Original Research Article

Compatibility of indigenous *Trichoderma* spp. with selected fungicides and insecticides

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 (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 ofcopper 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 relativelyhighercompatibilitywith 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 challenges to crop production, leading to considerable yield losses (Dignam *et al*., 2022). Fungi belonging to the genus *Trichoderma* are widely recognized for its ability to manage soil-borne plant pathogens. *Trichoderma* species are among the most frequently isolated soil fungi, with nearly all soil types containing 10^1 to 10^3 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 integration of *Trichoderma* strains into 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 impacts 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 about 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.,* 2020). 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. MATERIAL 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^{-5} were made, and using the pour plate method, one milli litre of each of the dilutions 10^{-2}, 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*. three *T.koningiopsis and one T.lixii isolate.*

2.2Compatibility 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 doublestrength 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 doublestrength 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 percentgrowth inhibition was calculated using the formula.Different concentrations evaluated are provided in Table 1.

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

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

UNDER PEER REVIEW

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, isolatesTr 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 hydroxidbut 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,

UNDER PEER REVIEW

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).

This suggests that the application of mancozeb should be carefully regulated when using *T. harzianum* in biocontrol strategies. *T. koningiopsis*showed more resilience to mancozeb, with only a moderate inhibition (62.47 to 64.84%) of radial growth at different tested concentrations in a study conducted by Lezama *et al.* (2023). 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. When it comes to Hexaconazole, it has been found to be highly inhibitory to *Trichoderma* species, with cent per cent inhibition of radial growth at all concentrations,particularlyduringthe initial incubation periods (Singh *et al.,* 2021).

Table 1. Pesticides used at different concentrations for compatibility study

Pesticide	Active ingredient	Concentration (%)		
		Low	Recommended	High
Kocide 2000	Copper hydroxide	0.1 %	0.15 %	0.2 %
Indofil M-45	Mancozeb	0.25 %	0.3 %	0.35 %
Bavistin	Carbendazim 50 WP	0.15 %	0.2 %	0.25 %
Contaf plus	Hexaconazole 5 SC	0.15 %	0.2 %	0.25 %
Axel-350	Metalaxyl 35 WS	0.05 %	0.1 %	0.15 %
Radar	Chlorpyriphos 20 EC	0.05 %	0.06 %	0.07 %
Marshal	Carbosulfan 25 EC	0.04 %	0.05 %	0.06 %

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

Sl. No.	Isolate	Per cent inhibition against copper hydroxide			Per cent inhibition against mancozeb		
		0.1 %	0.15 %	0.2 %	0.25 %	0.3 %	0.35 %
1	Tr 5	51.85^{b}	68.89^{b}	73.70^{de}	100.00^{a}	100.00^{a}	100.00^{a}
2	Tr 12	69.26^{a}	77.41^{a}	84.44^{a}	100.00^{a}	100.00^{a}	100.00^{a}
3	Tr 37	0.00^{d}	23.33^{e}	24.08^{g}	100.00^{a}	100.00^{a}	100.00^{a}
4	Tr 38	71.48^{a}	76.30^{a}	78.52^{bc}	61.48^{c}	67.41^{b}	69.63^{b}
5	Tr 40	70.00^{a}	73.70^{ab}	80.37^{ab}	100.00^{a}	100.00^{a}	100.00^{a}
6	Tr 41	66.67^{a}	72.22^{ab}	78.89^{bc}	100.00^{a}	100.00^{a}	100.00^{a}
7	Tr 43	48.15^{b}	56.30^{c}	70.00^{e}	20.00^{e}	44.44^{c}	67.04^{b}
8	Tr 48	37.78^{c}	44.82^{d}	70.00^{e}	22.22^{de}	27.04^{d}	31.11^{d}
9	Tr 52	0.00^{d}	15.93^{f}	36.30^{f}	24.07^{d}	41.48^{c}	42.96^{c}
10	Tr 55	0.00^{d}	4.45^{g}	25.92^{g}	4.82^{f}	17.78^{e}	22.22^{e}

UNDER PEER REVIEW

11	KAU	68.15[a]	74.44[ab]	75.18[cd]	84.07[b]	100.00[a]	100.00[a]
12	Control	0.00	0.00	0.00	0.00	0.00	0.00

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

Sl. No.	Isolate	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 %
1	Tr 5	100.00[a]	100.00[a]	100.00[a]	100.00	100.00	100.00	64.45[d]	81.85[d]	87.04[cd]
2	Tr 12	100.00[a]	100.00[a]	100.00[a]				65.19[d]	86.30[bc]	90.00[b]
3	Tr 37	75.56[c]	80.37[c]	81.48[c]				41.11[f]	54.07[f]	88.89[bc]
4	Tr 38	100.00[a]	100.00[a]	100.00[a]				72.22[c]	84.44[c]	89.26bc
5	Tr 40	100.00[a]	100.00[a]	100.00[a]				75.19[bc]	84.44[c]	100.00[a]
6	Tr 41	100.00[a]	100.00[a]	100.00[a]				56.30[e]	81.11[d]	89.26bc
7	Tr 43	100.00[a]	100.00[a]	100.00[a]				72.96[c]	81.11[d]	86.67[d]
8	Tr 48	100.00[a]	100.00[a]	100.00[a]				88.52[a]	100.00[a]	100.00[a]
9	Tr 52	76.67[b]	81.48[b]	84.45[b]				42.96[f]	57.04[e]	79.26[e]
10	Tr 55	73.33[d]	76.30[d]	80.37[d]				42.96[f]	56.67[e]	79.26[e]
11	KAU	100.00[a]	100.00[a]	100.00[a]				78.15[b]	86.67[b]	100.00[a]
12	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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

Sl. No.	Isolate	Per cent inhibition against chlorpyriphos			Per cent inhibition against carbosulfan		
		0.05 %	0.06 %	0.07 %	0.04 %	0.05 %	0.06 %
1	Tr 5	36.30[f]	36.30[d]	58.15[d]	0.00[b]	0.00[e]	0.00[e]
2	Tr 12	31.11[g]	30.37[g]	43.70[g]	0.00[b]	0.00[e]	0.00[e]
3	Tr 37	44.81[d]	50.37[e]	54.07[ej]	0.00[b]	3.70[bc]	22.22[a]
4	Tr 38	42.22[e]	52.22[e]	55.56[de]	0.00[b]	2.59[cd]	0.00[e]
5	Tr 40	22.22[i]	30.37[g]	32.59[i]	1.48[b]	11.85[a]	11.48[b]
6	Tr 41	20.37[i]	30.37[g]	40.74[h]	0.00[b]	0.00[e]	0.00[e]
7	Tr 43	31.48[g]	39.26[f]	48.15[f]	0.00[b]	0.00[e]	2.22[d]
8	Tr 48	27.78[h]	29.63[g]	33.70[i]	0.00[b]	0.00[e]	0.00[e]
9	Tr 52	57.04[b]	72.59[a]	74.82[a]	0.00[b]	0.00[e]	0.00[e]
10	Tr 55	62.59[a]	66.67[b]	68.15[b]	0.00[b]	1.85[d]	0.00[e]
11	KAU	48.52[c]	60.74[c]	64.07[c]	4.07[a]	4.07[b]	5.56[c]
12	Control	0.00	0.00	0.00	0.00	0.00	0.00

UNDER PEER REVIEW

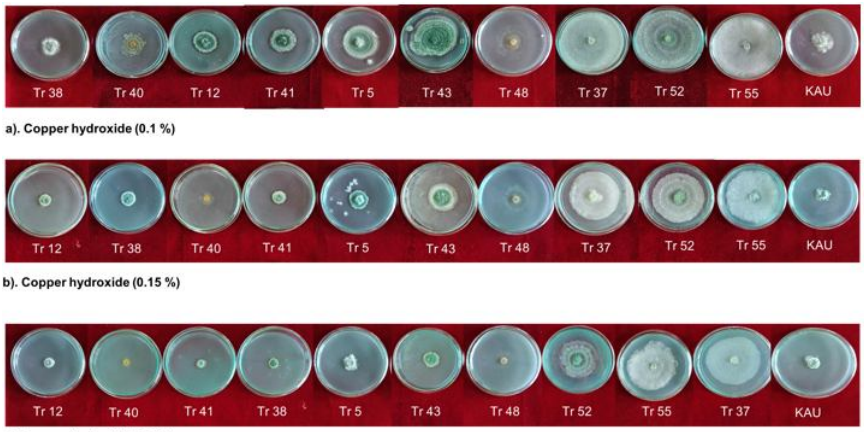

Fig. 1.Compatibility of native isolates of *Trichoderma* spp. with copper hydroxide

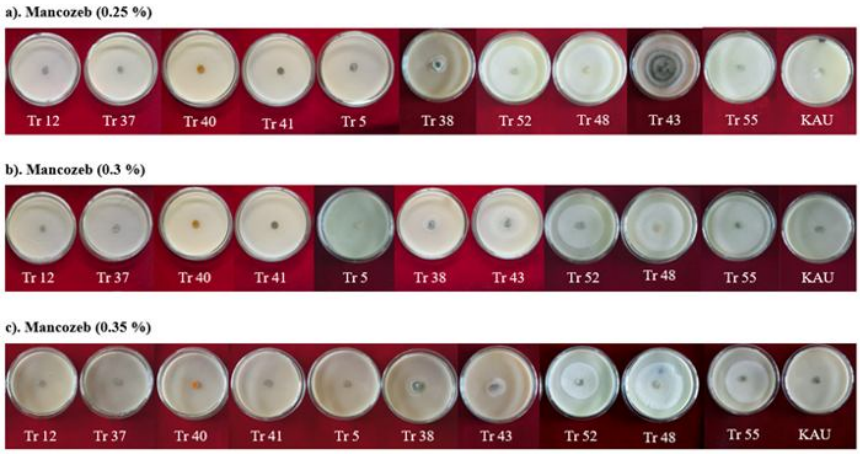

Fig.2.Compatibility of native isolates of *Trichoderma* spp. with mancozeb

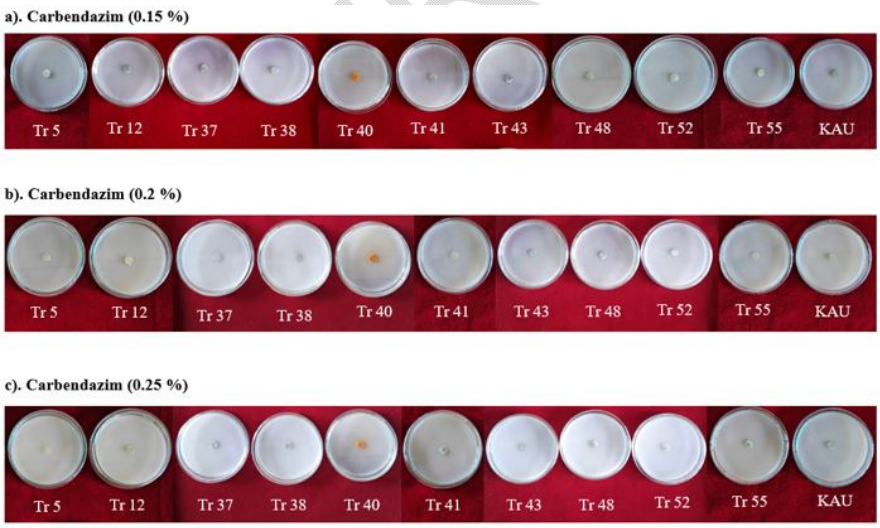

Fig.3.Compatibility of native isolates of *Trichoderma* spp. with carbendazim

UNDER PEER REVIEW

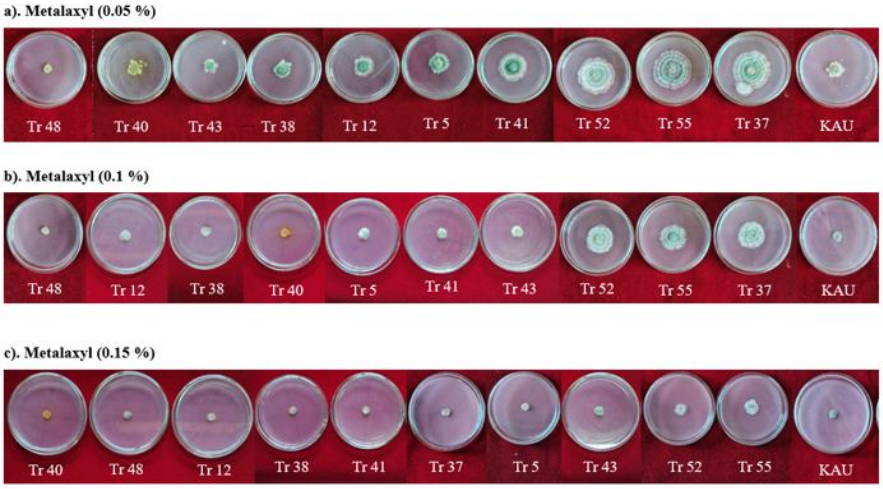

Fig.4.Compatibility of native isolates of *Trichoderma* spp. with metalaxyl

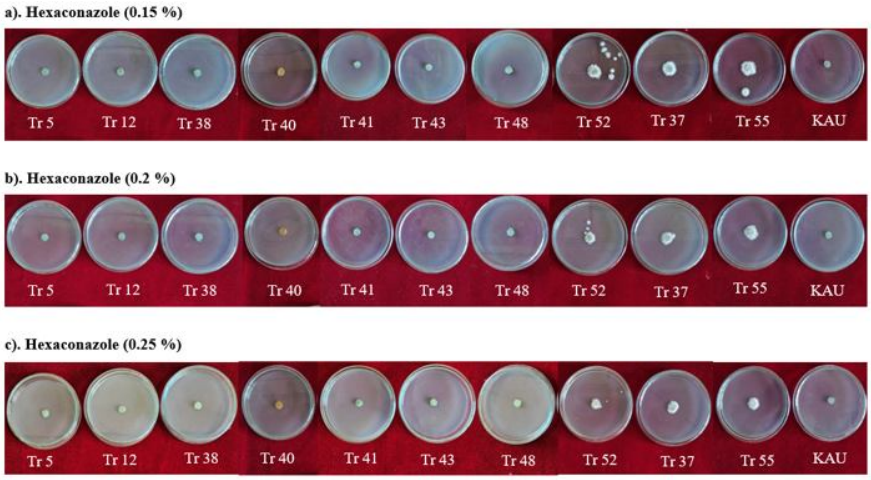

Fig.5.Compatibility of native isolates of *Trichoderma* spp. with hexaconazole

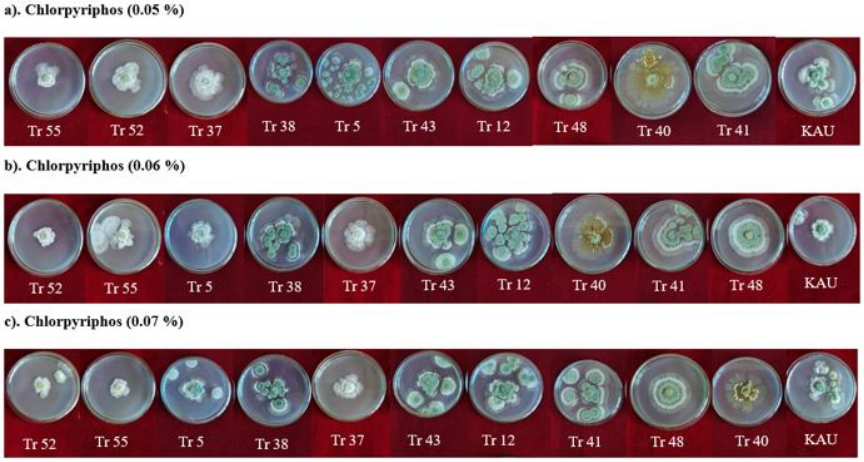

Fig.6.Compatibility of native isolates of *Trichoderma* spp. with chlorpyriphos

UNDER PEER REVIEW

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

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.

REFERENCES

1. Dignam, B.E., Marshall, S.D., Wall, A.J., Mtandavari, Y.F., Gerard, E.M., Hicksofit in dairyin, E., Cameron, C., Aalders, L.T., Shi, S., and Bell, N.L. 2022. Impacts of soil-borne disease on plant yield and farm pr g soils. *J. Sustain. Agric. Environ. 1*(1), pp.16-29.
2. Harman G. E., Howell C. R., Viterbo, A., Chet I., Lorito, M. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*. 2:43–56.
3. Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., and Lorito, M. 2008. *Trichoderma*–plant–pathogen interactions. *Soil Biol. Biochem.* 40(1): 1-10.
4. Singh, V., Keswani, C., Ray, S., Upadhyay, R. S., Singh, D. P., Prabha, R., Sarma, B. K., and Singh, H.B. 2019. Isolation and screening of high salinity tolerant *Trichoderma* spp. with plant growth property and antagonistic activity against various soilborne phytopathogens. *Arch. Phytopathol. Plant Prot.* 52(7-8): 667-680.
5. Maheshwary, N. P., Gangadhara Naik, B., AmoghavarshaChittaragi, M., Naik, S. K., and Nandish, M. 2020. Compatibility of *Trichoderma asperellum* with fungicides. *Pharma Innov. J*. 9: 136-140.
6. Ramanagouda, G. and Naik, M. K. 2021. Compatibility studies of indigenous *Trichoderma* isolates with pesticides. *Indian Phytopathol.* 74(1): 241-248.
7. Singh, M., Singh, R., Mishra, P., Sengar, R. S., and Shahi, U. P. 2021. *In-vitro* compatibility of *Trichoderma harzianum* with systemic fungicides. *Int. J. Chem. Stud*. 9(1): 2884-2888.
8. Maurya, S., Rai, D., Dubey, S., and Pal, R. K. 2020. Compatibility of *Trichoderma harzianum* with different fungicides under *in vitro*. *Int. J. Chem. Stud*. 8: 2946-2952.

UNDER PEER REVIEW

9. Elad, Y., Chet, I., and Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparasitica*. 9: 59-67.
10. Zentmeyer, G. A. 1955. A laboratory method for testing soil fungicides with *Phytophthora cinnamomii*, a test organism. *Phytopathol.* 45: 398-404.
11. Sarkar, S., Narayanan, P., Divakaran, A., Balamurugan, A., and Premkumar, R. 2010. The *in vitro* effect of certain fungicides, insecticides, and biopesticides on mycelial growth in the biocontrol fungus *Trichoderma harzianum*. *Turk. J. Biol.* 34(4): 399-403.
12. Rai, D., Bisht, K. S., and Tewari, A. K. 2016. *In-vitro* effect of newer fungicides on mycelial growth in biocontrol fungus *Trichoderma harzianum* (Th 14). *J. Hill Agric.* 7(1): 162-164.
13. Bhale, U.N. and Rajkonda, J.N.2015. Compatibility of fungicides and antagonistic activity of *Trichoderma* spp. against plant pathogens. *Biosci. Methods*. *6*(3): 1-9.
14. Lezama, C. P., Romero-Arenas, O., Valencia de Ita, M.D.L.A., Rivera, A., Sangerman Jarquín, D.M., and Huerta-Lara, M.2023. *In Vitro*study of the compatibility of four species of *Trichoderma* with three fungicides and their antagonistic activity against *Fusarium solani*. *Hortic. 9*(8): 905.

UNDER PEER REVIEW