

Original Research Article

Evaluation of fungicides and their compatibility with *Trichoderma asperellum* for management of against *Rhizoctonia bataticola* (Taub) Butler causing dry root rot ~~of in~~ groundnut and their compatibility with *Trichoderma asperellum*

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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 *Rhizoctonia bataticola*, the causal agent of dry root rot in groundnut was evaluated. ~~The antifungal activity was assessed at various concentrations using the poisoned food technique. Among the tested fungicides, Hexaconazole 4% + Zineb 68% WG exhibited the highest efficacy, with 100% inhibition of mycelial growth at all concentrations tested. Furthermore, the compatibility Tests of 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% of growth inhibition ~~of in~~ *T. asperellum* growth. However, a "compatibility" value of 64.12% 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, while the other fungicides tested were totally incompatible with showed 100 percent ~~inhibition~~ inhibition of *T. asperellum* growth, suggesting incompatibility with the biocontrol agent at the tested concentrations.~~

Keywords: Dry root rot, *Rhizoctonia bataticola*, *Trichoderma*, Fungicides, poisoned food technique, ~~compatibility~~ compatibility.

1. INTRODUCTION

~~Worldwide the leading producer of groundnut (*Arachis hypogaea* L.) is China, with a production of 17.57 million tonnes, followed by India with 6.73 million tonnes in 2019-20. Groundnut (*Arachis hypogaea* L.) is the most cultivated oil seed crop in India and second to soybean in production. 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.

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Among biotic factors, ~~pathogenic attacks~~ pathogens on groundnut plants ~~can lead to cause~~ significant yield losses ~~in the field as well as 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* The pathogen 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.

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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 (BCAs), such as *Trichoderma* spp., 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.

MATERIAL AND METHODS

2.1 Isolation of pathogen

The test pathogen ~~*Rhizoctonia*~~ *R. 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~~ sub culturing during the study.

Comment [GN3]: How was the identity of pathogen confirmed? Mention the source.

2.2 Isolation 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 and isolation of *Trichoderma* was conducted done using through 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.~~

Comment [GN4]: If more than one species were isolated, please mention their taxonomic identity.

Comment [GN5]: How was the identify confirmed? Mention the source.

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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 culture plates were subsequently incubated at a temperature of 28 ± 2°C for a period of 72 hours, during which colony development was regularly monitored.

Comment [GN6]: Please mention the name of the selective medium

2.3 Fungicides

In the present investigation four systemic fungicides viz Carbendazim 12%+ Mancozeb 63% WP, Tebuconazole 25% WG, Hexaconazole 4% + Zineb 68 % WG, Tebuconazole 50%+Trifloxystrobin 25% 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)

Comment [GN7]: In the table, the mycelial growth is represented in cm.

2.4 Evaluation of effective fungicide and Efficacy of fungicides against *R. bataticola* dry root rot pathogen and potential compatibility with *Trichodermaasperellum*

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 30 ml of the autoclaved PDA medium. This poisoned medium was then evenly distributed into three petri plates to solidify, 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 $28 \pm 2^\circ\text{C}$, 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*:

All the fungicides were completely inhibitory to *R. bataticola* at concentrations from 250-1000ppm. Of the four fungicides tested, in the present study, four fungicides viz, Carbendazim 12%+ Mancozeb 63% WP, Tebuconazole 25% WG, Hexaconazole 4% + Zineb 68 % WG, Tebuconazole 50%+Trifloxystrobin 25% 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 as well as Tebuconazole 50% +Trifloxystrobin 25% (100, 250, 500, 1000) exhibited complete inhibition of the mycelial growth of the pathogen recording more than 95% efficacy. This indicates that the fungicide was highly effective in suppressing the growth of *R. bataticola* at all concentrations tested.

Comment [GN8]: ppm?

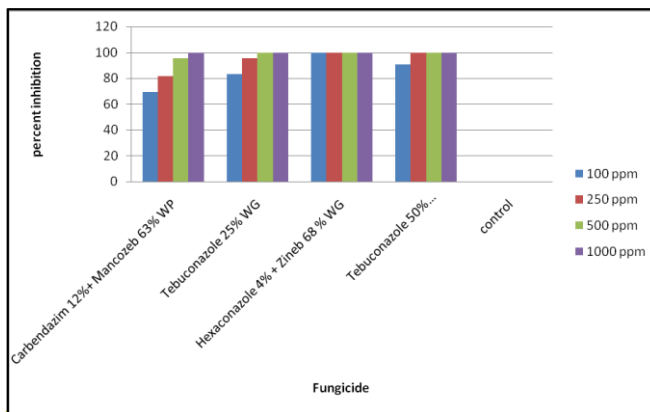
Among the four different concentrations tested, the fungicide Carbendazim 12%+ Mancozeb 63% exhibited the highest inhibition of 95.56 and 100 per cent was observed at 500 and 1000 ppm, respectively while Tebuconazole 25% at 250, 500, and 1000 ppm recorded complete inhibition (100%) of the mycelial growth of *R. bataticola* (Table 1). 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% +Trifloxystrobin 25% 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 ppm recorded 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.

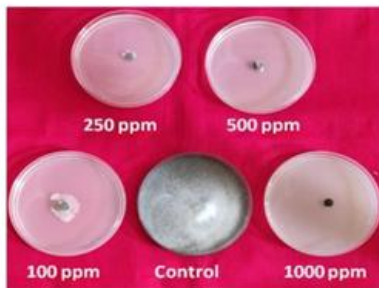
Concentration	1.504	0.740	0.523
Fungicide x concentration	3.364	1.655	1.170



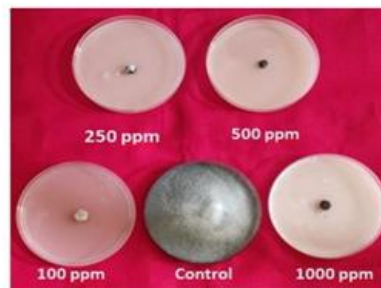
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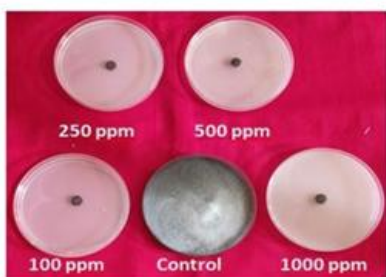
Fig 1 Effect of different concentrations of fungicide on the mycelial growth of *R. bataticola*



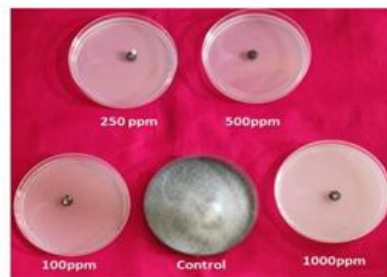
Carbendazim 12%+ Mancozeb 63%



Tebuconazole 25%



Hexaconazole 4% + Zineb 68 %



Tebuconazole 50%+Trifloxystrobin 25%

Plate1:Effect of fungicides on the mycelial growth of *Rhizoctoniabataticola*

3.2COMPATIBILITY OF FUNGICIDES WITH POTENTIAL *TRICHODERMA SP.*

~~Moderate compatibility of 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~~

Comment [GN19]: In few places, it is mentioned as *Trichoderma asperellum* and in here, it is mentioned as *Trichoderma sp.* Follow uniformity.

~~Hexaconazole 4% + Zineb 68 % at 100 ppm with the bioagent was observed shown as 38.88% inhibition. the 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 However, the compatibility value at 500 ppm was 5.56%, indicating lower compatibility and stronger inhibitory effects.~~

~~Carbendazim 12% + Mancozeb 63% All other fungicides tested at all four concentrations at all tested concentrations (100, 250, 500, and 1000 ppm), the fungicide recorded complete completely arrested inhibition (100%) of the mycelial growth of the bioagent. indicating This indicates that there was no in compatibility between the bioagent and the fungicide at all the tested concentrations. Patel and Biswas (2016) had also observed the compatibility of Hexaconazole+Zineb with *Trichoderma*.~~

Comment [GN20]: Concentration?

~~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% + Trifloxystrobin 25%) 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*.~~

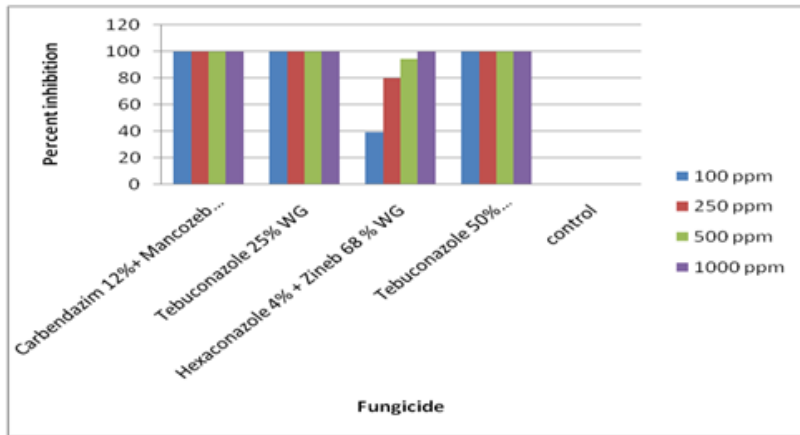
Table 2 COMPATIBILITY OF FUNGICIDES WITH POTENTIAL *TRICHODERMA ASPERELLUM*

S.no	Fungicide	Concentration (ppm)	Mycelial growth(cm)	#Per cent inhibition
1	Carbendazim 12%+	100	0	100 (90.00)
	Mancozeb 63% WP	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	0.66	0.46
concentration	1.20	0.59	0.41
Fungicide x concentration	2.69	1.32	0.93



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

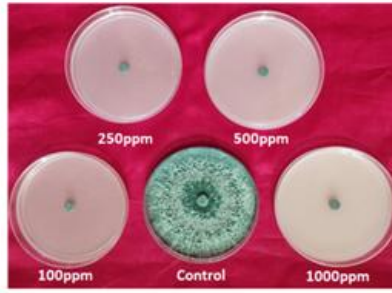
Comment [GN23]: Please mention the name consistently: either *T.asperellum* or *Trichoderma* sp.

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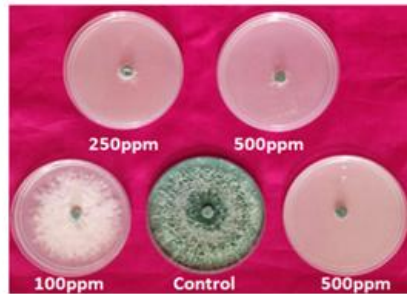
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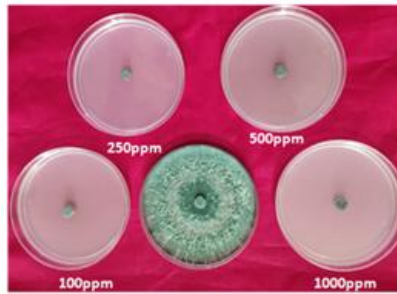
Carbendazim 12%+ Mancozeb 63%



Tebuconazole 25%



Hexaconazole 4% + Zineb 68 %



Tebuconazole 50%+Trifloxystrobin25%

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

Comment [GN24]: Is it *T.asperellum* or *Trichoderma* sp.?

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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, tested against the dry root rot pathogen *R. bataticola*, exhibited strong inhibitory effect on the mycelial growth at 250-1000ppm concentrations while, against the pathogen *Rhizoctonia bataticola*, 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 combination, displaying complete inhibition at all concentrations even at a lower dose of 100ppm. All fungicides except Carbendazim 12%+ Mancozeb 63% WP at the lowest dose of 100ppm, were completely incompatible. 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 at 100ppm has the potential for use in integrated disease management strategies against *R. bataticola*, as it effectively controls *R. bataticola* while still displaying moderate 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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