copper oxychloride. Thus, the present finding suggest that compatible fungicides copper oxychloride can be used with *T. viride* in an integrated disease management practices for managing soil borne pathogens.

Keywords: Compatibility, Fungicides, Sensitivity, Sporulation, *Trichoderma*

Fungi of the genus *Trichoderma* have emerged as the most powerful biocontrol for management of soil-borne plant diseases (Kumar *et al.* 2020a). *Trichoderma* have long been known as bio–control agent for plant diseases, become a valuable part of agricultural disease control (Kumar *et al.* 2013, Singh *et al*. 2017, Zaidi *et al*. 2018). Soil-borne diseases are often difficult to manage by use of chemicals fungicides alone. Chemical fungicides are one of effective method to control the soil-borne plant pathogens is an established fact but extensive use of broad–spectrum compound to soil-borne pathogens, some are non-degradable resulted a variety of harmful and undesirable effect on soil health, hence their use are discouraged. Integrated disease management (IDM) of soil-borne pathogens is only way of reducing severe impact of chemical pesticides. Thus, studies on compatibility of *Trichoderma* to commonly available commercial pesticides will be helpful in developing IDM modules. Presently, various studies have been conducted on compatibility of bio-control agent with chemical and botanicals (Kumar *et al.* 2020c). To develop an effective IDM programme, compatibility of potential bio-agent with fungicides and organic cake, i.e. neem cake is more essential (Kumar *et al.* 2017). Combining antagonist with synthetic and non-synthetic chemicals eliminated chance of resistance development and reduces loads fungicides application. Keeping these things in view, laboratory experiment was conducted to test the possibility of combining *T. viride* with fungicides and neem cakes. Long term goal is to develop an effective IDM for management soil-borne plant diseases management and preventing resistance development in pathogens to chemicals. Thus, integrating chemical resistance *Trichoderma* strains has an importantrals in framework of IDM for management of soil borne diseases.

MATERIALS AND METHODS

Present experiment was conducted during 2018 and 2019 at Department of Plant Pathology, Bihar Agricultural University (BAU), Sabour, Bhagalpur, Bihar. Culture of *T. viride* was collected from Plant Pathology Department, multiplied and maintained on Potato Dextrose Agar (PDA) slants for further investigation.

Compatibility of Trichoderma with fungicides: Nine fungicides of 3 categories, i.e. systemic (Carbendazim 50 WP, Hexaconazole 5% EC and Thiophanate methyl 70 WP), protective (Mancozeb 75 WP, Copper oxy-chloride 50 WP and Propineb 70WP) and mixture of systemic and protective (Carbendazim 12%+Mancozeb 63%, Metalaxyl 8% +Mancozeb 64 WP and Iprodione 25%+ Carbendazim 25% WP) were evaluated to study the

Present address: [1]Bihar Agricultural University, Sabour, Bhagalpur, Bihar; [2]ICAR-Research Complex for Eastern Region, Patna, Bihar; [3]ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana. *Corresponding author e-mail: rakeshbhu08@gmail.com.

sensitivity against *T. viride*. 15 ml of stock solution of 2000 µg/ml strength of copper oxychloride (COC), mancozeb, propineb, carbendazim+mancozeb, metalaxyl+mancozeb and iprodione + carbendazim and 5000 µg/ml strength of carbendazim, hexaconazole and thiophanate methyl were prepared. To desired concentration of fungicides in medium, amount of stock solution to be added in PDA was calculated as: $C_1V_1 = C_2V_2$
where, C_1, Concentration of stock solution (µg/ml); C_2, Concentration of fungicides (µg/ml); V_1, Volume (ml) of the stock solution to be added; V_2, Measured volume (ml) of PDA in which fungicides was to be added.

Required amount of stock solution was poured in 60 ml of sterilized molten PDA to get the final concentration of 200, 500 and 1000 µg/ml of COC, mancozeb, propineb (Antracol) of protective group and carbendazim +mancozeb, metalaxyl+mancozeb and iprodione + carbendazim and 25, 50 and 100 µg/ml of carbendazim, hexaconazole and thiophanate methyl. PDA medium poisoned with different fungicides poured into sterilized petridishes @ 20 ml/plate

Table 1 Sensitivity of *T. viride* to different fungicides at lower concentrations

Fungicides	Concentration (µg/ml)	Colony diameter (mm)* Incubation period		Growth inhibition over check (%)	
		48 hr	72 hr	48 hr	72 hr
Systemic					
Carbendazim (Bavistin)	25	0.00	0.00	100 (90.00)	100 (90.00)
	50	0.00	0.00	100 (90.00)	100 (90.00)
	100	0.00	0.00	100 (90.00)	100 (90.00)
Hexaconazole (Contaf)	25	0.00	0.00	100 (90.00)	100 (90.00)
	50	0.00	0.00	100 (90.00)	100 (90.00)
	100	0.00	0.00	100 (90.00)	100 (90.00)
Thiophanatemethyl (ROKO)	25	32.67	40.67	56.33 (48.81)	54.64 (47.67)
	50	24.67	34.67	67.25 (55.09)	61.34 (51.56)
	100	15.67	23.33	79.19 (62.67)	73.98 (59.34)
Non-systemic					
Mancozeb (Indofil M-45)	200	65.67	87.33	12.65 (20.64)	02.58 (09.02)
	500	54.33	76.33	27.85 (31.84)	14.86 (22.66)
	1000	49.00	69.33	34.91 (36.21)	22.67 (28.43)
Copper oxychloride (Blitox 50)	200	35.67	57.67	52.64 (46.52)	35.68 (36.68)
	500	23.33	47.00	68.99 (56.17)	47.58 (43.61)
	1000	16.33	36.67	78.30 (62.64)	59.11 (50.25)
Propineb (Antracol)	200	36.00	67.67	52.18 (46.25)	24.53 (29.69)
	500	28.00	58.67	62.83 (52.44)	34.57 (36.01)
	1000	24.67	52.33	68.59 (55.91)	41.63 (40.18)
Systemic + Contact					
Carbendazim+Mancozeb (Companion)	200	0.00	0.00	100 (90.00)	100 (90.00)
	500	0.00	0.00	100 (90.00)	100 (90.00)
	1000	0.00	0.00	100 (90.00)	100 (90.00)
Metalaxyl+Mancozeb (Master)	200	38.00	65.33	99.33 (44.73)	27.13 (31.38)
	500	30.33	59.33	59.74 (50.62)	33.83 (35.56)
	1000	25.33	50.33	66.34 (54.55)	43.86 (41.48)
Iprodione + Carbendazim (Quintal)	200	0.00	0.00	100 (90.00)	100 (90.00)
	500	0.00	0.00	100 (90.00)	100 (90.00)
	1000	0.00	0.00	100 (90.00)	100 (90.00)
PDA as control		75.33	90.00		
CD (P=0.05)		1.48	2.03	1.61	1.47
CV		4.41	3.79	1.49	1.58

*Average of 3 replications; ** Figure in parenthesis are Sin angular transformation value.

and allowed to solidify. After solidification 5 mm disc of *T. viride* cut from 3 days old culture were centrally inoculated. PDA medium not amended with any fungicide but inoculated with test fungus served as control and all treatment replicated thrice. All plates were incubated in a BOD incubator at 28±1^{0}C. Observations on colony diameter were recorded after 48 and 72 h of incubation. Per cent (%) inhibition of growth (I) over check was calculated Vincent (1927).

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

where, I, Per cent (%) inhibition; C, Colony diameter in control (mm); T, Colony diameter in the treatment (mm).

Per cent data were transformed in angular transformation for statistical analysis. Data were statistically analyzed using completely randomized design (CRBD).

Effect of fungicides on sporulation: Number of spores of *T. viride* was recorded at different fungicide concentrations were recorded after 72 h of incubation. 5 mm disc of *T. viride* was cut with sterilized cork borer from each replication, washed with camel brush in 2 ml of water and again in 3 ml of water. Finally, 5 ml fungal spore suspension was obtained. Ten observations were taken from each replication and number of spores/ml of suspension was counted using haemocytometer.

RESULTS AND DISCUSSION

Nine fungicides, i.e. carbendazim, hexaconazole, thiophanate methyl, mancozeb (Indofil M-45), copper oxychloride, mancozeb, propineb (non-systemic), carbendazim+mancozeb, metalaxyl+ mancozeb, iprodione+carbendazim were evaluated at lower concentrations than recommended dose against *T. viride* to study the tolerance by antagonist by poison food techniques on PDA media. Systemic fungicides were evaluated at 25, 50 and 100 μg/ml, whereas mixture of protective and systemic at 200, 500 and 1000 μg/ml, respectively. Complete inhibition of growth of *T. viride* was noted in carbendazim, hexaconazole, carbendazim+mancozeb and iprodione + carbendazim at all concentration (Table 1). Among all protective fungicides, copper oxychloride was the least toxic to *T. viride*. Inhibition of growth of *T. viride* was 2.58, 14.86 and 22.67% at 200, 500 and 1000 μg/ml respectively. Propineb ranked the second and showed 24.53, 34.57 and 41.63% inhibition of growth of *T. viride* at 200, 500 and 1000 μg/ml respectively. Thiophanate methyl, mancozeb and mixture of metalaxyl + mancozeb showed 27.1 to 73.9% inhibition of growth of *T. viride* (Fig 1).

Sporulation of *T. viride* completely inhibited by carbendazim, hexoconazole, carbendazim + mancozeb and iprodione + carbendazim at all concentrations (Table 2). Though, sporulation was affected by all the fungicides, but in COC, inhibition of sporulation was minimum, i.e. 19.71, 37.06 and 53.91 at 200, 500 and 1000 μg/ml, respectively. Sporulation of *T. viride* was also good at all three concentrations of thiophanate methyl, i.e. 20.2, 28.6 and 41.6% at 25, 50 and 100 μg/ml, respectively. In remaining fungicides, percent inhibition of spore production by *T. viride* was influenced by fungicides and ranged from 32.01–80.9%. Since, minimum inhibitory effect on growth and spore production had noted in COC, it may be safely integrated with *T. viride* in IDM studies. Copper

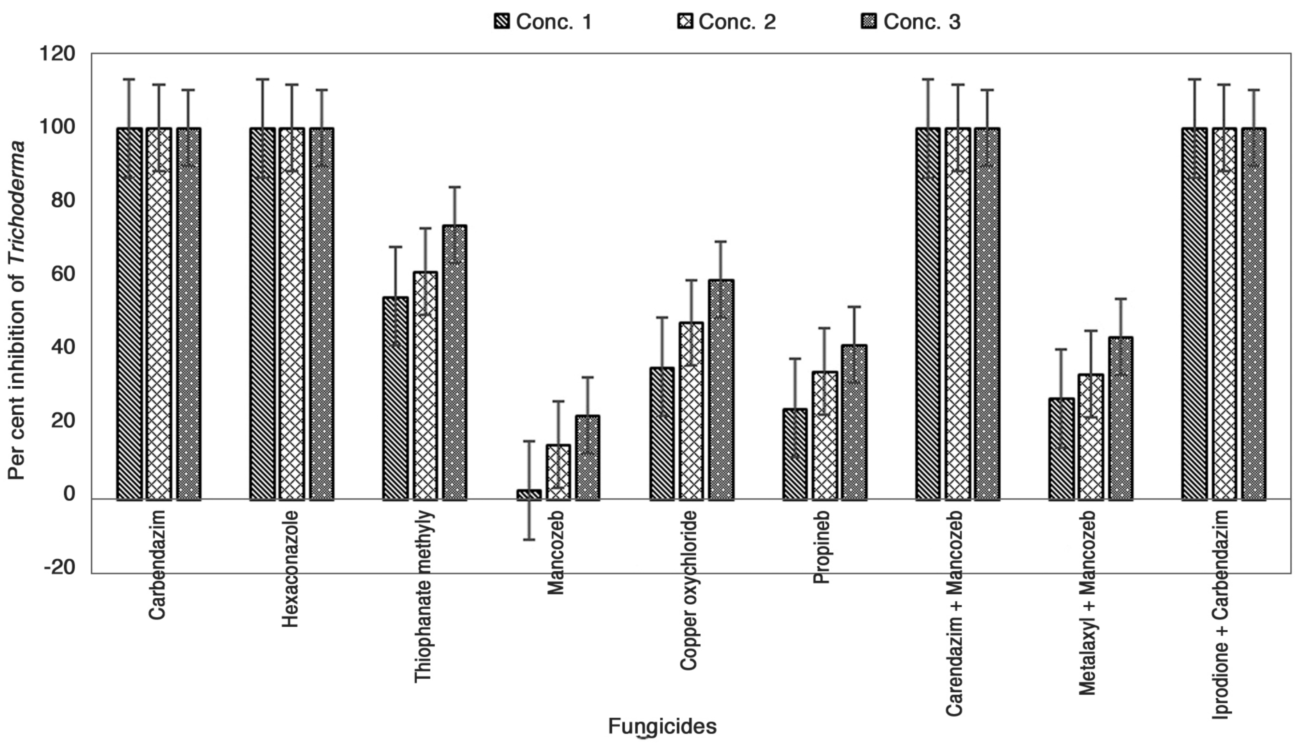

Fig 1 Effect of fungicides on growth of *Trichoderma viride.*

Table 2 Effect of fungicides on sporulation of *T. viride*

Fungicides	Concentration (µg/ml)	No. of spores/ml ($\times 10^6$)	Inhibition of spore production over check (%)
Systemic			
Carbendazim (Bavistin)	25	0.00	0.00
	50	0.00	0.00
	100	0.00	0.00
Hexaconazole (Contaf)	25	0.00	0.00
	50	0.00	0.00
	100	0.00	0.00
Thiophanate methyl (ROKO)	25	9.47	20.21
	50	8.47	28.64
	100	6.93	41.61
Non-systemic			
Mancozeb (Indofil M-45)	200	4.53	61.83
	500	3.47	70.76
	1000	2.27	80.87
Copper oxychloride (Blitox50)	200	9.53	19.71
	500	7.47	37.06
	1000	5.47	53.91
Propineb (Antracol)	200	8.07	32.01
	500	4.93	58.46
	1000	3.07	74.13
Systemic+Contact			
Carbendazim + Mancozeb (Companion)	200	0.00	0.00
	500	0.00	0.00
	1000	0.00	0.00
Metalaxyl + Mancozeb (Master)	200	4.47	62.34
	500	3.20	73.04
	1000	2.53	78.68
Iprodione + Carbendazim (Quintal)	200	0.00	0.00
	500	0.00	0.00
	1000	0.00	0.00
PDA as control		11.87	
CD (P=0.05)		0.36	
CV		6.40	

*Average of 3 replications

oxychloride was least toxic fungicides followed by propineb to growth of *T. viride in vitro.* Hence, these fungicides may be integrated safely as seed treatment along with bio-agent *T. viride*. Carbendazim either alone or in mixture proved to be highly toxic to *T. viride or* should not combine for seed or soil application along with bio-control agent (Kumar *et al*. 2020b).

Desai and Kulkarni (2004) revealed that carbendazim, chlorpyriphos and thiram were inhibitory to *T. harzianum* at 500, 1000 and 2000 ppm. Captan and Metalaxyl at 500 ppm were comparatively safe to *T. harzianum*. Bagwan (2010) also reported that thiram (0.2%), mancozeb (0.2%) and copper oxychloride (0.2%) were found to be compatible and comparatively safer to *T. harzianum* and *T. viride*. Madhavi *et al.* (2011) reported contact fungicides, viz. pencycuron and propineb were noted to be fully compatible with *T. viride*. Wafa Khirallah *et al.* (2016) showed that mepanipyrim was highly (92–81%) to moderately (48.5%) compatible with conidia germination of *Tcomp* (strains of *T. harzianum*) as increasing concentrations. Carbendazim fungicides inhibited to growth and sporulation of *T. viride*. Thiophanate methyl, mancozeb, propineb and COC restricted spore formation of *T. viride*. However, minimal effect on spore production by *T. viride* was recorded in COC, which had least effect on radial growth. Thus, COC may be safely integrated with *T. viride* in integrated management studies.

In the present study, compatibility of various systemic, non-systemic and mixture of fungicides were tested against *T. viride*. Insensitivity of *Trichoderma* was measured with different fungicide concentration. Among the fungicides, copper oxychloride was least toxic to growth and sporulation of *T. viride*. Thus, copper oxychloride was considered as safe fungicide and recommended in IDM module for soil borne diseases in addition to biological control measures.

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