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Compatibility of *Trichoderma asperellum* with fungicides

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Abstract
Fungicides applied for the management of plant diseases have a toxic effect on the biological control agents. For effective disease management, the activity of biological control agents should not be hampered by the application of fungicides. To find the compatibility of bioagents with the fungicides, we have evaluated the different fungicides against *Trichoderma asperellum in vitro*. Among contact fungicides, *Trichoderma* was compatible with copper hydroxide, copper oxychloride and mancozeb, and least compatible with captan. Whereas among systemic fungicides, metalaxyl was compatible at all the concentrations whereas tebuconazole, propiconazole and carbendazim were incompatible. Among combination fungicides, metalaxyl-M + mancozeb was found to be highly compatible whereas other combination fungicides tested were incompatible with *Trichoderma.*

Keywords: Compatibility fungicides applied management *Trichoderma asperellum*

1. Introduction
Biological control using *Trichoderma* spp has been practised worldwide (Mukhopadhyay, 1987) [19]. The use of several *Trichoderma* species has been well documented against economically important soil born plant pathogens (Cook and Baker, 1983; Chet, 1987; Raghuchander *et al*., 1997; Anitha and Tripathi, 2000; Mukerjee *et al.*, 2001) [7, 6, 24, 2, 18]. The successful reports of biocontrol activity of *Trichoderma* species were attributed to the mycoparasitism and antibiosis activity (Aluko and Hering, 1970; Chet, 1987; Elad and Kapat, 1999) [1, 6, 9], competition (Harman, 2000; Howell *et al.*, 2000) [11, 13], production of chitinases and glucanases enzymes (Metcalf and Wilson, 2001) [16], induction of defence responses (Yedidia *et al*., 1999) [31] and metabolism of germination stimulants (Howell, 2002) [12].
The management of plant diseases by employing a potential biological control agent (BCA) is always preferred over hazardous chemicals (Pandey *et al*., 2006) [21]. The pathogens can be effectively managed if the fungicide used is compatible with the bioagents without causing any toxic effect (Papavizas and Lumsden, 1980) [80]. The use of BCAs with a compatible fungicide may suppress the disease similar to the application of hazardous fungicide at a higher dose (Monte, 2001) [17]. Application of compatible synthetic chemicals with BCAs reduces the development of fungicidal resistance (Wedajo, 2015) [30].
Therefore it is required to find the compatible antagonist with commonly used fungicides in the management of plant diseases having no deleterious effect on the native BCAs. There are reports on the *Trichoderma* strains tolerant to fungicides (Papavizas *et al*., 1982). The use of combination fungicides that are compatible with the bioagents forms an effective IDM strategy by protecting seeds and seedlings from the soilborne diseases (Dubey and Patil, 2001) [8]. Many of the fungicides that are commonly used by the farmers destroy the native BCAs. To find the safe fungicide, we have evaluated the contact, systemic and combination fungicides in our present study.

2. Materials and method
2.1 Isolation of *Trichoderma* spp from rhizosphere soil
The rhizosphere soil was collected from the Shivamogga district of Karnataka, India at a depth ranging from 15-20 cm, by removing the top two cm surface soil. A working sample of 10 g soil was taken from the composite soil sample and mixed with 90 ml of sterilized distilled water. From this suspension, the *Trichoderma* spp was isolated using the serial dilution technique.

From 10^{-3} and 10^{-4} dilutions, one ml suspension was transferred into each of the three Petri plates containing the *Trichoderma* selective media (TSM) (Elad *et al.*, 1980) [10] by giving a gentle whirling motion to the plate and incubated at room temperature. After the growth of *Trichoderma*, the probable colonies were picked up from culture and observed under the microscope. The resulted *Trichoderma* isolates were subcultured using single spore isolation to get pure culture, and these cultures were transferred to potato dextrose agar slants and stored in the refrigerator at 4 ℃ for further studies. The culture was further confirmed through ITS sequencing and found as *T. asperellum* (accession number: MH383521).

2.2 Fungicides

In the present investigation we have used various contact (Captan 50% WP, Copper hydroxide 53.8% W/W, Copper oxychloride 50% WP, Mancozeb 80% WP, Propineb 70% WP) systemic (Metalaxyl 35% WS, Azoxystrobin 25% SC, Tebuconazole 25.9% EC, Propiconazole 25% EC, Carbendazim 50% WP) and combination fungicides (Metalaxyl-M 4% + Mancozeb 64% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Carbendazim12% + Mancozeb 63% WP.

2.3 Poison food technique

The quantified fungicide was prepared and mixed with the molten potato dextrose agar (PDA). Approximately 20 ml of poisoned medium was poured into sterilized Petri plates. By using the sterilized cork borer, the mycelial disc of approximately 5.00 mm diameter was dissected out from five days old culture and transferred to the centre of Petri plates. The plate containing PDA without any fungicide served as control. The experiment was replicated thrice, and the inoculated plates were incubated at 27±1°C temperature. The radial growth was measured after the maximum growth attainment was observed in control. The fungicide efficacy was expressed as per cent inhibition of mycelial growth over control (Vincent, 1947).

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

Where, I = per cent inhibition, C = growth of *Trichoderma* in the control plate, T = growth of *Trichoderma* in treatment plate

3. Results and discussion

In sustainable agriculture, improved crop production technologies coupled with plant protection strategies, play a vital role in plant disease management, thereby enhancing the production and productivity of the crops. In-plant protection, pesticides (fungicides, insecticides and herbicides) have proved their potential to combat insect pests and diseases. Simultaneously, biological control agents, especially *Trichoderma* spp. are gaining popularity for the management of plant diseases and pests and become an essential component of integrated disease management (IDM) strategy. In the framework of IDM, *Trichoderma* spp. are frequently applied in combination with various pesticides. Therefore, compatibility of *Trichoderma* with various pesticides as well as their tolerance to various dosages of the pesticides needs to be explored for economic and eco-friendly management of plant diseases/insects-pests. Hence, we have evaluated the efficacy of five contact fungicides, five systemic fungicides and four combination fungicides at different concentrations by poison food technique for the compatibility with *Trichoderma asperellum*.

Among contact fungicides tested (Fig. 1), *T. asperellum* showed compatible with copper hydroxide, copper oxychloride and mancozeb, and moderately compatible with propineb whereas, least compatible with captan (Table 1). These results were similar with the results of Bagwan (2010) [3], Bindu *et al.* (2011) [5], Ranganathaswamy *et al.* (2012) [27] and Vasundara *et al.* (2015) [29] who reported that mancozeb was compatible with *Trichoderma* species.

Among systemic fungicides (Fig. 2), metalaxyl was compatible at all concentrations, and azoxystrobin was moderately compatible, whereas, tebuconazole, propiconazole and carbendazim were incompatible (Table 2). Our findings are in agreement with the Bagwan (2010) [3] and Bindu *et al.* (2011) [5], where they reported the incompatibility of tebuconazole with *Trichoderma.* Pandey and Upadhyay (1998) [20] also reported that *T. viride* and *T. harzianum* could not be used with carbendazim even at 10 µg/ ml concentration, whereas they found the bioagent compatibility with thiram up to 50 µg /ml.

Among combination fungicides (Fig. 3), metalaxyl-M (4%) + mancozeb (64%) was found to be highly compatible whereas, tebuconazole (50%) + trifloxystrobin (25%), azoxystrobin (18.2%) + difenoconazole (11.4%) and carbendazim (12%) + mancozeb (63%) was incompatible with *Trichoderma* (Table 3). A similar observation was made by Patil *et al.* (2012) [4], who reported that metalaxyl-M + mancozeb was compatible with the growth of *Trichoderma* species. Kumhar *et al.* (2016) reported that carbendazim + mancozeb was not compatible with *Trichoderma*, which is on par with the results obtained.

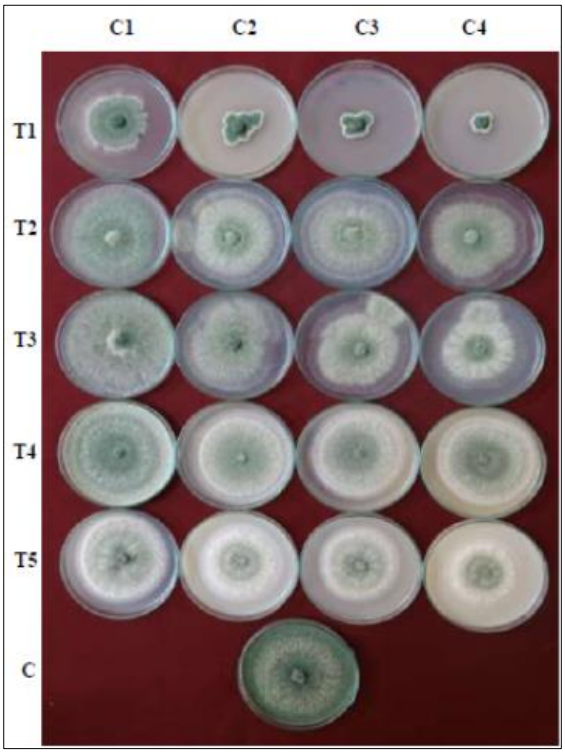

Fig 1: Effect of contact fungicides on the mycelial growth of *Trichoderma asperellum.*

T1= Captan; T2= Copper hyderoxide; T3= Copper oxychloride; T4= Mancozeb; T5= Propineb; C= Control;

C1=500 ppm; C2=1000 ppm; C3= 1500 ppm; C4= 2000 ppm.

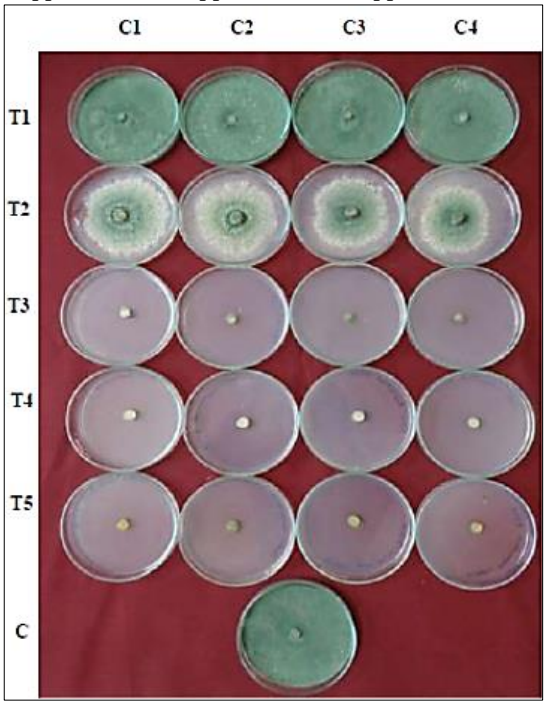

Fig 2: Effect of systemic fungicides on the mycelial growth of *Trichoderma asperellum*.

T1= Metalaxyl; T2= Azoxystrobin; T3= Tebuconazole; T4= Propiconazole; T5= Carbendazim; C= Control; C1=5 ppm; C2=25 ppm; C3= 30 ppm; C4= 100 ppm.

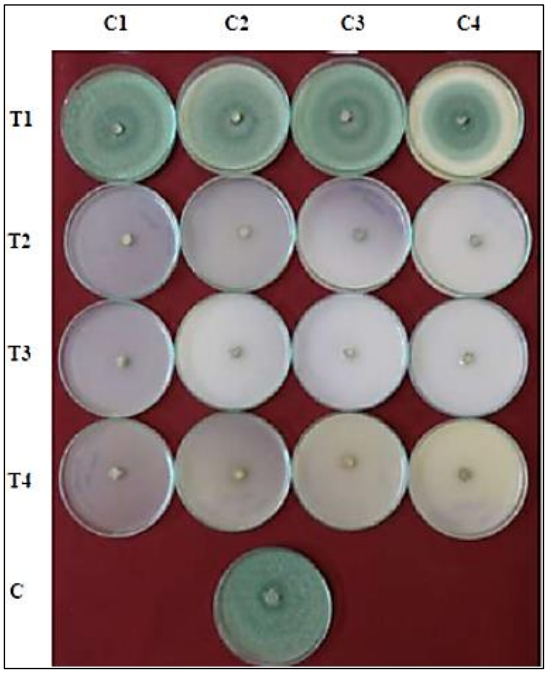

Fig 3: Effect of combination fungicides on the mycelial growth of *Trichoderma asperellum*.

T1= Metalaxyl + Mancozeb; T2= Tebucconazole + Trifloxystrobin; T3= Azoxystrobin 18.2% + Difenoconazole 11.4% SC; T4= Carbendazim12% + Mancozeb 63% WP; T5= Control; C1=1000 ppm; C2=1500 ppm; C3= 2000 ppm; C4= 2500ppm.

Table 1: *In vitro* evaluation of contact fungicides on the growth of *Trichoderma asperellum*

S. No.	Fungicides	Inhibition per cent #				Mean
		Concentrations (ppm)				
		500	1000	1500	2000	
1.	Captan 50% WP	51.10 (45.65)*	75.92 (60.64)	86.66 (68.61)	93.70 (75.50)	76.85
2.	Copper hydroxide 53.8% W/W	0.74 (4.94)	5.92 (14.1)	7.96 (16.40)	12.59 (20.79)	6.80
3.	Copper oxychloride 50% WP	0.00 (0.00)	4.81 (12.68)	11.48 (19.82)	18.89 (25.77)	8.80
4.	Mancozeb 80% WP	0.00 (0.00)	4.81 (12.68)	10.74 (19.14)	12.59 (20.79)	7.04
5.	Propineb 70% WP	11.48 (19.81)	21.11 (27.37)	33.33 (35.28)	50.74 (45.45)	29.17
6.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
			S.Em±		CD at 1%	
	Fungicides (F)		0.32		1.19	
	Concentrations (C)		0.26		0.98	
	F X C		0.65		2.39	

*Figures in parenthesis are arc sine transformed values
Mean of three replications

Table 2: *In vitro* evaluation of systemic fungicides on the growth of *Trichoderma asperellum*

S. No.	Fungicides	Inhibition per cent #				Mean
		Concentrations (ppm)				
		5	25	50	100	
1.	Metalaxyl 35% WS	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
2.	Azoxystrobin 25% SC	10.37 (18.79)	17.77 (24.94)	22.96 (28.64)	38.14 (38.16)	22.31
3.	Tebuconazole 25.9% EC	100 (90.05)	100 (90.05)	100 (90.05)	100 (90.05)	100
4.	Propiconazole 25% EC	100 (90.05)	100 (90.05)	100 (90.05)	100 (90.05)	100
5.	Carbendazim 50% WP	100 (90.05)	100 (90.05)	100 (90.05)	100 (90.05)	100
6.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
			S.Em±		CD at 1%	
	Fungicides (F)		0.08		0.31	
	Concentrations (C)		0.07		0.25	
	F X C		0.16		0.61	

*Figures in parenthesis are arc sine transformed values
Mean of three replications

Table 3: *In vitro* evaluation of combination fungicides on the *Trichoderma asperellum* growth

S. No.	Fungicides	Inhibition per cent #				Mean
		Concentrations (ppm)				
		1000	1500	2000	2500	
1	Metalaxyl-M 4% + Mancozeb 64% WP	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	12.03 (20.31)	3.01
2	Tebuconazole 50% + Trifloxystrobin 25% WG	100 (90.05)	100 (90.05)	100 (90.05)	100 (90.05)	100
3	Azoxystrobin 18.2% +Difenoconazole 11.4% SC	100 (90.05)	100 (90.05)	100 (90.05)	100 (90.05)	100
4	Carbendazim12% + Mancozeb 63% WP	100 (90.05)	100 (90.05)	100 (90.05)	100 (90.05)	100
5	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
			S.Em±		CD at 1%	
	Fungicides (F)		0.09		0.34	
	Concentrations (C)		0.08		0.31	
	F X C		0.18		0.69	

*Figures in parenthesis are arc sine transformed values

Mean of three replications

In the present study, incompatibility of *Trichoderma* with various contact, systemic and combination-fungicides may be due to their higher concentrations used as well as tolerance potential of the test native *Trichoderma.* The findings are in agreement with the earlier reports of several workers (Madhusudhan *et al.,* 2010; Rakholiya *et al.,* 2010; Ranganathswamy *et al.,* 2012; Sreeja and Girija, 2015; Rai *et al.,* 2016) [15, 26, 27, 28, 25]. The inhibitory effect of fungicides may be due to the direct effect of the toxic chemicals on the *Trichoderma* cells and spores. The differential response of antagonistic microorganism to various fungicides may be due to their ability to degrade chemicals and inherent resistance to most fungicides (Papavizas, 1985) [22].

The per cent of compatibility decreased with an increase in the concentration of fungicide. Reduced amount of fungicide can weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist. Therefore, rather than applying these chemicals alone, it is imperative to use *Trichoderma* in combination with fungicides at the lower concentration for effective management of fungal pathogens since they do not have a side effect on the environments.

4. Conclusions

Trichoderma was compatible with copper hydroxide, copper oxychloride, mancozeb and metalaxyl and least compatible with captan whereas it was incompatible with tebuconazole, propiconazole and carbendazim. Among combination fungicides, metalaxyl + mancozeb was found to be highly compatible whereas tebuconazole + trifloxystrobin, azoxystrobin + difenoconazole and carbendazim + mancozeb were incompatible.

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