Original Research Article

Evaluation of fungicides against *Rhizoctonia bataticola* (Taub)Butler causing dry root rot of groundnut and their compatability with*Trichoderma asperellum*

Comment [E1]: Evaluation of fungicides against Rhizoctonia bataticola (Taub) Butler and their compatibility with Trichoderma asperellum in peanu

ABSTRACT

The study aimed to evaluate the efficacy of four systemic and combination fungicides, namely Carbendazim 12% + Mancozeb 63% WP, Tebuconazole 25% WG, Hexaconazole 4% + Zineb 68% WG, and Tebuconazole 50% + Trifloxystrobin 25% WG, against *Rhizoctoniabataticola*, the causal agent of dry root rot in groundnut. The antifungal activity was assessed at various concentrations using the poison food technique. Among the tested fungicides, Hexaconazole 4% + Zineb 68% WG exhibited the highest efficacy, with 100% inhibition of mycelial growth at all concentrations.Furthermore, the compatibility of these fungicides with *Trichoderma asperellum* a potential biocontrol agent, was investigated. The results revealed that Hexaconazole 4% + Zineb 68% WG caused 38.88% inhibition of *T. asperellum* growth. However, a "compatibility" value of 61.12% indicated a moderate level of compatibility between the fungicides and the bioagent.In conclusion, the tested fungicides showed strong inhibitory effects against *R. bataticola*, making them promising candidates for managing dry root rot in groundnut crop. Hexaconazole 4% + Zineb 68% WG was found to be most effective fungicide. On the other hand, the other fungicides tested showed 100 percent inhibition of *T. asperellum* growth, suggesting incompatibility with the biocontrol agent at the tested concentrations.

Keywords: Dry root rot, Rhizoctoniabataticola, Trichoderma, Fungicides,poisoned food technique, compatability,

1. INTRODUCTION

Groundnut (*Arachishypogaea* L.) is a significant oilseed crop grown globally, valued for its high oil content and nutritional profile. In india, it holds the first position in terms of cultivation area and the second position in terms of production after soybean. The leading producer of groundnut is China, with 17.57 million tonnes, followed by India with 6.73 million tonnes in 2019-20. Despite its importance, groundnut productivity in many developing countries remains low due to various production constraints, including both biotic and abiotic stresses.

Among biotic factors, pathogenic attacks on groundnut plants can lead to significant yield losses and reduce seed quality during post-harvest storage. One such pathogen is *Rhizoctonia bataticola*, a soil-borne fungus responsible for causing dry root rot in groundnut plants. The disease, also known as "dry wilt," is particularly severe in regions like Rajasthan, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, and Maharashtra, where dry conditions prevail during the rain-fed season. *Rhizoctonia bataticola* can survive on or within seeds and persists in the soil in the form of black sclerotia, which are abundantly produced on infected tissues and disseminated during tillage operations.

Chemical fungicides are commonly used to control *R. bataticola*and manage the disease. However, the use of fungicides may have negative effects on beneficial soil microorganisms, such as *Trichoderma* sp., which are crucial for sustainable agriculture. Employing potential biological control agents (BCAs) is preferred over hazardous chemicals for managing plant diseases. The effectiveness of BCAS can be enhanced if compatible fungicides are used, as it can lead to disease suppression similar to using hazardous fungicides at higher doses. Using compatible synthetic chemicals with BCAs can also help reduce the development of fungicidal resistance.

Moreover, the compatibility with *Trichoderma* sp. suggests the potential for their use in integrated disease management strategies, maintaining a balance between disease control and the preservation of beneficial soil microorganisms. By utilizing compatible fungicides with BCAS, the management of dry root rot in groundnut can be improved, reducing the reliance on hazardous chemicals and promoting more environmentally friendly and effective disease control practices.

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MATERIAL AND METHODS

2.1 ISOLATION OF PATHOGEN

The test pathogen *Rhizoctonia bataticola*was isolated from root portions of the dry root rot affected plants. The pathogen Rhizoctonia bataticola causing dry root rot in groundnut was isolated using the tissue segment method, as described by Rangaswamy and Mahadevan in (1999). Small tissue pieces (2-5 mm diameter) comprising

infected root portions and some healthy tissue were cut with a sterile scalpel. These tissue pieces were surface sterilized with 1% sodium hypochlorite for 1 minute, followed by three washes with sterile distilled water. The sterilized tissue bits were then placed on preplated potato dextrose agar (PDA) medium and incubated at 28 ± 2°C. Periodic observation allowed the growth of the fungus to be monitored. Pure cultures were obtained using the single hyphal tip method, maintaining the isolates on PDA medium through regular subculturing during the study.

2.2Isolation of *Trichoderma*spp from rhizosphere soil

Composite soil samples were collected from the rhizosphere of healthy groundnut plants with the aim of isolating *Trichoderma* spp. This isolation was conducted using the serial dilution technique, as outlined by Johnson and Curl in (1972). The soil samples were dried in the shade and then utilized for the serial dilution process. To achieve a 10^{-1} dilution, 10 grams of the soil were dissolved in 90 ml of sterile distilled water. From this dilution, 1 ml of the soil suspension was taken and added to 9 ml of sterile distilled water to obtain a 10^{-2}dilution. This process was repeated, creating further dilutions of $10^{-2}$and 10^{-6}, which were utilized to isolate the fungi.

For the isolation of the fungi, the appropriate final dilution of 10^{-4} (fungi) was added at a rate of 1 ml per sterile Petri plate. *Trichoderma* selective medium was then poured into the Petri plates and gently rotated to ensure the even distribution of the soil suspension within the medium. The plates were subsequently incubated at a temperature of 28 ± 2°C for a period of 72 hours, during which colony development was regularly monitored.

2.3FUNGICIDES

In the present investigation foursystemic fungicides *viz* Carbendazim 12%+ Mancozeb 63% WP, Tebuconazole 25% WG, Hexaconazole 4% + Zineb 68 % WG, Tebuconazole 50%+Trifloxystrobin25% WG were used to test their efficacy against *R. bataticola*at four different concentrations(100, 250, 500, 1000ppm) using poison food technique ((Nene and Thapliyal,1993)

Percent inhibition of mycelial growth of dry root rot pathogen=C-T/C*100

Where C=mycelial growth in control(mm)
T=mycelial growth in treatment(mm)

2.4 Evaluation of effective fungicide and agents against dry root rot pathogen and potential *Trichoderma asperellum*

For each treatment, a volume of 30 ml of double-strength potato dextrose agar (PDA) medium was measured and poured into a 100 ml conical flask, which was then autoclaved. In a separate sterile container, the specified concentration of fungicide was dissolved in 30 ml of sterile distilled water at lukewarm temperature. The fungicide solution was thoroughly mixed with the autoclaved PDA medium. This poisoned medium was then evenly distributed into three petri plates, which were considered as three replications, and left to solidify. To inoculate the test pathogen *R. bataticola*, 6mm discs were cut from the periphery of an actively growing colony using a sterile cork borer, and each disc was transferred to the center of each petri plate containing the poisoned medium. To maintain a control, fungal discs were placed in petri plates containing untreated medium without any chemical. The inoculated petri plates were incubated at a temperature of 28±2°C, and observations were recorded to determine the percentage inhibition when *R. bataticola* completely covered the plate in the control.

3. RESULTS AND DISCUSSION

3.1 *In vitro* evaluation of fungicides against *R.bataticola*:

In the present study, four fungicides *viz*., Carbendazim 12%+ Mancozeb 63% WP, Tebuconazole 25% WG, Hexaconazole 4% + Zineb 68 % WG, Tebuconazole 50%+Trifloxystrobin25% WG were used to test their efficacy against *R. bataticola*at four different concentrations using poison food technique. The effect of different concentrations of the fungicides on mycelial growth of *R. bataticola*was tested and the per cent inhibition was calculated. The data pertaining to the results was presented in the Table .1

The fungicide Hexaconazole 4% + Zineb 68 % WG (100, 250, 500, 1000) exhibited complete inhibition of the mycelial growth of the pathogen. This indicates that the fungicide was highly effective in suppressing the growth of *R.bataticola* at all concentrations tested.

Among the four different concentrations tested, the fungicide Carbendazim 12%+ Mancozeb 63% exhibited the highest inhibition of 100 per cent was observed at 1000 ppm. This concentration displayed the maximum inhibitory effect on the mycelial growth of the pathogen. At 500 ppm, the fungicide also exhibited significant inhibition with 95.56 per cent growth inhibition. The inhibition was slightly reduced to 81.48 per cent at 250 ppm. The lowest inhibition was observed at 100 ppm, with 69.26 per cent inhibition.

Tebuconazole 50% +Trifloxystrobin25% exhibited complete inhibition (100%) of the mycelial growth of *R. bataticola*at 250, 500, and 1000 ppm. These concentrations displayed the highest inhibitory effects on the pathogen. At 100 ppm, the inhibition was slightly reduced to 95.56 per cent , but it still showed significant inhibitory activity.

Tebuconazole 25% at 250, 500, and 1000 ppmrecorded complete inhibition (100%) of the mycelial growth of *R. bataticola*. These concentrations exhibited strong inhibitory effects on the pathogen. At 100 ppm, the inhibition was slightly lower but still significant, with 90.74 per cent inhibition.

The overall results indicate that all tested fungicides, including Carbendazim 12% + Mancozeb 63% WP, Tebuconazole 25% WG, Hexaconazole 4% + Zineb 68% WG, and Tebuconazole 50% + Trifloxystrobin 25% WG, showed strong inhibitory effects on the mycelial growth of *R. bataticola*. The effectiveness of each fungicide varied with concentration, with higher concentrations generally resulting in higher inhibition rates.

Maruthi et al (2017) reported that. Tebuconazole exhibited 100 per cent inhibition at a concentration of 0.1%, while propiconazole, difenconazole, carbendazim, and hexaconazole showed 100 per cent inhibition at a slightly higher concentration of 0.15%.

Similarly Agale *et al.*(2018) reported that carbendazim 50% WP at 500 ppm, carboxin 37.5% + thiram 37.5% WP at 1500 ppm, and carbendazim 12% WP + mancozeb 63% WP at 2000 ppm. Additionally, thiophanate methyl 70% WP showed 93.57 per cent inhibition, captan 50% WP showed 89.48% inhibition, and hexanconazole 5% EC showed 87.62 per cent inhibition of mycelial growth.

Similar reports were given by the *Tekade* et al. (2021) where he observed cent cent per cent inhibition in Carbendazim, Curzet M-8, Ridomil – MZ, Propiconazole, Dithane M-45, Thiram, Carbendazim + Mancozeb, and Zineb + Hexaconazole.

Table 1 *IN VITRO* EVALUATION OF FUNGICIDES AGAINST *R. BATATICOLA*:

SNo.	Fungicide	Concentration (ppm)	Mycelial growth (cm)	Per cent inhibition[#]	
1	Carbendazim 12%+ Mancozeb 63% WP	100	2.766	69.26	(56.33)
		250	1.66	81.48	(64.51)
		500	0.4	95.56	(77.83)
		1000	0	100.0	(90.00)
2	Tebuconazole 25% WG	100	1.5	83.33	(65.91)
		250	0.4	95.56	(77.83)
		500	0	100.00	(90.00)
		1000	0	100.00	(90.00)
3	Hexaconazole 4% + Zineb 68 % WG	100	0	100.00	(90.00)
		250	0	100.00	(90.00)
		500	0	100.00	(90.00)
		1000	0	100.00	(90.00)
4	Tebuconazole 50% +Trifloxystrobin25%	100	0.83	90.74	(72.28)
		250	0	100.00	(90.00)
		500	0	100.00	(90.00)

	WG	1000	0	100.00	(90.00)
7	Control	---	9.00	0.00	(0.00)

*Figures in parenthesis are arc sine transformed values. # Mean of three replications

	C.D.	SE(d)	SE(m)
Fungicide	1.682	0.828	0.585
Concentration	1.504	0.740	0.523
Fungicide × concentration	3.364	1.655	1.170

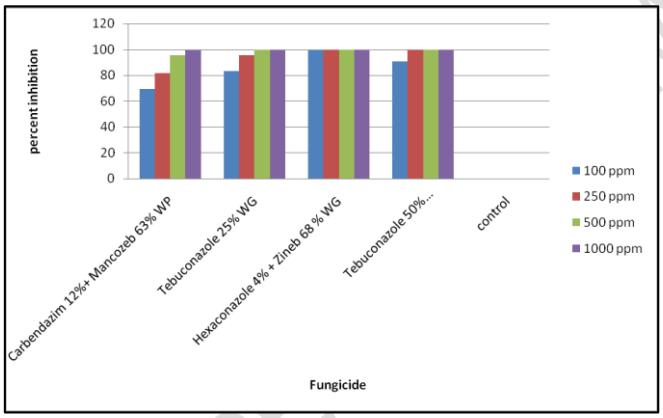

Fig 1 Effect of different concentrations of fungicide on the mycelial growth of *R. bataticola*

Plate1: Effect of fungicides on the mycelial growth of *Rhizoctonia bataticola*

3.2 COMPATIBILITY OF FUNGICIDES WITH POTENTIAL *TRICHODERMA* SP.

In the experiment comparing different fungicides at various concentrations against a potential *Trichoderma* isolate to determine compatibility, the results regarding the mycelial growth inhibition of the bioagent are as follows

Hexaconazole 4% + Zineb 68 % at 100 ppm shown 38.88% inhibition. The "compatibility" value at this concentration was 61.12%, indicating a moderate level of compatibility between the fungicide and the bioagent. At 250 ppm the mycelial growth was significantly reduced with 79.62% inhibition. The compatibility value at this concentration was 20.38%, suggesting lower compatibility but higher inhibitory effects. At 500 ppm the mycelial growth was further reduced to 94.44%. The compatibility value at 500 ppm was 5.56%, indicating lower compatibility and stronger inhibitory effects.

Carbendazim 12% + Mancozeb 63% at all tested concentrations (100, 250, 500, and 1000 ppm), the fungicide recorded complete inhibition (100%) of the mycelial growth of the bioagent. This indicates that there was no compatibility between the bioagent and the fungicide at all the tested concentrations.

Tebuconazole 25% when tested at concentrations (100, 250, 500, and 1000 ppm), the fungicide was recorded with complete inhibition (100%) of the mycelial growth of the bioagent. There was no compatibility between the bioagent and the fungicide at any concentration.

Similar to the other fungicides ,Tebuconazole 50% + Trifloxystrobin 25% when tested at concentrations (100, 250, 500, and 1000 ppm) exhibited complete inhibition (100%) of the mycelial growth of the bioagent. There was no compatibility observed between the bioagent and the fungicide at any concentration.

Overall, the results indicate that Hexaconazole 4% + Zineb 68 % WG showed varying degrees of compatibility and inhibitory effects at different concentrations, while the other fungicides (Carbendazim 12% + Mancozeb 63%, Tebuconazole 25%, and Tebuconazole 50%+ Trifloxystrobin25%were found to be show complete inhibition of the

mycelial growth of the *Trichoderma* at all tested concentrations, suggesting no compatibility between the bioagent and these fungicides.

Patel and Biswas (2016) studied about the compatability and reported that Hexaconazole+Zineb was found compatible to with *Trichoderma.*

Table 2 COMPATIBILITY OF FUNGICIDES WITH POTENTIAL *TRICHODERMA ASPERELLUM*

S.no	Fungicide	Concentration (ppm)	Mycelial growth(cm)	#Per cent inhibition	
1	Carbendazim 12%+ Mancozeb 63% WP	100	0	100	(90.00)
		250	0	100	(90.00)
		500	0	100	(90.00)
		1000	0	100	(90.00)
2	Tebuconazole 25% WG	100	0	100	(90.00)
		250	0	100	(90.00)
		500	0	100	(90.00)
		1000	0	100	(90.00)
3	Hexaconazole 4% + Zineb 68 % WG	100	8	38.88	(38.58)
		250	2.66	79.62	(63.17)
		500	0.83	94.44	(76.37)
		1000	0	100	(90.00)
4	Tebuconazole 50% +Trifloxystrobin25% WG	100	0	100	(90.00)
		250	0	100	(90.00)
		500	0	100	(90.00)
		1000	0	100	(90.00)
7	Control	---	9	0	(0.00)

*Figures in parenthesis are arc sine transformed values. # Mean of three replications

	C.D.	SE(d)	SE(m)
Fungicide	1.34	o.66	0.46
concentration	1.20	0.59	0.41
Fungicide × concentration	2.69	1.32	0.93

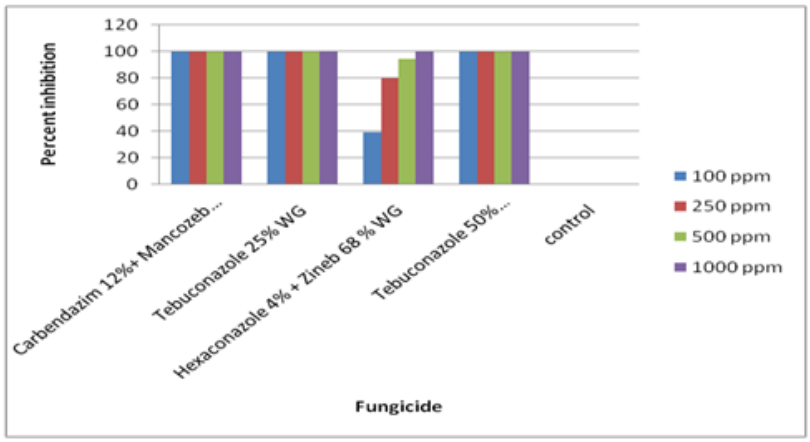

Fig 2 Effect of different doses of fungicide on the mycelial growth inhibition of *Trichoderma* sp.

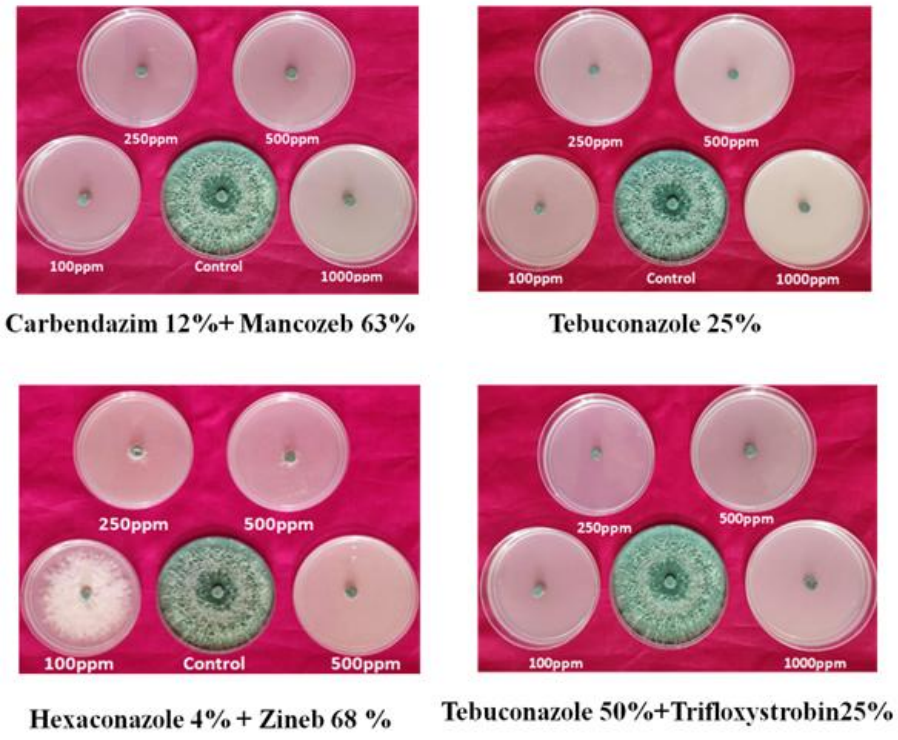

Plate 2 : Effect of fungicides on the mycelial growth of *Trichoderma* sp.

4. CONCLUSION

In conclusion, the study evaluated the efficacy of four fungicides, Carbendazim 12%+ Mancozeb 63% WP, Tebuconazole 25% WG, Hexaconazole 4% + Zineb 68% WG, and Tebuconazole 50% + Trifloxystrobin 25% WG, against the pathogen *Rhizoctoniabataticola*, responsible for causing dry root rot in groundnut. The fungicides exhibited strong inhibitory effects on the mycelial growth of *R. bataticola*, with Hexaconazole 4% + Zineb 68% WG being the most effective, displaying complete inhibition at all concentrations.

Additionally, the study assessed the compatibility of these fungicides with a potential Trichoderma isolate, a biocontrol agent. The results indicated that Hexaconazole 4% + Zineb 68% WG demonstrated varying degrees of compatibility and inhibitory effects at different concentrations. However, the other fungicides, including Carbendazim 12% + Mancozeb 63%, Tebuconazole 25%, and Tebuconazole 50% + Trifloxystrobin 25%, showed complete inhibition of Trichoderma mycelial growth at all tested concentrations, suggesting no compatibility between the fungicides and the bioagent.These findings suggest that Hexaconazole 4% + Zineb 68% WG has the potential for use in integrated disease management strategies, as it effectively controls *R. bataticola* while still displaying moderate

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compatibility with *Trichoderma*. However, further research and field trials are necessary to validate the practical applicability and sustainability of using these fungicides in controlling dry root rot in groundnut crops while preserving beneficial soil microorganisms.Overall, the study emphasizes the importance of assessing the compatibility of fungicides with biocontrol agents, as using compatible chemicals can enhance disease management while reducing the development of fungicidal resistance. By adopting integrated approaches, we can improve disease control, promote sustainable agriculture, and maximize groundnut productivity in regions where dry root rot poses a significant challenge.

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