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COMPATIBILITY ASSESSMENT OF INDIGENOUS *TRICHODERMA* STRAINS WITH FUNGICIDE MOLECULES THROUGH *IN VITRO* ANALYSIS

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ABSTRACT

A study on compatibility of native strains of *Trichoderma* sp. with fungicides was ascertained at the Department of Plant Pathology, Dr Kalam Agricultural College, Kishanganj (under BAU, Sabour, Bhagalpur) during 2022-23. The compatibility studies revealed that among the eight fungicides tested (each @ 100, 250, and 500 ppm), carbendazim 50% WP, Propiconazole 25 EC, and Tebuconazole 25.9% EC were incompatible to all *Trichoderma* isolates. However, only three fungicides viz., Metalaxyl 4% + Mancozeb 64% WP, Mancozeb 75% WP and Copper oxychloride 50 % WP were found to be compatible and Azoxystrobin 250 EC, Azoxystrobin 18.2% W/W + Difenconazole 11.4% W/W was moderately compatible with all the test *Trichoderma* isolates. The compatible fungicides and doses of the test fungicides may be recommended for the farmers to manage plant diseases under integrated disease management practices.

Keywords **:** Biological control, Compatibility, Pesticides, Disease management.

Introduction

Trichoderma is a genus of asexually reproducing saprophytic fungus that is found in almost all temperate to tropical soils, decaying plant tissues, and root ecosystems. *Trichoderma* spp. are effective biocontrol agents because they are potent antibiotic producers, rapid growers, prolific spore producers, and strong opportunistic invaders (Benitez *et al*. 2004). *Trichoderma* has a distinctive morphology that includes phialids, hyaline or green conidia carried in slimy heads, tufted or postulate conidiophores, and repeatedly branching conidiophores (Bisset 1984).

The genus *Trichoderma* is a member of the class Sordariomycetes, order Hypocreales, family Hypocreaceae, and phylum Ascomycetes. More than 200 years ago, Persoon (1794) in Germany published the first description of the genus *Trichoderma*. *Trichoderma* as bio-control agent was first reported by Weindling in 1932. Tulasne brothers described Hypocrea, a teleomorph of *Trichoderma*, in 1865. (Gams and Bissett, 2002). *Trichoderma* is an effective bio-control agent because of their high reproductive capacity, efficiency in nutrient uptake, ability to modify the rhizosphere, ability to be aggressive against plant pathogenic fungi, and effectiveness in promoting plant growth and defense mechanisms. This fungus, in addition to being used as a bio-control agent, serves as an excellent growth stimulant, solubilizes nutrients, produces numerous hydrolytic enzymes, and induces resistance in plants to various abiotic and biotic stresses. (Harman, 2011).

Commercial preparations of *Trichoderma* species have been utilised for biological management of fungal-induced plant diseases. *Trichoderma harzianum* is the active component in the product TRICHODEX, which is used to treat apple postharvest rot, and it is coupled with *T. polysporum* in the product BINAB-T, which is used to treat wound decay and wood rot.

(Ricard, 1981) The most widely utilised species in biological control are *T. harzianum and T. virens.* (Papavizas, 1985). *T. reesei's* capacity to break down cellulosic materials by producing cellulase enzymes has led to commercial use (Kubicek *et al*., 1996). Because of their wide metabolic capabilities and intense competitive character, they were excellent colonisers of their environments. (Gams and Bissett, 2002).

Trichoderma biocontrol mechanism involves coiling around the host, the production of appressoria and the disintegration of the host cell wall, antibiosis, and competition for resources such as space and nutrients. (Tiwari *et al.,* 2021)

T. virens, T. atroviride, and T. reesei were the first species in the genus to have their genome sequenced, allowing for more in-depth research of the genus *Trichoderma.* (Guzman *et al*., 2023). *Trichoderma* secondary metabolites can activate disease-fighting systems inside plants and protect them against infections. Exogenous indicators that might be utilised to identify and monitor specific *Trichoderma* isolates in agro-ecosystems include glucuronidase (GUS), green fluorescent protein (GFP), hygromycin B phosphotransferase (HygB), and generating genes. (Manzar *et al*., 2022).

Materials and Methods

Soil samples were collected from the rhizosphere soil of Zone II of Bihar and were sieved, shade dried and used for isolation of *Trichoderma using* serial dilution and pour plate technique. Further, sub-cultured on the PDA medium to get the pure cultures using hyphal tip technique (Tuite, 1969) and their pure cultures were preserved in the refrigerator and used for further experiments.

Compatibility of *Trichoderma spp*. with fungicides

In the present study eight fungicides, were selected for testing the compatibility with fungal biocontrol agents at various concentrations, by applying Poisoned food technique (Nene and Thapliyal, 1993), and using PDA as basal culture medium.

Required quantity of fungicides for each chemical was calculated using the formula, $N_1V_1 = N_2V_2$. In order to achieve the correct concentrations, the necessary quantity of the test fungicides was mixed with autoclaved and chilled (to room temperature) PDA medium in conical flask. Poisoned PDA with the test fungicides was put aseptically into petri plates (20 ml per plate). Three replications were maintained for each of the test fungicides and its test concentrations using CRD (Completely Randomized Design). After solidification of PDA medium, each plate was individually and aseptically inoculated with 5.0 mm mycelial disc taken from an actively growing mycelium of seven-day-old pure culture of *Trichoderma* sp. such as TR 1, TR 2, TR 3, TR 4, TR 5, TR 6 and incubated for four days at a temperature of 28°C. Petri plates poured with PDA (without any fungicides) and inoculated separately with TR 1, TR 2, TR 3, TR 4, TR 5, TR 6 and were maintained as untreated control.

Assessment of growth

Colony growth of *Trichoderma* isolates was assessed by measuring the diameter (mm) of mycelial growth using the measuring scale after four days of incubation. Per cent inhibition of colony growth was calculated by using the formula given by Vincent (1947).

$$I = [(C-T) / C] * 100$$

Were,

I = Percent growth inhibition.

C = Colony diameter in control (mm).

T = Colony diameter in treatment (mm).

Statistical analysis

The mycelial growth diameter and percentage inhibition of *Trichoderma* isolates were analyzed using OPSTAT software, which conducted a statistical examination of factorial CRD data.

Table 1: Treatment details of compatibility study of *Trichoderma* sp. with fungicides

Treatment detail	
Name of Fungicide	**Concentration (ppm)**
Mancozeb 75% WP	100, 250, 500
Propiconazole 25 EC	100, 250, 500
Tebuconazole 25.9% EC	100, 250, 500
Copper oxychloride 50% WP	100, 250, 500
Carbendazim 50% WP	100, 250, 500
Azoxystrobin 18.2% W/W + Difenconazole 11.4% W/W	100, 250, 500
Azoxystrobin 250 EC	100, 250, 500
Metalaxyl 4% + Mancozeb 64% WP	100, 250, 500
Control (untreated)	

Results and Discussion

The result revealed that among the eight test fungicides, four systemic fungicides *viz.,* Carbendazim 50% WP, Propiconazole 25 EC, Tebuconazole 25.9% EC, Azoxystrobin 250 EC and two contact fungicides (Mancozeb 75% WP, Copper oxychloride 50% WP) and two combi- products (Azoxystrobin 18.2% W/W +

Difenconazole 11.4% W/W, Metalaxyl 4% + Mancozeb 64% WP) at three concentrations viz., 100, 250, 500 ppm evaluated against *Trichoderma* isolates under *in vitro* conditions indicated that lower concentration (100-250ppm) of fungicides were found to be highly compatible with *Trichoderma* sp. as compared to higher concentration (500 ppm). Among, eight fungicides tested Carbendazim 50% WP, Propiconazole 25 EC, and Tebuconazole 25.9% EC were incompatible with all the *Trichoderma* isolates at all concentrations (100, 250 and 500 ppm) by inhibiting cent per cent mycelial growth of all the isolates. The data are presented in the Table 2, 3, and 4.

At 100 ppm

Metalaxyl 4% + Mancozeb 64% WP, Mancozeb 75% WP and Copper oxychloride 50% WP were found to be compatible with all the isolates (Tr 1, Tr 2, Tr 3, Tr 4, Tr 5 and Tr 6) showing 0% inhibition and growth of colony was 90 mm. In Azoxystrobin 250 EC treated plates, isolate Tr 2 showed maximum inhibition 11.11% followed by Tr 6 (10.74%), Tr 1 (5.93%), Tr 4 (4.07%) and Tr 3 (3.93%) at 100 ppm concentration.

In case of Azoxystrobin 18.2% W/W + Difenconazole 11.4% W/W Isolate Tr 4 showed maximum inhibition (73.70%) followed by Tr 2 (25.56%), Tr 5 (22.22%), Tr 6 (13.70) and Tr 3 (13.70) at 100 ppm. Carbendazim 50% WP, Propiconazole 25 EC, and Tebuconazole 25.9% EC were incompatible with all the *Trichoderma* isolates at 100 ppm (Table 2, Fig 1).

At 250 ppm

Most of the tested *Trichoderma* isolates (Tr 1, Tr 2, Tr 3 and Tr 4) were found compatible with (Metalaxyl 4% + Mancozeb 64% WP) and Mancozeb 75% WP. Tr 2 and Tr 3 were found compatible with copper oxychloride 50% WP. While all the *Trichoderma* isolates Tr 1 to Tr 6 were completely inhibited by Carbendazim 50% WP, Propiconazole 25 EC, and Tebuconazole 25.9% EC indicating non-compatibility with these fungicides at 250 ppm. All the tested *Trichoderma* isolates showed partial compatibility with Azoxystrobin 18.2% W/W + Difenconazole 11.4% W/W (Table 3, Fig 2).

At 500 ppm

Trichoderma isolates Tr 1, Tr 2, Tr 3, Tr 5 and Tr 6 were found to be compatible with Metalaxyl 4% + Mancozeb 64% WP with less than 10% inhibition of colony growth. Tr 1 and Tr 4 were found to be compatible with Mancozeb 75% WP with less than 20% inhibition. Isolates Tr 1, Tr 2, Tr 5 and Tr 6 were performed better than less than 20% inhibition with Copper oxychloride 50% WP. With Azoxystrobin 250 EC Tr 3 and Tr 6 found compatible with less than 20% inhibition. All the *Trichoderma* isolates were found non-compatible with Carbendazim 50% WP, Propiconazole 25 EC, and Tebuconazole 25.9% EC with Cent percent inhibition. Data is shown in (Table 4, Fig 3).

Table 2: Compatibility of *Trichoderma* isolates with different fungicides at 100 ppm

Trichoderma Isolates	Metalaxyl 4% + Mancozeb 64 % WP		Mancozeb 75 % WP		Copper oxychloride 50 % WP		Azoxystrobin 250 EC		Azoxystrobin 18.2 % W/W+ Difenconazole 11.4 % W/W		Carbendazim 50 % WP		Tebuconazole 25.9 % EC		Propiconazole 25 EC	
	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)
Tr 1	90.00	0.00	90.00	0.00	90.00	0.00	84.67	5.93	90.00	0.00	0.00	100.00	0.00	100.00	0.00	100.00
Tr 2	90.00	0.00	90.00	0.00	90.00	0.00	80.00	11.11	67.00	25.56	0.00	100.00	0.00	100.00	0.00	100.00
Tr 3	90.00	0.00	90.00	0.00	90.00	0.00	86.47	3.93	86.67	3.70	0.00	100.00	0.00	100.00	0.00	100.00
Tr 4	90.00	0.00	90.00	0.00	90.00	0.00	86.33	4.07	23.67	73.70	0.00	100.00	0.00	100.00	0.00	100.00
Tr 5	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	70.00	22.22	0.00	100.00	0.00	100.00	0.00	100.00
Tr 6	90.00	0.00	90.00	0.00	83.33	7.41	80.33	10.74	77.67	13.70	0.00	100.00	0.00	100.00	0.00	100.00
Control	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.0	0.00	90.00	0.00
CD @ 1 %	N/S		N/S		2.47		4.571		3.26		0.782		1.156		1.744	
C.V.	Nil		Nil		1.545		3.003		2.62		3.439		5.086		7.673	

*Average of three replications

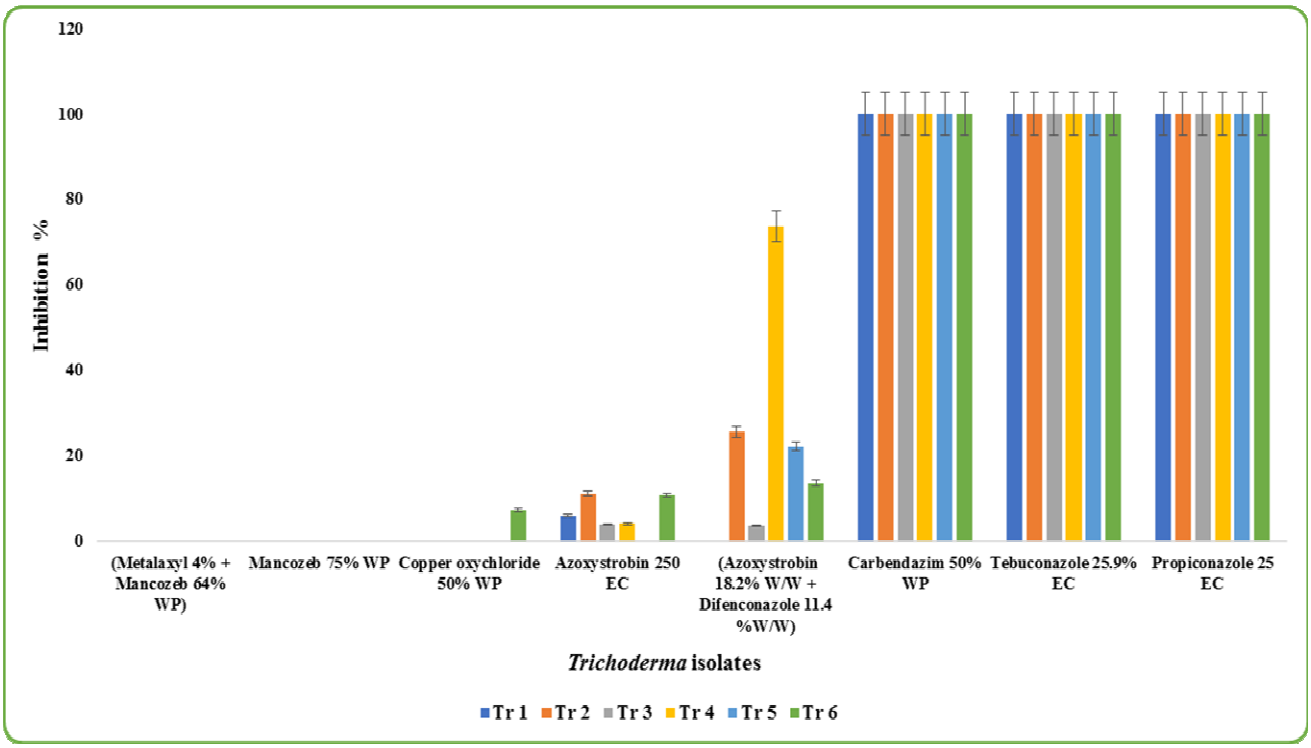

Fig. 1: Tolerance of *Trichoderma* isolates with different fungicides at 100 ppm

Table 3: Compatibility of *Trichoderma* isolates with different fungicides at 250 ppm

Trichoderma Isolates	Metalaxyl 4%+Mancozeb 64 % WP		Mancozeb 75% WP		Copper oxychloride 50% WP		Azoxystrobin 250 EC		Azoxystrobin 18.2 % W/W + Difenconazole 11.4 % W/W		Carbendazim 50% WP		Tebuconazole 25.9 % EC		Propiconazole 25 EC	
	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)
Tr 1	90.00	0.00	90.00	0.00	83.00	7.78	62.33	30.74	27.33	69.63	0.00	100.00	0.00	100.00	0.00	100.00
Tr 2	90.00	0.00	90.00	0.00	90.00	0.00	77.67	13.70	25.67	71.48	0.00	100.00	0.00	100.00	0.00	100.00
Tr 3	90.00	0.00	90.00	0.00	90.00	0.00	83.33	7.41	45.00	50.00	0.00	100.00	0.00	100.00	0.00	100.00
Tr 4	90.00	0.00	90.00	0.00	35.33	60.74	44.67	50.37	23.00	74.44	0.00	100.00	0.00	100.00	0.00	100.00
Tr 5	87.17	3.70	86.67	3.70	88.50	1.67	80.67	10.37	16.67	81.48	0.00	100.00	0.00	100.00	0.00	100.00
Tr 6	83.33	7.04	83.67	7.04	80.33	10.74	79.33	11.85	32.67	63.70	0.00	100.00	0.00	100.00	0.00	100.00
Control	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00
C.D@1%	2.705		3.498		3.321		4.312		1.427		N/A		N/A		N/A	
C.V.	1.701		2.2		2.371		3.361		2.795		2.7		2.9		2.3	

*Average of three replications

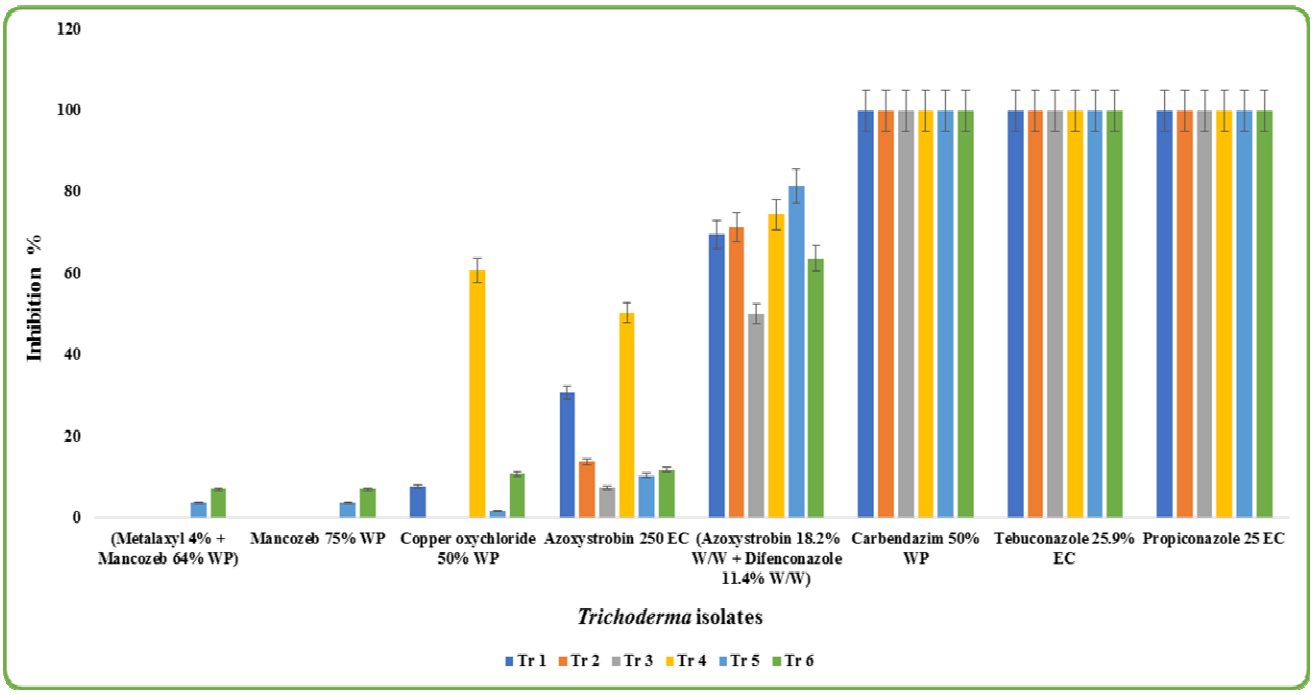

Fig. 2: Tolerance of *Trichoderma* isolates with different fungicides at 250 ppm

Table 4: Compatibility of *Trichoderma* isolates with different fungicides at 500 ppm

Trichoderma Isolates	Metalaxyl 4% + Mancozeb 64 % WP		Mancozeb 75 % WP		Copper oxychloride 50 % WP		Azoxystrobin 250 EC		Azoxystrobin 18.2 % W/W + Difenconazole 11.4 % W/W		Carbendazim 50 % WP		Tebuconazole 25.9 % EC		Propiconazole 25 EC	
	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)	G (mm)*	I (%)
Tr 1	90.00	0.00	74.67	17.04	75.33	16.30	59.33	34.07	23.00	74.44	0.00	100.00	0.00	100.00	0.00	100.00
Tr 2	86.67	3.70	29.00	67.78	76.00	15.56	66.00	26.67	14.00	88.44	0.00	100.00	0.00	100.00	0.00	100.00
Tr 3	88.40	1.78	46.00	48.89	90.00	0.00	78.33	12.96	12.00	86.67	0.00	100.00	0.00	100.00	0.00	100.00
Tr 4	56.67	37.04	82.33	8.56	30.33	66.30	20.90	76.78	12.17	86.48	0.00	100.00	0.00	100.00	0.00	100.00
Tr 5	84.00	6.67	38.33	57.41	88.00	2.22	70.33	21.85	6.93	92.30	0.00	100.00	0.00	100.00	0.00	100.00
Tr 6	80.67	10.37	40.00	55.56	79.67	11.48	76.67	14.81	18.00	80.00	0.00	100.00	0.00	100.00	0.00	100.00
Control	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00
C.D@1%	4.713		1.573		2.87		2.916		0.846		1.14		1.323		0.962	
C.V.	3.232		1.691		2.179		2.618		3.276		5.03		5.821		4.233	

*Average of three replications

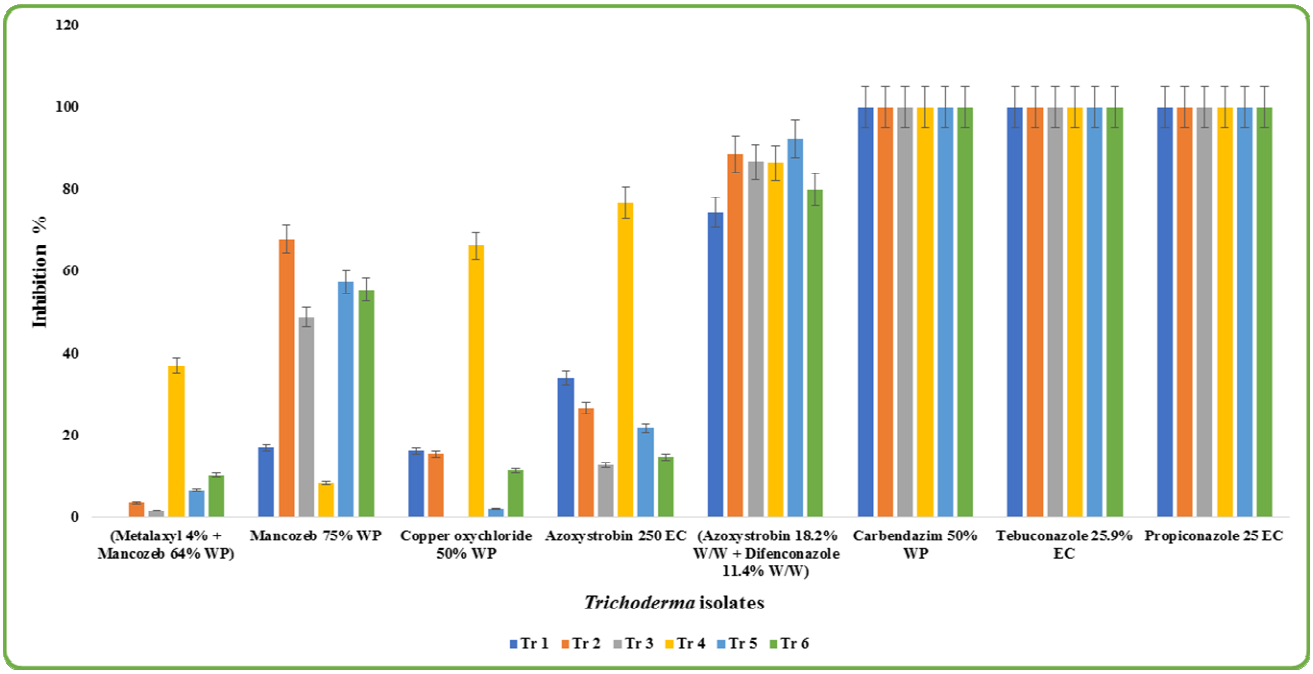

Fig. 3: Tolerance of *Trichoderma* isolates with different fungicides at 500 ppm

The results were closely supported with the findings of Tomer *et al*. (2018) who conducted an experiment to test the compatibility of four fungicides viz. Mancozeb, Thiram, Carboxin and Propiconazole with *Trichoderma harzianum* at 25, 50, 75 and 100 ppm and found that Mancozeb was highly compatible with all the four concentrations. Thiram was less compatible than Mancozeb and Carboxin and Propiconazole was toxic and incompatible with *Trichoderma harzianum.* Mishra *et al*. (2019) evaluated nine fungicides for their compatibility with bioagent *Trichoderma viride.* He observed that Mancozeb showed least inhibition of 42.96% at 200 ppm. Five other fungicides namely Azoxystrobin, Tebuconazole, Hexaconazole, Carbendazim, Propiconazole completely inhibited the growth and hence not compatible with *Trichoderma viride*. Kumar *et al*. (2019) revealed that *T. viride* was not compatible with Carbendazim 50%WP, Propiconazole 25%EC and Hexaconazole 5%EC even at 50 ppm concentration and 100 per cent inhibition in growth of *T. viride* was observed at 50 ppm and above concentrations. Thus the findings of current study is supported by the findings of Kumar *et al*. . Maheshwary *et al*. (2020) tested the compatibility of *Trichoderma asperellum* with different fungicides where they found that *Trichoderma asperellum* was most compatible with Copper hydroxide, Copper oxychloride, Metalaxyl, Mancozeb and least compatible with Captan. Whereas it was incompatible with Carbendazim, Tebuconazole and Propiconazole. Among combination fungicides Metalaxyl-M + Mancozeb was extremely compatible with *Trichoderma asperellum*. These findings are in accordance with current findings with respect to compatibility of Metalaxyl + Mancozeb, Mancozeb, Copper oxychloride and incompatibility of Carbendazim, Tebuconazole and Propiconazole with *Trichoderma* isolates. Poudel *et al*. (2023) reported that 100 ppm of Copper oxychloride and 100,200 and 300 ppm of Mancozeb and Metalaxyl + Mancozeb was found to be compatible with *T. harzianum* and highest inhibition (100%) was observed in Carbendazim + Mancozeb, Carbendazim and Hexaconazole even at lower concentration of 100 ppm. These findings also support the findings of present studies.

Conclusion

Among eight tested fungicides (each @ 100, 250, and 500 ppm), Carbendazim 50% WP, Propiconazole 25 EC, and Tebuconazole 25.9% EC showed 100 per cent inhibition of mycelial growth indicating the incompatibility of these fungicides with all tested *Trichoderma* isolates. However, only three fungicides viz., Metalaxyl 4% + Mancozeb 64% WP, Mancozeb 75% WP and Copper oxychloride were found to be compatible with all the test *Trichoderma* isolates at lower concentrations of 100 and 250 ppm. While Azoxystrobin 250 EC, Azoxystrobin 18.2% W/W + Difenconazole 11.4% W/W showed moderately compatibility with all test *Trichoderma* isolates at all concentrations. The compatible fungicides may further screen at field conditions and can be used for sustainable and eco-friendly management of crop diseases under integrated disease management

practices in order to reduce the use of harmful and higher doses of inorganic fungicides.

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Competing Interests

Authors have declared that no competing interests exist.

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