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Compatibility of Fungicides with Potent *Trichoderma* Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The sensitivity of biological control agents were tested against fungicides that are used to treat plant diseases. Application of fungicides should not interfere with biological control agents ability to effectively manage disease. *Trichoderma* is an aggressive coloniser of soil and plant roots that can flourish in a variety of environmental circumstances. It also functions as a natural bioagent to shield plants against infection by soil-borne fungal infections. Evaluation of the various fungicides against five potent *Trichoderma* isolates *in vitro* was done in order to determine the compatibility of bioagents with the fungicides was performed at the Department of Plant Pathology, College of Agriculture, JNKVV, Jabalpur. *Trichoderma* was highly compatible with the following contact, systemic, and combi fungicides: Imbrex (Fluxapyroxad EC), Curzate M8 (Cymoxanil 8%+ Mancozeb 64% WP), Vitavax (Carboxin 37.5% + Thiram 37.5% DS) and Seedkot (Thiram 75% WS), while it proved just moderately compatible with Amistar top (Azoxystrobin 18.2% + Difenoconazole 11.4% SC) and Headline (Pyraclostrobin 20% WG). It showed least compatibility with Nativo (Tebuconazole 50%+ Trifloxystrobin 25% WG), Taqat (Captan 70%+ Hexaconazole 5% WP), Folicur (Tebuconazole 25.9% EC), and Saaf (Carbendazim 12% + Mancozeb 63% WP).

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1. INTRODUCTION

Plant diseases can be managed using a variety of biological control agents (BCAs). These consist of bacteria, fungi, and actinomycetes. The most significant BCAs are found in the genus *Streptomycetes*, *Bacillus* species, *Pseudomonas* species, and *Trichoderma* species. A promising alternative to decreasing modern agriculture's heavy reliance on expensive chemical fungicides, which not only pollute the environment but also encourage the emergence of resistant strains, is biological control of plant infections [1].

Mycoparasitism, antibiosis, competition for nutrients or space, increased root and plant development, induced resistance, solubilization and sequestration of inorganic nutrients, and inactivation of the pathogens' enzymes are just a few of the most current mechanisms [2]. In addition to their capacity for biocontrol, BCAs also exhibit traits like competence in the rhizosphere, resistance to fungicides, saprophytic competitiveness, tolerance of extremes in temperature, adaptability to various environmental conditions, good searching abilities, host specificity, high reproduction rates, short life cycles, adaptability, and the capacity to maintain themselves while reducing host populations [3] have demonstrated that *Trichoderma viride* displaced the yam tuber's naturally occurring microflora.

Achieving sustainable global food security will be a challenging task with the growing human population and shifting global food consumption patterns brought on by climate change [4]. Therefore, management of plant diseases should focus on eco-friendly measures and use of bioagents for plant disease management will certainly serve as a crucial strategy to achieve it. However, combined use of bioagent along with need-based fungicide may result in better control of plant diseases. Therefore, compatibility of different fungicide with bioagent shall lead to identification of safe fungicide to be used in combination with potential bio-agent like *Trichoderma* species. IDM strategies that combine fungicides and suitable bio agents shield seeds and seedlings from soil- and seed-borne pathogens [5]. It may be more effective to treat disease and manage soil-borne diseases if appropriate bio agents are used with fungicides [6]. Similar disease control would be accomplished by combining BCAs and fungicides as opposed to using more fungicides alone [7]. By combining antagonists with synthetic compounds, the potential for resistance development is avoided, and the need for fungicide application is decreased. Therefore, it is suggested to determine whether prospective bio agents are compatible with widely used fungicides for the environmentally friendly approach to treat tea diseases. Understanding the effects of fungicides on the pathogen and the antagonists would help in the selection of fungicides and fungicide resistant antagonists through compatibility studies *in vitro*. Fungicides should have an inhibitory effect on the pathogen but shouldn't have a detrimental effect on the antagonists. Additionally, this approach might show even better control of resistant fungal pathogen strains and aid commercial producers in using less fungicides, reducing the amount of chemical residue in the marketed goods. Combining BCA sprays with small doses of fungicides afterward may help the formulations' antagonists and relative cost [8].

Trichoderma species is a globally identified successful bioagent which not only control plant diseases [9-11] but can also be used as biofertilizer [12] and in production of several secondary metabolites [13]. They are also helpful in plant growth promotion [14,15] and bioremediation [16]. Further, their use as a native isolate have proven better potential in the local area for successful bio-control agent after proper identification and characterization [17-19]. On a variety of crops, *Trichoderma* species have been shown to inhibit root infection by soil-borne diseases such as *Macrophomina phaseolina, Rhizoctonia solani, Fusarium* species, and *Pythium* species [20]. *Trichoderma* species can promote growth, which may or may not be essential for biological control [21]. Effective suppression of root-infecting fungi and root-knot nematodes has been demonstrated by *Trichoderma harzianum* [22,23]. Rhizome rot was inhibited by *Trichoderma harzianum*, which was isolated from the soil [24]. It also promoted plant growth and yield. According to reports, numerous *Trichoderma* species exhibit varying degrees of innate and/or induced resistance to various fungicides. In the context of this, research was done to see if it was possible to combine *Trichoderma* species with fungicides in a lab conditions. The long-term objective is to create an effective IDM package for controlling soil-borne plant diseases and arresting the spread of pathogens resistant to fungicides. A crucial component of integrated disease management is including chemical-resistant *Trichoderma* species. Utilizing tolerant species that keep infections under sufficient pressure to limit their growth can help to prevent disease to a greater extent. Tr-1 (*Trichoderma sp.*), Tr-5 (*T. harzianum*), Tr-7 (*T. yunnanense*), Tr-13 (*T. asperellum*) and Tr-15 (*T. asperellum*) were used as test organisms for the *in vitro* compatibility of different fungicides with the BCA.

2. MATERIALS AND METHODS

2.1 Isolation of *Trichoderma* Species from Rhizosphere Soil

The top two centimeters of surface soil were removed in order to collect the rhizosphere soil from the several agro climatic zones of Madhya Pradesh, India, at a depth ranging from 15-20 cm. One g of soil was removed from the composite soil sample and mixed with 9 ml of sterilized distilled water to create a working sample. The serial dilution method was used to separate *Trichoderma* species from this sample. One ml suspension was added to each of the three Petri plates containing the potato dextrose agar media (PDA) [25] from the 10^{-3} and 10^{-4} dilutions. The plates were gently whirled, and the mixture was then incubated at room temperature. After *Trichoderma* had grown, the most probable colonies were plucked from the culture and examined under a microscope. To obtain pure cultures, the resulting *Trichoderma* isolates were sub-cultured using single spore isolation. These pure cultures were then transferred to potato dextrose agar slants and kept in the refrigerator at 4°C for future research. All 16 *Trichoderma* isolates were evaluated for their ability to control the important soil-borne plant pathogens such as *Fusarium oxysporum* f. sp. *ciceri, Sclerotium rolfsii, Sclerotinia sclerotiorum, Rhizoctonia solani*, and *Rhizoctonia bataticola*. Five of the best performed *Trichoderma* isolates from this screening were chosen to assess their fungicide compatibility. Through ITS sequencing, the identity of these five chosen *Trichoderma* cultures as Tr-1 (*Trichoderma sp.*-OR150423), Tr-5 (*T. harzianum*- OR150424), Tr-7 (*T. yunnanense*- OR150425), Tr-13 (*T. asperellum*- OR150426) and Tr-15 (*T. asperellum*- OR150427) was further validated.

2.2 Fungicides

There were ten fungicides used in the research which were contact, systemic, and combi fungicides. The fungicides were Amistar Top (Azoxystrobin 18.2% + Difenoconazole 11.4% SC), Saaf (Carbendazim 12% + Mancozeb 63% WP), Seedkot (Thiram 75% WS), Taqat (Captan 70%+ Hexaconazole 5% WP), Curzate M8 (Cymoxanil 8%+ Mancozeb 64% WP), Nativo (Tebuconazole 50%+ Trifloxystrobin 25% WG), Headline (Pyraclostrobin 20% WG), Imbrex (Fluxapyroxad EC), Folicur (Tebuconazole 25.9% EC) and Vitavax (Carboxin 37.5% + Thiram 37.5% DS).

2.3 Compatibility of *Trichoderma* sp. with Fungicides under *In vitro* Conditions

Ten fungicides were tested for compatibility with the five *Trichoderma* isolates using the poisoned food method Dhingra and Sinclair [26]. For each treatment, 150 ml of PDA was taken in a 250 ml conical flask and sterilized. To achieve the appropriate concentration at lukewarm temperature, the test chemical was added to this medium in the quantity according to recommended doses and thoroughly mixed by shaking the flasks. Aseptically poured into sterilized Petri plates, the poisoned medium was allowed to solidify. Three replications were maintained for each treatment. With a sterile cork borer, mycelial discs (5 mm in diameter) of the test isolates of *Trichoderma*, Tr-1, Tr-5, Tr-7, Tr-13, and Tr-15 culture, were removed from the colony's periphery and placed in the center of poisoned media in each Petri plate. *Trichoderma* species discs (5mm) were placed onto Petri plates with untreated medium (i.e., without chemicals) to maintain appropriate controls. At 28°C in a BOD, all of the inoculated Petri plates were incubated. After five days of inoculation, the colony diameter of all isolates of *Trichoderma* species was measured in, with the average value being recorded. The following formula [27] was used to compute the percentage of inhibitions of the fungal mycelial growth.

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

PI = Percentage inhibition
C = Radial growth of the *Trichoderma* in control plate (cm)
T = Radial growth of the *Trichoderma* in treatments (cm)

3. RESULTS

It is highly challenging to manage soil-borne plant diseases using just one method of control. In order to control the diseases carried on by several soil borne pathogens, integrated disease management (IDM) is a viable strategy. Since biological control has grown to be one of the key elements of IDM, it is inevitable that biocontrol agents will frequently be applied alongside fungicides to plants, soil, or both, which could cause either synergism or antagonistic interactions between them. The compatibility of *Trichoderma* sp. with various fungicides regularly used in annual crops was assessed in this work *in vitro* because most commonly used fungicides have either positive, negative, or neutral effects on biocontrol agents.

3.1 Isolation and Identification of *Trichoderma* Species from Rhizosphere Soil

The good colonies of *Trichoderma* species were obtained from the 10^{-4} dilutions. The *Trichoderma* colonies were identified by their cultural, morphological characteristics. The *Trichoderma* colonies were dark green to yellowish white in colour and in some species typical ring formation was observed. The colonies were confirmed by observing them under microscope for the presence of typical phialides and the spores.

3.2 Compatibility of *Trichoderma* Species with Fungicides

Total five (Tr-1, Tr-5, Tr-7, Tr-13, and Tr-15) *Trichoderma* isolates were tested for compatibility with systemic, contact and broad spectrum fungicides at three distinct concentrations: 50 ppm, 100 ppm, and 150 ppm.

1. Amistar Top (Azoxystrobin 18.2% + Difenoconazole 11.4% SC)

Amistar Top was compatible (100%) with *Trichoderma* isolates Tr-15 and Tr-7 at 50, 100 and 150 ppm concentrations, and it inhibits the mycelial growth to 5.55% at 150 ppm in Tr-7, according to an *in-vitro* compatibility investigation of *Trichoderma* isolates with this fungicide (Table 1, Plates 1 & 2). However, in Tr-1, Tr-5, and Tr-13, the percentage inhibition of mycelial growth of *Trichoderma* rises from 50 to 150 ppm. Tr-13 exhibited inhibition of 19.63% (50 ppm), 24.81% (100 ppm), and 28.88% (150 ppm). In Tr-1 inhibition % ranges from 36.66 to 54.81%, and similarly in Tr-5 it ranges from 21.11to 34.81%. Comparing Tr-1 to other *Trichoderma* isolates and the control, this fungicide was found to be less compatible with Tr-1.

2. Saaf (Carbendazim 12% + Mancozeb 63% WP)

Saaf significantly suppressed the development of *Trichoderma* isolates Tr-1, Tr-7, and Tr-15 at all tested concentrations, according to an *in vitro* compatibility investigation of *Trichoderma* isolates with this fungicide (Table 2, Plates 1 & 2). As compared to isolate Tr-5, which grew up to 16 mm at 50 and 100 ppm and 2.33 mm at 150 ppm. The mycelial growth in Tr-13 was recorded 13.33 mm (50 ppm), 10.67 mm (100 ppm), and 0 mm (150 ppm). This reveals that even at concentrations of 50 ppm and higher, none of the isolates were compatible with Saaf.

Table 1. Compatibility of *Trichoderma* isolates with amistar top fungicide

Amistar Top (Azoxystrobin 18.2% + Difenoconazole 11.4% SC)						
Isolates	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)
Tr-1	57.00	36.66	53.67	40.36	40.67	54.81
Tr-5	71.00	21.11	66.00	26.66	58.67	34.81
Tr-7	90.00	0.00	90.00	0.00	85.00	5.55
Tr-13	72.33	19.63	67.67	24.81	64.00	28.88
Tr-15	90.00	0.00	90.00	0.00	90.00	0.00
Control	90.00	-	90.00	-	90.00	-
SEm±	0.72	-	1.43	-	0.90	-
CD 0.01%	3.11	-	6.16	-	3.89	-

Table 2. Compatibility of *Trichoderma* isolates with SAAF fungicide

Isolates	SAAF (Carbendazim 12% + Mancozeb 63% WP)					
	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial Growth (mm)	Percent inhibition (%)	Mycelial Growth (mm)	Percent Inhibition (%)	Mycelial growth (mm)	Percent Inhibition (%)
Tr-1	0.00	100.00	0.00	100.00	0.00	100.00
Tr-5	16.00	82.22	16.00	82.22	2.33	97.41
Tr-7	0.00	100.00	0.00	100.00	0.00	100.00
Tr-13	10.67	88.14	13.33	85.18	0.00	100.00
Tr-15	0.00	100.00	0.00	100.00	3.00	96.66
Control	90.00	-	90.00	-	90.00	-
SEm±	0.36	-	0.59	-	1.55	-
CD 0.01%	1.56	-	2.56	-	6.70	-

3. Seedkot (Thiram 75% WS)

The compatibility tests of *Trichoderma* isolates with the various tested concentrations of thiram (Table 3, Plates 1 & 2) revealed excellent results at all concentrations of 50 ppm, 100 ppm, and 150 ppm. Compared to the control, where Tr-2 at all doses showed growth of 90.00 mm. The isolate Tr-15 had 100% compatibility (90 mm growth) up to 100 ppm, but at 150 ppm, it began to fall a bit, with 85.33 mm (5.18% inhibition) of mycelial growth. The isolates Tr-13 and Tr-7 were shown to be 100% (90 mm) compatible at 50 ppm, however Tr-1 showed an inhibition of 18.88% mycelial growth. However, as the concentration of Thiram was increased from 100 ppm to 150 ppm, the percentage of growth inhibition for both isolates of *Trichoderma* continued to rise, reaching up to 3.33-15.18% inhibition in isolate Tr-13, 17.77-39.25% in isolate Tr-7, and 19.63-29.63% in isolate Tr-1, respectively.

4. Taqat (Captan 70%+ Hexaconazole 5% WP)

The compatibility assay of *Trichoderma* isolates was tested with three concentrations of Taqat, it revealed that Taqat significantly inhibited the growth of *Trichoderma* isolates at 50 ppm concentration, with percent inhibitions of Tr-5, Tr-13, Tr-1, Tr-15, and T7 recorded as 87.77%, 86.30%, 83.70%, 79.63, and 78.88%, respectively, in comparison to control (Table 4, Plates 1 & 2). All *Trichoderma* isolates showed decreased growth when Taqat concentration was increased from 100 to 150 ppm, and inhibition was observed for Tr-1(87.77-88.88 %), Tr-5(91.11-94.07 %), Tr-7(86.30-90.37%), Tr-13(91.11-94.44%), and Tr-15(88.52-92.58%), revealed incompatibility of *Trichoderma* with Taqat at all the concentrations tested.

5. Curzate M8 (Cymoxanil 8%+ Mancozeb 64% WP)

Curzate M8 was found to be compatible with all *Trichoderma* isolates up to 100 ppm in an *in vitro* compatibility testing of *Trichoderma* (Table 5, Plates 1 & 2). With the exception of Tr-5, which showed inhibition of 6.30% (50 ppm) and 15.92% (100 ppm), whereas, all isolates of *Trichoderma* showed full Petri plate growth of 90 mm at 50 and 100 ppm concentrations. This reveals their 100% compatibility at 50 and 100 ppm concentrations. However, the compatibility continued to decline when Curzate M8 concentration was further increased. It was found that *Trichoderma* isolates Tr-15, Tr-1, and Tr-5, respectively, each showed 10.74%, 11.47%, and 22.96% growth inhibition at 150 ppm concentration when compared to the control, while Curzate M8 was found to be 100% compatible with isolates Tr-7 and Tr-13 at 150 ppm concentration.

6. Nativo (Tebuconazole 50%+ Trifloxystrobin 25% WG)

Nativo's compatibility study showed that *Trichoderma* isolates could not grow well in the presence of this fungicide, even at lower concentration of 50 ppm (Table 6, Plates 1 & 2). Inhibition rates for Tr-1, Tr-5, Tr-7, Tr-13, and Tr-15 isolates were calculated to be 81.85%, 83.33%, 91.11%, 85.18%, and 75.55%, respectively. However, as the concentration of Nativo was increased from 50 ppm to 100 ppm, greater percentage of *Trichoderma* isolates' development was inhibited, reaching as high as 84.44% (Tr-1), 89.25% (Tr-5), 94.44% (Tr-7), 95.92% (Tr-13), and 88.14% (Tr-15), respectively. The colony growth of the *Trichoderma* isolates Tr-5 and Tr-13 was thoroughly examined and fully inhibited upon raising the concentration of Nativo from 100 ppm to 150 ppm, while Tr-1, Tr-15, and Tr-7 each shown 90.74%, 90.74%, and 94.44% inhibition, respectively.

7. Headline (Pyraclostrobin 20% WG)

Headline compatibility test (Table 7, Plates 1 & 2) showed that there was a 1.85-48.52% reduction in growth of the isolates of *Trichoderma* starting at 50 ppm concentration compared to control. The colony diameter continued to decline as Headline concentration was raised. As compared to the control, *Trichoderma*'s growth was inhibited by a percentage ranging from 1.47 to 57.77% at 100 ppm and 6.66 to 58.88% at 150 ppm. This demonstrates that Headline and isolates of *Trichoderma* are only moderately compatible.

8. Imbrex (Fluxapyroxad EC)

Fluxapyroxad was found to be highly compatible with all *Trichoderma* isolates in an *in vitro* compatibility study, with the exception of Tr-5 isolate, which exhibits mycelial growth of 84 mm, 77.33 mm, and 67.67 mm at 50 ppm, 100 ppm, and 150 pm, respectively (Table 8, Plates 1 & 2). From a concentration of 100 ppm (89 mm) to 150 ppm (83 mm), Fluxapyroxad began to inhibit *Trichoderma* growth in Tr-7. At doses of 100 to 150 ppm, it was found that the growth rates of the *Trichoderma* isolates Tr-1, Tr-13, and Tr-15 did not show any significant reduction. The results revealed that *Trichoderma* and Fluxapyroxad are extremely compatible up to 150 ppm concentrations with most of the isolates.

Table 3. Compatibility of *Trichoderma* isolates with Seedkot fungicide

Isolates	Seedkot (Thiram 75% WS)					
	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial Growth (mm)	Percent Inhibition (%)	Mycelial Growth (mm)	Percent Inhibition (%)	Mycelial Growth (mm)	Percent Inhibition (%)
Tr-1	73.00	18.88	72.33	19.63	63.33	29.63
Tr-5	90.00	0.00	90.00	0.00	90.00	0.00
Tr-7	89.00	1.11	74.00	17.77	54.67	39.25
Tr-13	90.00	0.00	87.00	3.33	76.33	15.18
Tr-15	90.00	0.00	90.00	0.00	85.33	5.18
Control	90.00	-	90.00	-	90.00	-
SEm±	0.53	-	2.63	-	1.86	-
CD 0.0 1%	2.27	-	11.35	-	8.04	-

Table 4. Compatibility of *Trichoderma* isolates with Taqat fungicide

Isolates	Taqat (Captan 70%+ Hexaconazole 5% WP)					
	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibitio n (%)
Tr-1	14.67	83.70	11.00	87.77	10.00	88.88
Tr-5	11.00	87.77	8.00	91.11	5.33	94.07
Tr-7	19.00	78.88	12.33	86.30	8.66	90.37
Tr-13	12.33	86.30	8.00	91.11	5.00	94.44
Tr-15	18.33	79.63	10.33	88.52	6.67	92.58
Control	90.00	-	90.00	-	90.00	-
SEm±	0.62	-	0.56	-	0.47	-
CD 0.0 1%	2.69	-	2.42	-	2.04	-

Table 5. Compatibility of *Trichoderma* isolates with Curzate M8 fungicide

	Curzate M8 (Cymoxanil 8%+ Mancozeb 64% WP)					
Isolates	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)
Tr-1	90.00	0.00	90.00	0.00	79.67	11.47
Tr-5	84.33	6.30	75.67	15.92	69.33	22.96
Tr-7	90.00	0.00	90.00	0.00	90.00	0.00
Tr-13	90.00	0.00	90.00	0.00	90.00	0.00
Tr-15	90.00	0.00	90.00	0.00	80.33	10.74
Control	90.00	-	90.00	-	90.00	-
SEm±	0.59	-	0.59	-	0.41	-
CD 0.01%	2.56	-	2.56	-	1.76	-

Table 6. Compatibility of *Trichoderma* isolates with Nativo fungicide

	Nativo (Tebuconazole 50%+ Trifloxystrobin 25% WG)					
Isolates	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)
Tr-1	16.33	81.85	14.00	84.44	8.33	90.74
Tr-5	15.00	83.33	9.67	89.25	0.00	100.00
Tr-7	8.00	91.11	5.00	94.44	5.00	94.44
Tr-13	13.33	85.18	3.67	95.92	0.00	100.00
Tr-15	22.00	75.55	10.67	88.14	8.33	90.74
Control	90	-	90	-	90	-
SEm±	1.86	-	1.65	-	0.38	-
CD 0.01%	8.06	-	7.13	-	1.66	-

Table 7. Compatibility of *Trichoderma* isolates with Headline fungicide

	Headline (Pyraclostrobin 20% WG)					
Isolates	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)
Tr-1	88.33	1.85	86.67	3.70	84.00	6.66
Tr-5	46.33	48.52	38.00	57.77	37.00	58.88
Tr-7	74.00	17.77	65.67	27.03	37.33	58.52
Tr-13	73.67	18.14	72.00	20.00	57.00	36.66
Tr-15	88.33	1.85	88.67	1.47	77.67	13.70
Control	90.00	-	90.00	-	90.00	-
SEm±	1.01	-	0.94	-	1.56	-
CD 0.01%	4.36	-	4.07	-	6.73	-

Table 8. Compatibility of *Trichoderma* isolates with Imbrex fungicide

Isolates	Imbrex (Fluxapyroxad EC)					
	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial Growth (mm)	Percent Inhibition (%)	Mycelial Growth (mm)	Percent Inhibition (%)	Mycelial Growth (mm)	Percent Inhibition (%)
Tr-1	90	0.00	88	2.22	90	0.00
Tr-5	84	6.66	77.33	14.07	67.67	24.81
Tr-7	90	0.00	89	1.11	83	7.77
Tr-13	90	0.00	89.33	0.74	90	0.00
Tr-15	90	0.00	89.67	0.36	90	0.00
Control	90	-	90	-	90	-
SEm±	0.47	-	1.20	-	0.86	-
CD 0.0 1%	2.04	-	5.19	-	3.72	-

Table 9. Compatibility of *Trichoderma* isolates with Folicur fungicide

Isolates	Folicur (Tebuconazole 25.9% EC)					
	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)
Tr-1	10.67	88.14	8.33	90.74	0.00	100
Tr-5	22.33	75.18	14.00	84.44	8.67	90.36
Tr-7	8.67	90.36	7.33	91.85	0.00	100
Tr-13	7.67	91.47	5.33	94.07	0.00	100
Tr-15	20.33	77.41	11.33	87.41	0.00	100
Control	90.00	-	90.00	-	90.00	-
SEm±	0.73	-	0.49	-	0.36	-
CD 0.0 1%	3.16	-	2.12	-	1.56	-

Table 10. Compatibility of *Trichoderma* isolates with Vitavax fungicide

Isolates	Vitavax (Carboxin 37.5% + Thiram 37.5% DS)					
	Concentrations					
	50 ppm		100 ppm		150 ppm	
	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)	Mycelial growth (mm)	Percent inhibition (%)
Tr-1	89	1.11	63	30.00	36	60.00
Tr-5	60	33.33	10	88.88	6.67	92.58
Tr-7	88.33	1.85	74	17.77	60.33	32.96
Tr-13	90	0.00	80	11.11	54.67	39.25
Tr-15	89.67	0.36	89.33	0.74	87	3.33
Control	90	-	90	-	90	-
SEm±	1.25	-	0.64	-	0.79	-
CD 0.0 1%	5.42	-	2.76	-	3.43	-

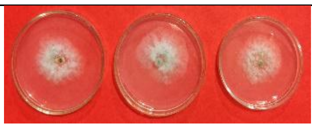
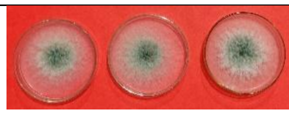
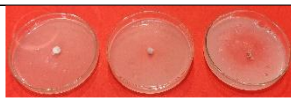
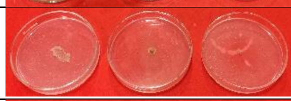
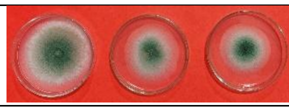
9. Folicur (Tebuconazole 25.9% EC)

With all of the *Trichoderma* isolates, the fungicide Folicur did not work very well. According to Table 9, (Plates 1 & 2) the percent growth inhibition ranged from 75.18 to 91.47% at 50 ppm, 84.44 to 94.07% at 100 ppm, and 90.36 to 100% at 150 ppm. At all concentrations, isolate wise compatibility falls off begins from at Tr-5, Tr-15, Tr-1, Tr-7, and Tr-13, respectively.

10. Vitavax (Carboxin 37.5% + Thiram 37.5% DS)

Trichoderma might develop with Vitavax at lower concentrations of 50 ppm (Table 10, Plates 1 & 2) according to a compatibility investigation. It was determined that the growth of the *Trichoderma* isolates Tr-13, Tr-15, Tr-1, Tr-7, and Tr-5 was inhibited by 0%, 0.36%, 1.11%, 1.85%, and 33.33%, respectively. However, when Vitavax concentration increased from 50 ppm to 150 ppm, the percent inhibition of *Trichoderma* isolates also increased, reaching values of 0.74-88.88% and 0.33-92.58%, respectively. Among all the isolates there was no significant decrease in mycelium growth was seen in Tr-15, indicating that isolate Tr-15 performed remarkably well against Vitavax and it was extremely compatible with Vitavax.

Plate 1. Compatibility of *Trichoderma* isolates with different fungicides at various concentrations

Fungicides	Tr-1			Tr-5			Tr-7		
Conc. (ppm)	50	100	150	50	100	150	50	100	150
Amistar Top									
Saaf									
Seedkot									
Taqat									
Curzate M8									
Nativo									
Headline									
Imbrex									
Folicur									
Vitavax									

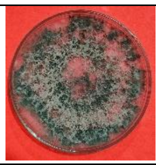
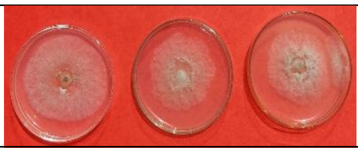
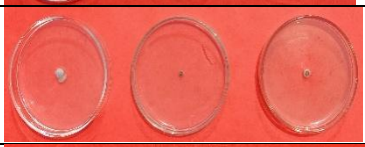
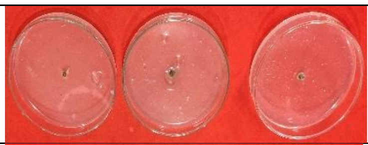
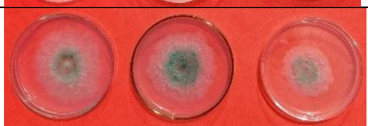
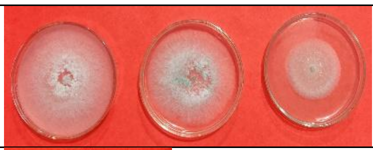
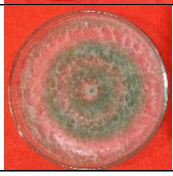
Fungicides	**Tr-1**			**Tr-5**			**Tr-7**		
Conc. (ppm)	**50**	**100**	**150**	**50**	**100**	**150**	**50**	**100**	**150**
Control									

Plate 2. Compatibility of *Trichoderma* isolates with different fungicides at various concentrations

Fungicides	**Tr-13**			**Tr-15**		
Conc. (ppm)	**50**	**100**	**150**	**50**	**100**	**150**
Amistar Top						
Saaf						
Seedkot						
Taqat						
Curzate M8						
Nativo						
Headline						
Imbrex						
Folicur						

Fungicides	Tr-13			Tr-15		
Conc. (ppm)	**50**	**100**	**150**	**50**	**100**	**150**
Vitavax						
Control						

4. DISCUSSION

Synthetic compounds combined with antagonists decrease the need for fungicide application and eliminate the possibility of resistance development. *Trichoderma* species that were tested for compatibility in the present study was found to be highly compatible with the following contact, systemic, and combi fungicides: Imbrex (Fluxapyroxad EC), Curzate M8 (Cymoxanil 8%+ Mancozeb 64% WP), Vitavax (Carboxin 37.5% + Thiram 37.5% DS) and Seedkot (Thiram 75% WS), while it proved just moderately compatible with Amistar top (Azoxystrobin 18.2% + Difenoconazole 11.4% SC) and Headline (Pyraclostrobin 20% WG). It showed least compatibility with Nativo (Tebuconazole 50%+ Trifloxystrobin 25% WG), Taqat (Captan 70%+ Hexaconazole 5% WP), Folicur (Tebuconazole 25.9% EC), and Saaf (Carbendazim 12% + Mancozeb 63% WP).

Understanding the impact of fungicides on the pathogen and the antagonists would help in the selection of fungicides and fungicide resistant antagonists through compatibility studies in vitro. Fungicides should have an inhibitory effect on the pathogen but shouldn't have a negative effect on the antagonists. Fungicides were able to totally inhibit *Trichoderma* species from growing at concentrations higher than the one (100 ppm) utilized in the current study. As opposed to using fungicide and the fungal antagonists separately, the combination of biological control agents and widely used fungicides reduced seed infection positively. The efficiency of the biological control agent could be further improved when it was applied with the recommended fungicide and used at a lower concentration. The results of this screening will aid in the choice of biological control agents that can be employed in combination with a lesser dosage of particular fungicides to control plant pathogenic fungus.

Maheshwary et al. [28] observed that *Trichoderma* is incompatible with different contact, systemic, and combination fungicides may be related to the greater quantities applied as well as the test native *Trichoderma* under capacity for tolerance. Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, and Carbendazim 12% + Mancozeb 63% WP were all determined to be completely incompatible with *Trichoderma*. These conclusions are supported by the current study's findings. The results support previous research from various researchers [4,29-32]. The direct harmful chemical's impact on *Trichoderma* cells and spores may be the cause of the fungicides' inhibitory effects. Due to their capacity to break down compounds and innate resistance to most fungicides, antagonistic microorganisms may react differently to different fungicides [6]. Azoxystrobin was moderately compatible with systemic fungicides, but tebuconazole, propiconazole, and carbendazim were incompatible. Their findings concur with those made by Bagwan [33] and Bindu et al. [34], who revealed that tebuconazole is incompatible with *Trichoderma*.

Azoxystrobin was shown to be extremely compatible with *T. harzianum* and *T. viride* among all the fungicides (0.00% inhibition at three tested concentrations). The fungicide with the highest inhibition was carbendazim. Ranganathswamy et al., [4] also observed that *Trichoderma* sp. was compatible with azoxystrobin.

Manadhar et al., [35] tested compatibility with fungicides where they found that Carbendazim, Saaf had complete inhibitory effect on all *Trichoderma*, irrespective of the isolates tested. While the fungicide Aver green (Cymoxanil 8%+ Mancozeb 64% WP) which has similar active ingredient as curzate M8 fungicide used in present study was found to be compatible with all

Trichoderma isolates. These results are similar with the results found in present study.

Kumar et al*.,* [36] findings indicate fungicide compatibility analysis of *Trichoderma* which revealed that with thiram 75%WP *T. viride* is more than 70 % compatible upto 100 ppm. According to earlier findings, agrochemicals and bio control agents that can withstand a particular degree of fungicides were combined to eradicate illnesses [37]. Similar to this, Bagwan [33] observed that *Trichoderma harzianum* and *Trichoderma viride* are compatible with thiram, copper oxychloride, and Mancozeb at 0.2%. The use of microorganisms that combat plant pathogenic fungus is risk-free because fungicides frequently have negative effects on non-target species [20].

Compatibility tests were conducted under *in vitro* condition to find out safer fungicides. For this different fungicide were tested against *Trichoderma* isolates, results indicate that among the fungicides tested, *Trichoderma* was most sensitive to captan, tebuconazole, vitavax, propiconazole and chlorothalonil [33].

Laboratory tests were done by Wedajo [38] to determine whether certain *Trichoderma* species may be used with fungicides. The findings showed that both *Trichoderma* species were 50% compatible with both fungicides at the curzate (400 ppm) and sancozeb (600 ppm) concentrations that were chosen. As the concentration of both fungicides got higher, it was found that the percent inhibition of radial growth in AUT1 and AUT2 gradually increased. At concentrations greater than the 1000 ppm employed in their investigation, both fungicides were capable of totally suppressing the growth of both *Trichoderma* species.

It might be possible to reduce disease in a similar way to how more fungicides are used if biological control agents and fungicides were combined. A fungicide application is decreased and the possibility of resistance developing is eliminated when antagonists are used with synthetic and non-synthetic chemicals. Mancozeb 75%WP and pyraclostrobin were the two fungicides that performed best in earlier experiments on *T. viride* compatibility. In keeping with Somasekhara et al*.* [39].

Since *Trichoderma* species have no negative environmental effects, it is crucial to combine them with fungicides at lower concentrations rather than administering these chemicals alone to effectively manage fungal pathogens. Similar to this, [40] stated that the combination of biological control agents and frequently used fungicides demonstrated favourable relationship by reducing the seed infection as compared to fungicide and the fungal antagonists independently. According to Silimela and Korsten [41], the effectiveness of the biological control agent could be increased still further when used in conjunction with the suggested fungicide and at a lower concentration. In context with this, the antagonistic potential of *Trichoderma* species in terms of improved modes of action as well as increased hyper parasitism activity in the current investigation [42-45]. The results of this screening will aid in choosing biological control agents that can be employed in association with specific fungicides at decreased doses to control plant pathogenic fungus [46-50].

5. CONCLUSION

The current research demonstrates that various chemical fungicides both support and limit the growth of *Trichoderma* species For the integrated disease management of agricultural crops, it is possible to choose which fungicides are compatible with *Trichoderma* species and employ them in combination. It is conceivable for the chemical responses of *Trichoderma* isolates to vary, hence it is preferable to verify the compatibility of individual *Trichoderma* species before using them in integration rather than generalizing the impact of chemicals on *Trichoderma* growth. Pathogens can be instantly controlled and kept under control for a longer amount of time by using a combination of bioagent and chemical. To determine the impact on disease control, field tests combining the *Trichoderma* species and suitable fungicides from this study are required.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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