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Compatibility of multifacial isolates of *Trichoderma* species with six common fungicides used against soil-borne fungal pathogens

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The compatibility of six fungicides *viz*., azoxystrobin,carbendazim, chlorothalonil, propiconazole,metalaxyl and thifluzamide with six multi-facial biocontrol isolates of *Trichoderma* species *viz*., *Trichoderma harzianum* AMUTH-1*, T. harzianum* AMUTH-2, *T. harzianum* AMUTH-3, *T. asperellum* (= *T. viride*) AMUTV-1, *T. asperellum* AMUTV-3 and *T. virens* (= *Gliocladium virens*) AMUTS-1 were tested by the poisoned food method at six different concentrations (10, 25, 50, 100, 250 and 500 ppm). The highest concentration of fungicide at which the lowest inhibition of *Trichoderma* isolates was observed, is regarded as maximum tolerance concentration (MTC). Among the fungicides,the highest compatibility was observed with thifluzamide followed by metalaxyl and carbendazim at MTC value of 200-500 ppm.Among the *Trichoderma* isolates, *Trichoderma harzianum* AMUTH-1, *Trichoderma asperellum* AMUTV-1 and*Trichoderma harzianum* AMUTH-3 showed maximum compatibility with thifluzamide,metalaxyl and carbendazim.The lowest MTC was observed with propiconazole (10 ppm) followed by chlorothalonil (25 ppm) and azoxystrobin (50 ppm) with all *Trichoderma* isolates. The study demonstrated the high compatibility of three fungicides (thifluzamide,metalaxyl and carbendazim) with all *Trichoderma* isolates except *T. virens* AMUTS-1 and their MTC ranged from 200 to 500 ppm, which was significantly higher than the recommended doses of these fungicides. Hence, fungicidal contamination at the above mentioned concentration in the soil will not affect the effectiveness of the above *Trichoderma* isolates. However, the fungicides propiconazole, chlorothalonil and azoxy strobinare not advised to be applied in conjugation with biopesticides (*Trichoderma* isolates) under integrated disease management for soil-borne pathogens.

Keywords: Biocontrol,chemical control,fungicide sensitivity, plant-pathogenic fungi, poison food technique

INTRODUCTION

Soil-borne plant pathogenic fungi *viz*., *Fusarium oxysporum*, *Macrophomina phaseolina,Pythium aphanidermatum*, *Phytophthora* spp., *Sclerotinia sclerotiorum, Rhizoctonia solani*, etc. are incredibly damaging and severely reduce crop yields (Khan *et al.* 2021; Khan and Haque, 2022). Microbial antagonists can be a useful tool for controlling plant diseasese, specially those caused by soil-borne fungi (Mohiddin *et al.* 2010; Haque and Khan, 2023). *Trichoderma* species are cosmopolitan and till date 488 species have been identified (Moo-Koh et al. 2022).

A number of these species have received extensive research as biocontrol agents for plant pathogenic fungi (Li *et al.* 2019) and plant-parasitic nematodes (Haque and Khan 2022), and also serve in plant growth promotion (Khan and Mohiddin 2018). The fungicidal potential of *Trichoderma* species is increasingly being utilised to develop new and safer biocontrol agents against soil-borne pathogens such as *Pythium aphanidermatum* (Khan and Haque 2022), *Fusarium oxysporum* (Dubey *et al.* 2007), *Rhizoctonia solani* (Haque *et al.* 2018), *Macrophomina phaseolina* (Singh *et al.* 2012) and *Phytophthora* spp. (Osorio-HernÃ¡ndez 2016). Application of *Trichoderma* spp. are easier and safer to human beings, environment and non target organisms. *Trichoderma* species suppress the plant pathogens through various mechanisms such as mycoparasitism, antibiosis (Mohiddin *et al.* 2010), enzyme production (Khan and Mohiddin 2018) and induced systemic resistance (Haque and Khan, 2022).

Despite the well-known negative consequences on the environment and human health, chemical

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fungicides are still one of the best disease control strategies in plant protection. Several fungicides are commonly used against soil-borne diseases (Widmer, 2019). Among these, the broad spectrum fungicide azoxystrobin belonging to strobilurins group, is effective against ascomycetes, deuteromycetes,basidiomycetes and oomycetes. It interrupts the activity of electron transport chain and prevents the germination of fungal spores and halts the growth process of the fungus (Kanetis *et al.* 2007). The benzimidazole group compound, carbendazim is very effective against soil-borne fungi (Garcia *et al.* 2001). Chlorothalonil is a halogenated fungicide, belongs to the organochlorides group and is widely used against the diseases caused by *Phytophthora* spp. and *Alternaria* spp. It interferes with the enzyme systems of the fungus and prevents germination of spores (Kanetis *et al.* 2007). Metalaxyl belongs to phenylamine group (acylalanines) and used to control plant diseases caused by Oomycetes (Wong and Wilcox, 2001). It selectively interferes with DNA synthesis by inhibiting the growth of mycelium and the formation of spores and haustoria. Propiconazole belongs to the Triazoles group and is effective against rusts, mildew and blights pathogens by inhibiting the activity of 1,4á-sterol demethylase which prevents the production of essential ergosterols for fungal cell membranes.Thifluzamide is a succinate dehydrogenase inhibitor (SDH) fungicides with protective and curative action and effectively controls *Rhizoctonia solani* in various crops (Mu *et al*.2017).

A careful study of the available data revealed that *Trichoderma* species *viz*., *T. asperellum, T. harzianum, T. hamatum, T. virens* etc.have demonstrated their compatibility with most of the conventional fungicides i.e., captan, thiram (Mohiddin and Khan, 2013), mancozeb (Madhusndhan *et al.* 2010), and some new fungicides cyazofamid, myclobutanil, puraclostrobin, boscalid (Widemer, 2019) and fluopyram (Haque *et al.* 2023). However, the compatibility of most commonly used fungicides against soil-borne pathogens such asazoxystrobin,chlorothalonil, propiconazole, metalaxyl and thifluzamide have been partially explored with multifacial *Trichoderma* species, mainly because of unknown compatibility. Hence, potential prospects exist for their joint application with multifacial *Trichoderma* isolates for integrated disease mangement. With this background, the present investigation was carried out to check the compatibility of above these fungicides with six proven multifacial biocontrol isolates of *Trichoderma* species (Haque *et al.* 2018; Haque and Khan, 2022) based on their of minimum inhibitory concentration (MIC) and maximum tolerance concentration *in vitro* condition. Compatibility testing of the above six new fungicides with six potential *Trichoderma* species certainly adds new information on the integrated disease management, if they did not act mutually suppressive.

MATERIALS AND METHODS

Trichoderma isolates

Six indigenous *Trichoderma* isolates, *viz*., *Trichoderma harzianum* AMUTH-1 (NCBI GenBank Accessions no. KM435269), *T. harzianum* AMUTH-2 (NAIMCC, ICAR-NBAIM, Mau Accessions no. NAIMCC-F-04335), *T. harzianum* AMUTH-3 (NCBI accessions no. KY062569), *T. asperellum* (= *T. viride*) AMUTV-1 (NAIMCC accessions no. NAIMCC-F-04337), *T. asperellum* (= *T. viride*) AMUTV-3 (NCBI accessions no. KY062571) and*T. virens* (=*Gliocladium virens*) AMUTS-1 (NAIMCC accessions no. NAIMCC-F-04336) were previously identified and selected for this study based on their multifacial nature and biocontrol capability against sheath blight fungus, *Rhizoctonia solani* (Haque and Khan 2021), root-rot pathogen, *Pythium aphanidermatum* (Haque and Khan 2022) and rice root-knot nematode, *Meloidogyne graminicola* (Haque *et al*. 2018).

Fungicides and their doses

Six fungicides *viz*., azoxystrobin (Azoxycure™ 23 SC, Best Agrochem Pvt. Ltd., India), carbendazim (Bavistin™, 50 WP, Tata Holset, India), chlorothalonil (Kavach™ 75 WP, Syngenta India Ltd., India), metalaxyl (Himil Gold™ 35 WS, Insecticides India Ltd., India), propiconazole (Dhan™ 25 EC, Indofil Industries Ltd., India) and thifluzamide (Pulsar™ 24 SC, Insecticides India

Ltd., India) were obtained from an authorised dealer of pesticide in Aligarh, India. For compatibility, six concentrations *viz*., 10 ppm, 25 ppm, 50 ppm, 100 ppm 200 ppm, and 500 ppm of respective fungicides were prepared on active ingredient basis and used in this study.

Determination of minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) of nematicides against mycelial growth of Trichoderma isolates in vitro

The compatibility of six *Trichoderma* isolates with six fungicides were tested by the poisoned food technique (Dhingra and Sinclair, 1995). Liquid sterilized potato dextrose agar (PDA, Himedia, India™) medium was poured in Petri plates (12 cm dia.) having fungicides solution of 10, 25, 50, 100, 200 and 500 ppm concentration active ingredient basis. Thereafter, the medium was allowed to solidify. A mycelial disc (9 mm dia.) of *Trichoderma* isolates were placed in the middle of the Petri plates separately with five replicates for each concentration. An appropriate control plate was also maintained without adding fungicide. The plates were incubated in a BOD incubator at 27 ± 2°C temperature and the diameter (in mm) of colonies was recorded on the 7th day. The per cent inhibition of growth was calculated using the formula: PI = {(C – T) /C} X 100, where C is the growth of *Trichoderma* isolate (mm) in the control plate and T is the growth of *Trichoderma* isolate (mm) in the fungicide amended medium plate.

The lowest concentration of fungicide at which the maximum inhibition of *Trichoderma* isolates was observed, is regarded as minimum inhibitory concentration (MIC). Similarly, the highest concentration of fungicide at which the lowest inhibition of *Trichoderma* isolates was observed, is regarded as maximum tolerance concentration (MTC). The test was repeated two times with the same procedure to verify the data reproducibility.

Statistical analysis

ANOVA (analysis of variance) was used to process all the data using SPSS 11.0 for Windows-11. The differences between the data from the two repeated studies were non-significant at *P* d" 0.05, henceforth, the data were pooled (10 replicates per treatment). The data were analysed using a two-factor ANOVA, with the *Trichoderma* isolates as one factor and fungicides doses (ppm) as the second, and F-values were also calculated to recognize significant treatments (*Pd*" 0.05).

RESULTS AND DISCUSSION

Maximum tolerance concentration (MTC)

Among the fungicides, the highest compatibility in term of maximum tolerance concentration (MTC) was observed with thifluzamide (500 ppm) followed by metalaxyl (200 ppm) and carbendazim (200ppm) as revealed in Table1.The lowest MTC (10 ppm) was observed with propiconazole with all *Trichoderma* isolates followed by chlorothalonil and azoxystrobin except *T. harzianum* AMUTH-1(Table 1). Carbendazim exhibited surprising result and did not cause any significant inhibition to the mycelial growth of *Trichoderma* isolates up to 100 ppm. However, at 200 and 500 ppm, it caused 42-68% inhibition to *Trichoderma* isolates(Table 1).Whereas in the case of thifluzamide at the concentration of 500 ppm there was no effect on the mycelial growth of *Trichoderma* isolates except *T. virens* AMUTS-1 (Fig. 1). Chlorothalonil also inhibited the mycelial growth of *Trichoderma* isolates except *T. harzianum* AMUTH-1 up to 200 ppm (Table1). However, when the concentration of chlorothalonil was increased to 500 ppm, there was a significant increase in the mycelial growth inhibition of *T. harzianum* AMUTH-1 (Fig. 1).

Among *Trichoderma* isolates, *T. harzianum* AMUTH-1 showed the highest compatibility with most of the fungicides and the highest MTC value 500 ppm was recorded toward thifluzamide followed by metalaxyl (Fig. 1).The next MTC was observed with*T. asperellum* AMUTV-3 (500 ppm thifluzamide and 200 ppm metalaxyl), followed by *T. harzianum* AMUTH-3 (500 ppm thifluzamide and 200 ppm metalaxyl) and *T. asperellum* AMUTV-1 (500 ppm thifluzamide and 200 ppm metalaxyl). All the isolates of*Trichoderma* exhibited 100% inhibition at the lowest MTC (10 ppm) towards the fungicides propiconazole (Table

Table. 1: Determination of minimum inhibitory concentration (MIC) and maximum tolerance concentration (MTC) of six fungicides against six *Trichoderma* isolates *in-vitro*

Fungicides	*Trichoderma* isolates	Percent inhibition in the mycelial growth with different concentrations						LSD $P \leq 0.05$
		10 ppm	25 ppm	50 ppm	100 ppm	200 ppm	500 ppm	
Azoxystrobin	*T. asperellum* AMUTV-1	26.67	41.11	51.11	56.67	54.44	95.56	7.53
	T. asperellum AMUTV-3	22.22	24.44	43.33	44.44	57.78	100.00	8.51
	T. harzianum AMUTH-1	27.20	30.00	33.33	38.89	47.78	90.50	7.83
	T. harzianum AMUTH-2	31.11	48.89	51.11	54.13	55.56	92.22	7.03
	T. harzianum AMUTH-3	28.89	41.11	45.56	46.67	52.22	93.33	8.60
	T. virens AMUTS-1	25.56	41.11	48.89	50.00	52.22	100.00	8.07
Carbendazim	*T. asperellum* AMUTV-1	4.41	7.78	21.11	33.33	53.33	68.89	5.40
	T. asperellum AMUTV-3	0.00	3.33	15.56	23.33	44.44	67.78	5.67
	T. harzianum AMUTH-1	0.00	4.44	14.04	24.44	42.22	65.56	6.20
	T. harzianum AMUTH-2	0.00	2.22	11.11	25.45	48.89	68.80	5.77
	T. harzianum AMUTH-3	3.30	6.67	18.89	31.03	51.11	67.71	4.80
	T. virens AMUTS-1	6.67	12.20	30.00	44.44	58.89	72.22	6.57
Chlorothalonil	*T. asperellum* AMUTV-1	76.67	78.89	83.33	85.56	87.78	90.80	7.53
	T. asperellum AMUTV-3	28.89	65.56	80.00	81.11	84.44	90.00	7.51
	T. harzianum AMUTH-1	14.44	15.56	24.44	25.56	31.11	43.33	3.07
	T. harzianum AMUTH-2	77.78	80.03	82.22	83.33	88.68	91.11	8.03
	T. harzianum AMUTH-3	78.89	83.33	85.35	87.78	92.22	93.33	8.60
	T. virens AMUTS-1	62.22	73.33	76.67	83.33	84.44	92.22	8.83
Metalaxyl	*T. asperellum* AMUTV-1	16.67	23.33	12.22	5.56	10.00	28.89	2.02
	T. asperellum AMUTV-3	7.78	12.22	14.44	17.78	23.33	25.56	2.67
	T. harzianum AMUTH-1	0.00	4.44	6.67	8.60	9.16	16.07	1.40
	T. harzianum AMUTH-2	13.33	16.67	18.33	21.11	25.56	41.11	2.77
	T. harzianum AMUTH-3	16.67	18.89	19.09	21.11	24.17	42.22	2.80
	T. virens AMUTS-1	25.56	30.00	30.00	31.11	33.33	51.11	3.57
	T. asperellum AMUTV-1	100.00	100.00	100.00	100.00	100.00	100.00	-
	T. asperellum AMUTV-3	100.00	100.00	100.00	100.00	100.00	100.00	-
	T. harzianum AMUTH-1	100.00	100.00	100.00	100.00	100.00	100.00	-

Propiconazole	*T. harzianum* AMUTH-2	100.00	100.00	100.00	100.00	100.00	100.00	-
	T. harzianum AMUTH-3	100.00	100.00	100.00	100.00	100.00	100.00	-
	T. virens AMUTS-1	100.00	100.00	100.00	100.00	100.00	100.00	-
Thifluzamide	*T. asperellum* AMUTV-1	0.00	0.00	0.00	4.44	7.11	11.14	1.53
	T. asperellum AMUTV-3	10.00	6.11	12.22	14.15	17.21	19.22	1.51
	T. harzianum AMUTH-1	0.00	0.00	0.00	5.56	7.78	11.11	1.83
	T. harzianum AMUTH-2	0.00	0.00	3.30	9.52	15.56	17.78	2.03
	T. harzianum AMUTH-3	0.00	1.11	3.33	8.89	10.00	12.22	1.60
	T. virens AMUTS-1	31.11	44.44	47.78	52.22	53.25	55.56	4.07
LSD	(*P*≤0.05)	5.27	4.98	4.75	5.76	6.12	9.27	-
F values	*P*≤0.05)							
Trichoderma (T)	(df=5)	16.7	16.9	17.4	16.2	17.1	16.6	-
Dose (D)	(df=9)	6.2	5.8	NS	11.7	9.2	5.9	-
T x D	(df=44)	14.1	9.9	NS	6.2	4.6	NS	-

Data are means of ten replicates. *F*-values are significant otherwise not significant (NS) at *P*d”0.05.

1). Overall, the MTC order for *Trichoderma* isolates for thifluzamide were; *Trichoderma harzianum* AMUTH-1 (500 ppm) >*T. harzianum* AMUTH-3 (500 ppm)>*T.asperellum* AMUTV-1 (500 ppm) >*T. harzianum* AMUTH-2 (500 ppm) >*T. asperellum* AMUTV-3 (500 ppm) >*T. virens* AMUTS-1 (10 ppm) (Table 1).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of six fungicides substantially varied with the *Trichoderma* isolates (Table1). Among the fungicide,the MIC value of propiconazole was found lowest (10 ppm) against all *Trichoderma* isolates. Among the *Trichoderma* isolates,the MIC value of thifluzamide was found highest against *T.* AMUTH-1 (500 ppm) followed by *T. harzianum* AMUTH-3 (500 ppm), *T. asperellum* AMUTV-1(500 ppm), *T. harzianum* AMUTH-2 (200 ppm) >*T. asperellum* AMUTV-3 (200 ppm) and *T. virens* AMUTS-1 (10 ppm) (Table1). Similarly, the MIC value of metalaxyl was found highest against *T. harzianum* AMUTH-1 (500 ppm) followed by *T. aperellum* AMUTV-1 (500 ppm), *T. asperellum* AMUTV-3 (200 ppm), *T. harzianum* AMUTH-2 (200 ppm), *T. harzianum* AMUTH-3(200 ppm) and *T. virens* AMUTS-1 (10 ppm) (Table 1). In general, inhibition in the *Trichoderma* colonization exerted by the fungicide increased with an increase in the concentration (Table1). In the case of *T. harzianum* AMUTH-1, an increase in the chlorothalonil concentration from 100 ppm to 200 ppm significantly enhanced mycelial growth inhibition and reduced the MIC level (Table1).

The compatibility of six multi-facial *Trichoderma* isolates viz., *Trichoderma harzianum* AMUTH-1, *T. harzianum* AMUTH-2, *T. harzianum* AMUTH-3, *T. asperellum* (= *T. viride*) AMUTV-1, *T. asperellum* (= *T. viride*) AMUTV-3 and *T. virens* (= *Gliocladium virens*) AMUTS-1 against six fungicides revealed their higher tolerance toward three fungicides thifluzamide, metalaxyl and carbendazim up to 500 ppm. While the other three fungicides azoxystrobin, chlorothalonil and propiconazole showed significant inhibition of *Trichoderma* isolates at 10-500 ppm. The *Trichoderma* isolates, *T. harzianum* AMUTH-1, *T. asperellum* AMUTV-3, *T. harzianum* AMUTH-3, *T. asperellum* AMUTV-1 and *T. harzianum* AMUTH-2 were demonstrated to be most compatible against thifluzamide and did not exhibit any mycelial inhibition up to a concentration of 500 ppm, whereas, *T. virens* AMUTS-1 showed the lowest compatibility and

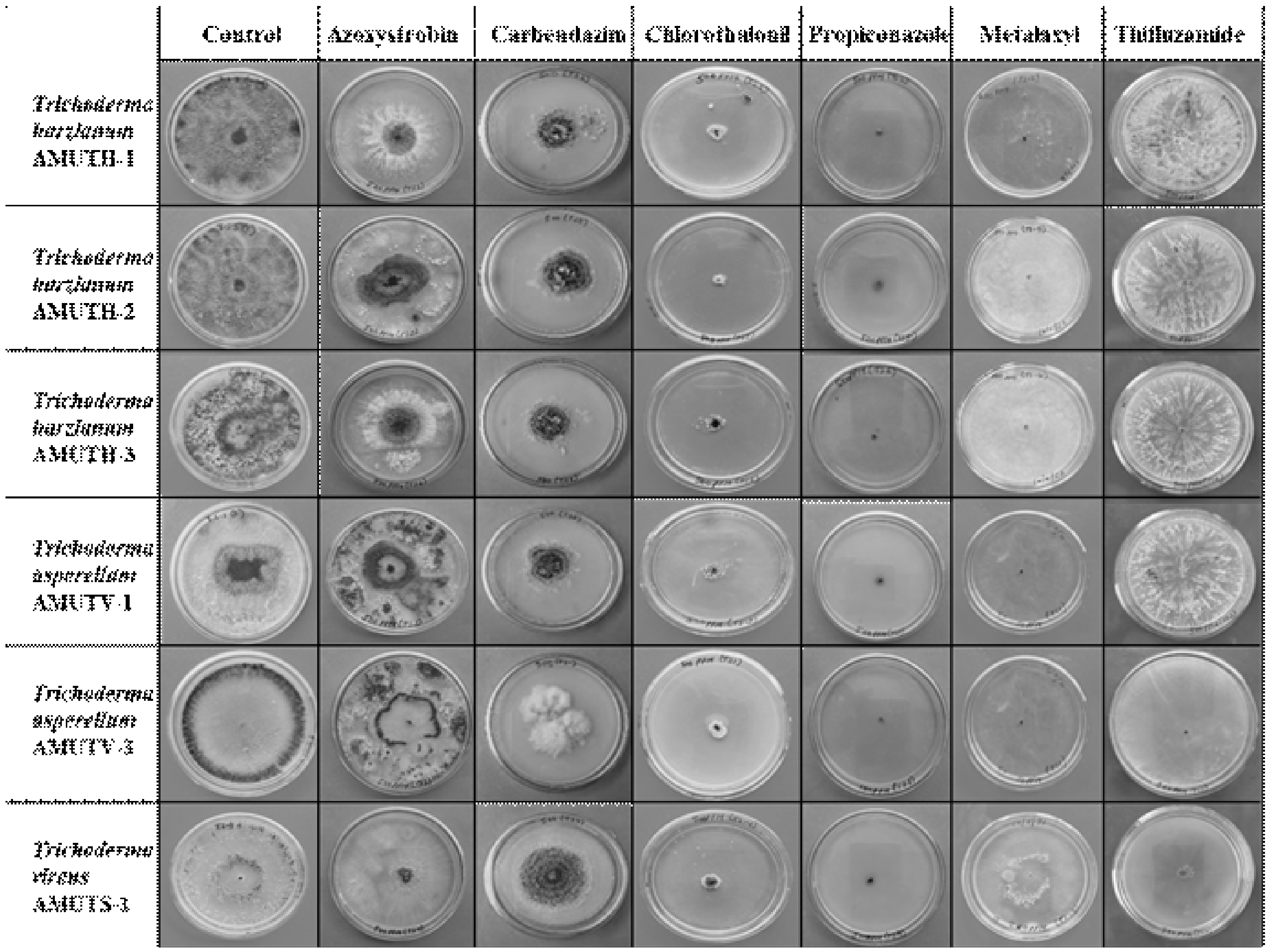

Fig. 1 : *In-vitro* compatibility of six fungicides with*Trichoderma* isolates at 500 ppm

31.11% of mycelial inhibition were recorded at 10 ppm concentration. Similarly, the *T. harzianum* AMUTH-1 exhibited the lowest mycelial inhibitionat 500 ppm of metalaxyl and carbendazim. The fungicides captan, thiram, and mancozeb have all been described as *T. harzianum*-tolerant even at greater concentrations up to 2000 mg/ml (Mohiddin and Khan, 2013). Somewhat comparable results with other fungicides have been recorded by other workers also (Vasundara *et al.* 2015; Singh *et al.* 2019). However, the response of chlorothalonil against all tested *Trichoderma* isolates were in contrast with the study conducted by Madhusndhan *et al.* (2010) which showed their higher compatibility with*Trichoderma viride* at 250 ppm.

Trichoderma species are opportunistic, avirulent plant symbionts and act as parasites or antagonists to many phytopathogens, particularly soil-borne plant pathogens (Mohiddin *et al*. 2010; Haque *et al*. 2018). So far, *Trichoderma* spp. are among the most effective biocontrol fungi and are commercially marketed as potent biopesticides, biofertilizer and also used in organic amendments (Moo-Koh *et al.* 2022).The varied response of *Trichoderma* isolates towards the MTC and MIC of tested fingicides may be attributed to their relative virulence. Varied virulence responses of *Trichoderma* isolates are generally expressed through different mechanisms such as rapid colonization/ competence (Mohiddin *et al.* 2010) and greater toxin production (Harman *et al*. 2004). Greater MTC value by *T. harzianum* AMUTH-1 than *T. asperellum* AMUTV-1 and *T. virens* AMUTS-1 against thifluzamide, metalaxyl and carbendazim may be due to the lower production of antifungal metabolites by the former isolates (Singh *et al.* 2012; Akrami and Yousefi 2015). A similar type of response of *Trichoderma* species against different fungicides also revealed by other workers (Madhusndhan *et al.* 2010; Vasundara *et al.* 2015; Singh *et al.* 2019; Widmer, 2019).

Overall, the compatibility tests revealed that the *Trichoderma* isolates exhibited more tolerance to thifluzamide and metalaxyl than the rest of the fungicides. Sharma *et al.* (2001) also found that *T. harzianum* was showing more tolerance to the fungicide metalaxyl when compared to carbendazim corroborating our result. Interestingly, at higher concentrations (500 ppm) of thifluzamide, *T. harzianum* AMUTH-1 and *T. asperellum* AMUTV-3 exhibited luxuriant mycelial growth and a significant decrease in growth inhibition was recorded. This may be due to the reason that the *Trichoderma* isolates might used the fungicide as a nutrient and hence can tolerate a much higher concentrations of chemicals as observed in the present study (Mohiddin and Khan, 2013).

We have demonstrated the compatibility of thifluzamide(up to 500 ppm), metalaxyl (up to 200 ppm) and carbendazim (up to 200 ppm) with six isolates of *Trichoderma* spp. except *T. virens* and their MTC were significantly higher than the recommended doses of all three fungicides. Thifluzamide showed higher compatibility than metalaxyl, carbendazim, azoxystrobin, chlorothalonil and propiconazole, and can be successfully used to manage soil-borne plant pathogens in various crops by integrating with *Trichoderma* isolates at recommended doses. The fungicidal contamination of metalaxyl and carbendazim up to 200 ppm concentration in the soil will also not affect the effectiveness of the above multifacial*Trichoderma* isolates except *T. virens* under integrated nematode management. The fungicides propiconazole, chlorothalonil and azoxystrobin are not advised to be applied in conjugation with biopesticides (*Trichoderma* isolates) under integrated disease management for soil-borne pathogens.However, multi-locational field trials are required to confirm their performance before recommendation for commercial use for soil-borne pathogens.

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