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# **Compatibility of Indigenous**  *Trichoderma* **spp. with Selected Fungicides and Insecticides**

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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**

**Aims:** Evaluation of compatibility of indigenous *Trichoderma* isolates obtained from different agro ecological units of Kasaragod district, Kerala, India, with commonly used soil fungicides and insecticides.

**Study Design:** CRD.

**Place and Duration of Study:** Department of Plant Pathology, College of Agriculture Padannakkad, between November 2022 and November 2023.

**Methodology:** Purposive sampling surveys were conducted in eighteen different locations within the agro ecological units *viz.,* AEU 2 (Northern coastal plain), AEU 7 (Kaipad lands), AEU 11 (Northern laterites), AEU 13 (Northern foot hills) and AEU 15 (Northern high hills) of Kasaragod district, Kerala, India. Native *Trichoderma* species were isolated in *Trichoderma* Selective Medium

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(TSM) by using dilution plate technique. The compatibility of promising indigenous *Trichoderma* isolates with commonly used fungicides at recommended concentrations *viz.,* copper hydroxide (0.15%), mancozeb (0.3%), carbendazim (0.2%), hexaconazole (0.2%) and metalaxyl (0.1%); and insecticides *viz.,* chlorpyriphos (0.06%) and carbosulfan (0.05%) was evaluated using poisoned food technique. Statistical analysis of the results obtained was carried out.

**Results:** At the lowest tested concentration of copper hydroxide (0.1%), isolates Tr 37, Tr 52, and Tr 55 demonstrated complete compatibility, without any growth inhibition. Isolates, Tr 5, Tr 12, Tr 37, Tr 40, and Tr 41 were highly susceptible to mancozeb, displaying 100% inhibition at all tested concentrations. Carbendazim was found inhibitory for all isolates at every concentration tested, resulting in complete growth inhibition. Hexaconazole showed a high level of inhibition, with inhibition rates exceeding 70% across all tested concentrations. Metalaxyl significantly suppressed radial growth across all concentrations, though isolates, Tr 37, Tr 52, and Tr 55 exhibited relatively low inhibition regardless of the concentration. The *Trichoderma* isolates were found relatively more compatible with insecticides chlorpyriphos and carbosulfan.

**Conclusion:** *T. harzianum* isolates exhibited a higher sensitivity to fungicides tested. *T. koningiopsis* isolates demonstrated relatively higher compatibility with all tested fungicides but were found to be affected by insecticides. All the isolates were found relatively more compatible with insecticides chlorpyriphos and carbosulfan.

*Keywords: Trichoderma; native isolates; compatibility; fungicides; insecticides.*

# **1. INTRODUCTION**

Soil and seed borne diseases pose significant challenge to crop production, leading to considerable yield losses (Dignam *et a*l., 2022). Fungi belonging to the genus *Trichoderma* are widely recognized for its ability to manage soilborne plant pathogens. *Trichoderma* species are among the most frequently isolated soil fungi, with nearly all soil types containing  $10<sup>1</sup>$  to  $10<sup>3</sup>$ culturable spores per gram of soil (Harman *et al.,* 2004). They employ direct and indirect mechanisms *viz.,* competition, mycoparasitism, antibiosis, induced systemic resistance and plant growth promotion to check the pathogenic growth (Vinale *et al*., 2008). The use of *Trichoderma* strains in integrated disease management (IDM) systems has emerged as a promising strategy (Singh *et al.,* 2019) against the soil borne pathogens. However, the widespread use of fungicides often negatively affects native bio control agents (Maheshwary *et al.,* 2020). Therefore, understanding the compatibility between *Trichoderma* isolates and agricultural chemicals, such as fungicides, insecticides, and herbicides, is crucial for optimizing their effectiveness in IDM programs.

Numerous studies have reported complete and partial compatibility of *Trichoderma* species with agricultural chemicals (Ramanagouda and Naik (2021); Singh *et al.,* 2021). *In vitro* compatibility studies serve as the primary approach for identifying compatible strains, which can be further utilized for formulating appropriate

management strategies. The integration of these bio control agents with fungicides can improve disease control and offer more efficient management of soil-borne diseases (Singh *et al.,* 2019). It also eliminates the chance of resistance development and reduces the frequency of application of fungicides (Maurya *et al*., 2020). Hence, it is important to evaluate the effects of pesticides on biocontrol agents. In light of this, a study was carried out to examine compatibility of native *Trichoderma* isolates with fungicides and insecticides under laboratory conditions. The overarching aim is to develop an effective integrated disease management (IDM) strategy for controlling soil borne plant diseases.

# **2. MATERIALS AND METHODS**

# **2.1 Isolation of Indigenous** *Trichoderma* **spp.**

Purposive sampling surveys were conducted in eighteen different locations within the agro ecological units *viz.,* AEU 2 (Northern coastal plain), AEU 7 (Kaipad lands), AEU 11 (Northern laterites), AEU 13 (Northern foot hills) and AEU 15 (Northern high hills) of Kasaragod district, Kerala, India. Composite soil samples collected from a depth of 15 cm were pooled, air dried and sieved through 2 mm mesh. Native *Trichoderma* species were isolated in *Trichoderma* Selective Medium (TSM) (Elad *et al.,* 1981) by using dilution plate technique. 10 g of soil sample was weighed out and added to 100 ml sterile water taken in a 250 ml conical flask. Dilutions up to 10<sup>5</sup> were made, and using the pour plate method, one millilitre of each of the dilutions 10<sup>-2</sup>, 10-3 , 10-4 and 10-5 was plated on TSM. The plates were then incubated at room temperature. The obtained *Trichoderma* isolates were sub cultured and maintained as pure culture. The promising isolates were identified using ITS sequencing which included six *T. asperellum* (Tr 5, Tr 12, Tr 38, Tr 41, Tr 43, and Tr 48), three *T. koningiopsis (*Tr 37, Tr 52, and Tr 55) *and one T.lixii isolate (*Tr 40).

# **2.2 Compatibility of** *Trichoderma* **spp. with Fungicides and Insecticides**

The compatibility of promising indigenous *Trichoderma* isolates with commonly used fungicides at recommended concentrations *viz.,* copper hydroxide (0.15%), mancozeb (0.3%), carbendazim (0.2%), hexaconazole (0.2%) and metalaxyl (0.1%); and insecticides *viz.,* chlorpyriphos (0.06%) and carbosulfan (0.05%) was evaluated using poisoned food technique (Zentmeyer, 1955). 50 ml of double strength PDA was measured into conical flasks. Fungicidal solution was prepared by dissolving the required amount of fungicide in 50 ml of sterile water, which was then mixed with the melted double strength PDA to achieve the desired final concentration. The poisoned media was poured into sterile Petri plates, and an 8 mm fungal disc of *Trichoderma* isolates was placed at the centre of each plate. Three replicates were maintained for each concentration, along with a control plate without the fungicidal solution. The radial growth of the isolates was recorded when the control plate reached full growth, and the percent growth inhibition was calculated using the formula. Different concentrations evaluated are provided in Table 1.

Per cent inhibition (I) = 
$$
\frac{(C - T)}{C} \times 100
$$

Where,

 $C =$  Radial growth of the isolate in the control plate (cm)

 $T =$  Radial growth of the isolate in the treatment plate (cm)

## **3. RESULTS AND DISCUSSION**

Indigenous *Trichoderma* spp. were isolated in *Trichoderma* selective medium using the dilution plate technique. Dilutions of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and

10-5 were selected for isolation based on preliminary trials. A total of fifty-five isolates were obtained, out of which ten promising isolates were selected based on *in vitro* antagonism. Six *T. asperellum* (Tr 5, Tr 12, Tr 38, Tr 41, Tr 43 and Tr 48), three *T. koningiopsis* (37, Tr 52 and Tr 55) and one isolate of *T. lixii* (Tr 40) along with a standard Kerala Agricultural University culture (*T. asperellum)* were evaluated for their *in vitro* compatibility with commonly used pesticides. We have evaluated the compatibility of two contact fungicides, three systemic fungicides and two insecticides with indigenous *Trichoderma* spp. Lower concentrations of the agro chemicals were found to be safer for the *Trichoderma* isolates than higher concentrations and the extent of inhibition depended on both the isolate and respective concentration.

At the lowest tested concentration of 0.1% copper hydroxide, isolates Tr 37, Tr 52, and Tr 55 demonstrated complete compatibility, without growth inhibition. Whereas, less than 50 per cent inhibition was recorded for isolates Tr 43 (48.15%) and Tr 48 (37.78%). Isolates Tr 37, Tr 52, and Tr 55 continued to show minimal inhibition even at higher concentrations. However, isolates Tr 12, Tr 38, Tr 40, and Tr 41 exhibited significant inhibition at 0.15% copper hydroxide, with inhibition levels exceeding 70 per cent. At 0.2%, Tr 12 and Tr 40 displayed the highest inhibition rates, reaching 84.44 per cent and 80.37 per cent respectively. Isolates, Tr 5, Tr 12, Tr 37, Tr 40, and Tr 41 were highly susceptible to mancozeb, displaying cent per cent inhibition at all concentrations tested. In contrast, Tr 55 exhibited the lowest inhibition levels. Isolates Tr 43, Tr 48, and Tr 52 showed moderate inhibition, which increased with higher concentrations. Carbendazim proved to be inhibitory for all isolates at every concentration tested, resulting in complete growth inhibition.

Hexaconazole showed a high level of inhibition, with inhibition rates exceeding 70 per cent across all tested concentrations. Isolates, Tr 5, Tr 12, Tr 38, Tr 40, Tr 41, Tr 43, and Tr 48 were completely inhibited at all concentrations (0.15%, 0.2%, and 0.25%). Metalaxyl significantly suppressed radial growth across all concentrations, though isolates, Tr 37, Tr 52, and Tr 55 exhibited relatively low inhibition, regardless of the concentration. The *Trichoderma* isolates were found relatively more compatible with insecticides. Tr 41 displayed the lowest inhibition (20.37%), closely followed by Tr 40 (22.22%) with 0.05 per cent chlorpyriphos. Carbosulfan was found to be the safest among the tested pesticides showing no inhibitory effect on most of the isolates. The isolate, Tr 40 showed slight inhibition (1.48%) at 0.04 per cent while no inhibition was observed for the remaining isolates. Isolates, Tr 5, Tr 12, Tr 38, Tr 41, Tr 48, Tr 52 and Tr 55 were completely compatible even at 0.06 per cent concentration.

The compatibility of *Trichoderma* species with various agrochemicals is a key consideration when integrating them into agricultural systems for disease management. Agricultural chemicals are essential tools for crop protection but can affect the efficacy of biocontrol agents. In this study, we explored the compatibility of native *Trichoderma* isolates with commonly used fungicides and insecticides, highlighting the variability in their responses to these chemicals. Sarkar *et al*. (2010) reported that contact fungicides exhibited lower toxicity when

compared to the systemic ones. The current study demonstrated relatively lower toxicity with copper hydroxide but carbendazim, a generally used systemic fungicide displayed complete inhibition of isolates. Rai *et al.* (2016) found that systemic fungicides such as hexaconazole, tebuconazole, and carbendazim displayed complete growth inhibition of *T. harzianum* at all tested concentrations. The findings of our study also align with the work of Maheshwary *et al.* (2020) who reported that *T. asperellum* isolate was compatible with copper hydroxide (93.2%), mancozeb (92.96%) and metalaxyl (100%) while tebuconazole, propiconazole, and carbendazim were found to be incompatible. Previous studies have demonstrated varying levels of tolerance to mancozeb across different *Trichoderma* species. *Trichoderma harzianum* was found to grow at lower concentrations of mancozeb, but higher concentrations (>8000 μg/ml) completely inhibited its growth (Bhale and Rajkonda, 2015).

#### **Table 1. Pesticides used at different concentrations for compatibility study**



#### **Table 2. Compatibility of native isolates of** *Trichoderma* **spp. with contact fungicides**



SI. No.	<b>Isolate</b>	Per cent inhibition against hexaconazole			Per cent inhibition against carbendazim			Per cent inhibition against metalaxyl		
		0.15%	0.2%	0.25%	0.25%	0.3%	0.35%	0.05%	0.1%	0.15%
	Tr 5	$100.00^a$	$100.00^a$	$100.00^a$				64.45 <sup>d</sup>	$81.85^{d}$	87.04 <sup>cd</sup>
2	Tr 12	$100.00^a$	$100.00^a$	$100.00^a$				65.19 <sup>d</sup>	$86.30^{bc}$	90.00 <sup>b</sup>
3	Tr 37	75.56c	80.37 <sup>c</sup>	81.48 <sup>c</sup>	100.00	100.00	100.00	$41.11$ <sup>f</sup>	54.07 <sup>f</sup>	88.89bc
4	Tr 38	$100.00^{\text{a}}$	$100.00^a$	$100.00^a$				72.22 <sup>c</sup>	$84.44^{\circ}$	89.26bc
5	Tr 40	$100.00^a$	$100.00^a$	$100.00^a$				75.19bc	84.44 <sup>c</sup>	$100.00^a$
6	Tr 41	$100.00^a$	$100.00^a$	$100.00^{\text{a}}$				56.30e	81.11 <sup>d</sup>	89.26bc
	Tr 43	$100.00^{\text{a}}$	$100.00^a$	$100.00^a$				72.96 <sup>c</sup>	81.11 <sup>d</sup>	86.67 <sup>d</sup>
8	Tr 48	$100.00^a$	$100.00^a$	$100.00^a$				88.52 <sup>a</sup>	$100.00^a$	$100.00^a$
9	Tr 52	76.67 <sup>b</sup>	81.48 <sup>b</sup>	84.45 <sup>b</sup>				42.96 <sup>f</sup>	57.04e	79.26 <sup>e</sup>
10	Tr 55	73.33 <sup>d</sup>	76.30 <sup>d</sup>	80.37 <sup>d</sup>				42.96 <sup>f</sup>	$56.67^{\circ}$	79.26 <sup>e</sup>
11	KAU	$100.00^a$	$100.00^a$	$100.00^a$				78.15 <sup>b</sup>	86.67 <sup>b</sup>	$100.00^a$
$12 \,$	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

**Table 3. Compatibility of native isolates of** *Trichoderma* **spp. with systemic fungicides**

# **Table 4. Compatibility of native isolates of** *Trichoderma* **spp. with insecticides**



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**Fig. 1. Compatibility of native isolates of** *Trichoderma* **spp. with copper hydroxide**



**Fig. 2. Compatibility of native isolates of** *Trichoderma* **spp. with mancozeb**



**Fig. 3. Compatibility of native isolates of** *Trichoderma* **spp. with carbendazim**

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#### **Fig. 4. Compatibility of native isolates of** *Trichoderma* **spp. with metalaxyl**



**Fig. 5. Compatibility of native isolates of** *Trichoderma* **spp. with hexaconazole**



**Fig. 6. Compatibility of native isolates of** *Trichoderma* **spp. with chlorpyriphos**

a). Carbosulfan  $(0.04, 96)$ 



**Fig. 7. Compatibility of native isolates of** *Trichoderma* **spp. with carbosulfan**

This suggests that the application of mancozeb should be carefully regulated when using *T. harzianum* in biocontrol strategies. In a study conducted by Lezama *et al.* (2023) *T. koningiopsis* showed more resilience to mancozeb, with a moderate inhibition (62.47 to 64.84%) of radial growth at different tested concentrations. Our study aligns with this finding, as we observed a complete inhibition of growth across all concentrations tested for certain *T. aesperellum* and *T. koningiopsis* isolates. According to Maurya *et al*. (2020), the growth inhibition of *T. harzianum* isolate Th-8 caused by fungicides *viz*., thiram, copper oxychloride, mancozeb, and metalaxyl at concentrations ranging from 100 ppm to 1000 ppm ranged from zero per cent to eighty per cent. Earlier reports by Ramanagouda and Naik (2021) suggested that insecticides like chlorpyriphos were highly compatible with *Trichoderma* isolates. Hexaconazole has been found to be highly inhibitory to *Trichoderma* species, with cent per cent inhibition of radial growth at all concentrations, particularly during the initial incubation periods (Singh *et a*l*.,* 2021).

### **4. CONCLUSION**

The native isolates of *Trichoderma* spp. were moderately compatible with copper hydroxide and metalaxyl. Carbendazim caused complete inhibition of growth at all tested concentrations. *T. koningiopsis* isolates were compatible with all tested fungicides but were found to be affected by Insecticides. *Trichoderma* isolates were found relatively more compatible with insecticides,

chlorpyriphos and carbosulfan. The overall findings of this study emphasize the importance of selecting fungicides carefully when deploying *Trichoderma* species in agricultural systems.

#### **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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