



Evaluation of Fungicides Against *Rhizoctonia bataticola* (Taub) Butler Causing Dry Root Rot of Groundnut and Their Compatability with *Trichoderma asperellum*

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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 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 using the poison food technique. Among the tested fungicides, Hexaconazole 4% + Zineb 68% WG exhibited the highest efficacy, with 100% inhibition of mycelial

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growth at all concentrations tested. Tests of compatibility of these fungicides with *Trichoderma asperellum* revealed that Hexaconazole 4% + Zineb 68% WG caused 38.88% of growth inhibition in *T. asperellum* while the other fungicides tested were totally incompatible with 100 percent inhibition.

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

1. INTRODUCTION

China stands as the primary global producer of groundnuts (*Arachis hypogaea* L.), having achieved a remarkable output of 17.57 million tonnes in 2019-20 followed by India, contributing 6.73 million tonnes during the same period according to data from the Agricultural Market Intelligence Centre at PJTSAU in 2022. In India, groundnut holds the status of being the most extensively cultivated oilseed crop and holds second place in production, trailing only behind soybeans. Despite its immense significance, the productivity of groundnut remains relatively low in numerous developing nations. This can be attributed to a range of constraints that affect production, encompassing both biotic and abiotic stresses.

Among biotic factors, pathogens on groundnut plants cause significant yield losses in the field as well as reduce seed quality during post-harvest storage. Among these diseases, dry root rot caused by *Rhizoctonia bataticola* is an emerging disease and has significant economic implications. It can cause substantial yield losses in groundnut crop (*Arachis hypogaea*). Although the fungus can infect all parts of the plant, it primarily targets the roots, stems, and pods. The first symptom of the disease is yellowing of the leaves which droop in next 2 or 3 days and withers off. The plant may wilt within a week after the appearance of first symptom. When stem is examined closely, dark lesions may be seen on the bark at the ground level. If the plants are pulled from soil, the basal stem and main root may show dry rot symptoms. The tissues are weakened and break off easily in advanced cases and sclerotial bodies may be seen scattered on the affected tissues [1].

Rhizoctonia bataticola is a warm dry weather pathogen, is responsible for economic losses to an extent of 80%. The disease is sporadic in occurrence and is particularly serious in Rajasthan, Uttar Pradesh, Tamil Nadu, Andhra Pradesh and Maharashtra where groundnut is cultivated mostly in rain fed and dry conditions prevailing during the season [2].

Chemical fungicides are commonly used to control *R. bataticola* and manage the disease. Owing to the adverse impacts of their usage and the emergence of resistance in agricultural pathogens, there has been a shift towards employing biological alternatives for disease management across different crops. In recent times bioagents have emerged as environmentally friendly and socially acceptable alternatives to traditional fungicides for controlling soil-borne diseases [3]. Consequently, *Trichoderma* spp. have gained prominence as microbial antagonists, finding application in the control of root diseases in diverse field crops [4].

However, the use of fungicides may also have negative effects on beneficial soil microorganisms (BCAS), such as *Trichoderma* spp., which are crucial for sustainable agriculture. 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.

2. MATERIALS AND METHODS

2.1 Isolation of Pathogen

The pathogen under examination, *R. bataticola*, was obtained from S.V. Agricultural College in Tirupati. It was extracted from the roots of plants afflicted with dry root rot using the tissue segment method [5] on Potato Dextrose Agar media and was identified based on morphological characters. Upon observation, the fungus that emerged from the root bits exhibited a prolific aerial mycelium that gradually changed colour from greyish brown to black. Pure cultures were obtained using the single hyphal tip method, maintaining the isolates on PDA medium through regular sub culturing during the study.

2.2 Isolation of *Trichoderma asperellum* from Rhizosphere Soil

Composite soil samples were collected from the rhizosphere of healthy groundnut plants

and isolation of *Trichoderma* was done through the serial dilution technique [6] on *Trichoderma* selective media. The culture plates were subsequently incubated at a temperature of $28 \pm 2^\circ\text{C}$ for a period of 72 hours, during which colony development was regularly monitored. The isolated *Trichoderma* sp. was identified as *Trichoderma asperellum* under the accession number OR357655.

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, 1000 ppm) using poisoned food technique [7].

Percent inhibition of mycelial growth of dry root rot pathogen = $\frac{C-T}{C} \times 100$

Where, C = mycelial growth in control (cm)
T = mycelial growth in treatment (cm)

2.4 Efficacy of Fungicides Against *R. bataticola* and Compatibility with *Trichoderma asperellum*

For each treatment, 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 autoclaved PDA medium. This poisoned medium was then evenly distributed into three petri plates to solidify, which were considered as three replications. A disc (6 mm) of *R. bataticola*, from the periphery of an actively growing colony 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-

1000 ppm. Of the four fungicides tested, Hexaconazole 4% + Zineb 68% WG as well as Tebuconazole 50% + Trifloxystrobin 25% (100, 250, 500, 1000 ppm) 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. Carbendazim 12% + Mancozeb 63% exhibited the highest inhibition of 95.56 and 100 per cent 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). Similar results from the study conducted by Maruti et al. (2017) provided insights into the efficacy of various fungicides against *R. bataticola* causing dry root rot of pigeon pea under *in vitro* conditions. Furthermore, the study investigated the effectiveness of systemic fungicides against *R. bataticola*. Tebuconazole showed 100 per cent inhibition at a concentration of 0.1%, while propiconazole, difenaconazole, carbendazim, and hexaconazole showed cent per cent inhibition at a slightly higher concentration of 0.15%.

Similarly, Agale et al. [8] evaluated the efficacy of several fungicides against *R. bataticola* of soy bean using *in vitro* poisoned food technique. All fungicides tested showed significant inhibition of mycelial growth compared to the untreated control. The most effective fungicides, with 100 per cent mycelial growth inhibition, were 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 per cent inhibition, and hexaconazole 5% EC showed 87.62 per cent inhibition of mycelial growth.

In the similar studies Tekade et al. [9] proved that cent per cent inhibition of *R. bataticola* obtained in Carbendazim, Curzet M-8, Ridomil – MZ, Propiconazole, Dithane M-45, Thiram, Carbendazim + Mancozeb, and Zineb + Hexaconazole. Next in order of merit fungicides like Tricyclozole + Mancozeb and Tridemorph recorded 98.50 per cent and 93.79 per cent inhibition respectively.

In the present investigation all the four fungicides were proved to be effective on mycelial growth inhibition. Similar results were provided by Bharani Deepan et al. [10] who investigated on the mode of action of different combination

fungicides and they revealed that tebuconazole and hexaconazole interfere with biosynthesis of sterols in cell membranes. They also reported that zineb in zineb 68% + hexaconazole 4% and mancozeb in mancozeb 50% + carbendazim 25% combination fungicide inactivates sulphahydryl groups in enzymes block

metabolism at krebs cycle whereas carbendazim disrupts spindle formation during cell division. Suthin et al. (2016) revealed that demethylation of C-14 by hexaconazole during ergosterol biosynthesis leads to accumulation of C-14 methyl sterols which controlled *Rhizoctonia solani* causing sheath blight of rice.

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% WG	100	0.83	90.74 (72.28)
		250	0	100.00 (90.00)
		500	0	100.00 (90.00)
		1000	0	100.00 (90.00)
5	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 x concentration	3.364	1.655	1.170

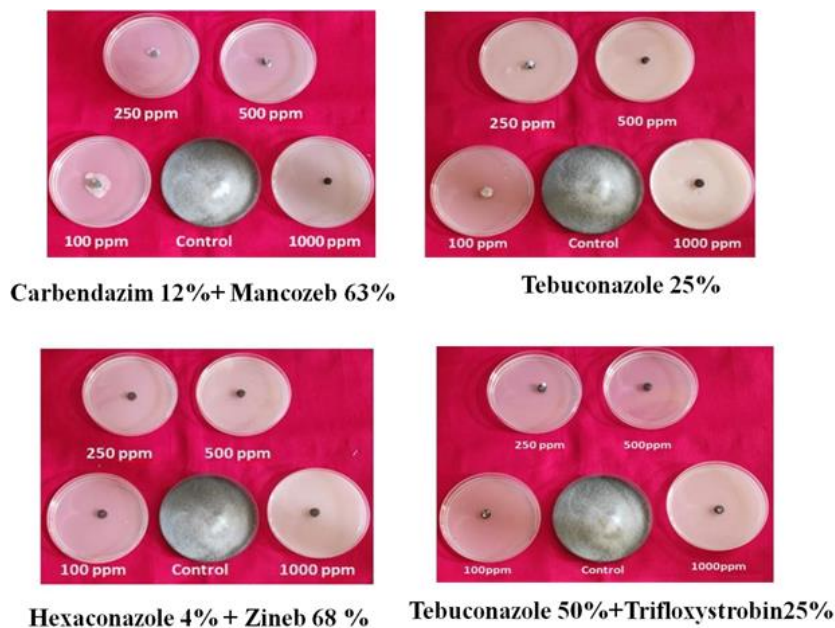


Plate 1. Effect of fungicides on the mycelial growth of *Rhizoctonia bataticola*

3.2 Compatibility of Fungicides with Potential *Trichoderma asperellum*

Moderate compatibility of Hexaconazole 4% + Zineb 68 % at 100 ppm with the bioagent was observed as the "compatibility" value at this concentration was 61.12%. However, the compatibility value at 500 ppm was 5.56%, indicating lower compatibility and stronger inhibitory effects. All other fungicides tested at all four concentrations completely arrested (100%) mycelial growth of the bioagent indicating incompatibility.

Kumar et al. [11] reported that Carbendazim 12% + Mancozeb 63% has completely inhibited mycelial growth of *T. asperellum* and had inhibited to cent per cent at 100,200,400 and 800 ppm.

Singh et al. [12] studied about the compatibility of different fungicides viz., Hexaconazole, Tebuconazole 50%+ Trifloxystrobin 25% WG, propiconazole and tebuconazole at 10, 15, 20 and 25 ppm for their compatibility with *T. harzianum* strain IRRI-1 using poisoned food technique. All the four concentrations of Tebuconazole 50%+ Trifloxystrobin 25% WG were highly compatible with low toxic effect against *T. harzianum* strain IRRI-1 *in vitro*. Nativo and Propiconazole were comparatively safer, than Hexaconazole and Tebuconazole which

exhibited acute toxicity for the growth of *T. harzianum* strain IRRI-1.

Similarly Sharma et al. [13] found that *Trichoderma harzianum* strain TCMS-14 was found to be completely compatible with sulphur (at 625ppm, 1250ppm and 2500 ppm), zineb (375ppm, 750ppm and 1500ppm).

These findings are in accordance with Bindu Madhavi et al. (2011) who reported that lower compatibility of *Trichoderma* with carbendazim + mancozeb (0.2%) as 67.4 per cent mycelial inhibition. Durga et al. (2014) also opined that Avtar (Zineb + Hexaconazole) was compatible with *T. koningi* at 0.25 per cent. Teertha et al. (2017) also reported that Avtar (Zineb 68% + Hexaconazole 4%) tested at 100 ppm inhibited *T. asperellum* to 76.4 per cent with 23.60 compatibility. Further, they reported that complete inhibition of radial growth of *T. asperellum* was recorded with tebuconazole 50% + trifloxystrobin 25% WG, azoxystrobin 18.2% + difenoconazole 11.4% SC, carbendazim 12% + mancozeb 63% at different concentrations of 1000ppm. Maheswary et al. (2020) and Vineela et al. [14] reported that carbendazim 12% + mancozeb 63% has inhibited radial growth of *T. viride*, *T. harzianum* and *T. hamatum* completely (100% inhibition) and was found to be incompatible with *Trichoderma* [15,16,17].

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
		250	0	100
		500	0	100
		1000	0	100
2	Tebuconazole 25% WG	100	0	100
		250	0	100
		500	0	100
		1000	0	100
3	Hexaconazole 4% + Zineb 68 % WG	100	8	38.88
		250	2.66	79.62
		500	0.83	94.44
		1000	0	100
4	Tebuconazole 50% +Trifloxystrobin25% WG	100	0	100
		250	0	100
		500	0	100
		1000	0	100
5	Control	---	9	0

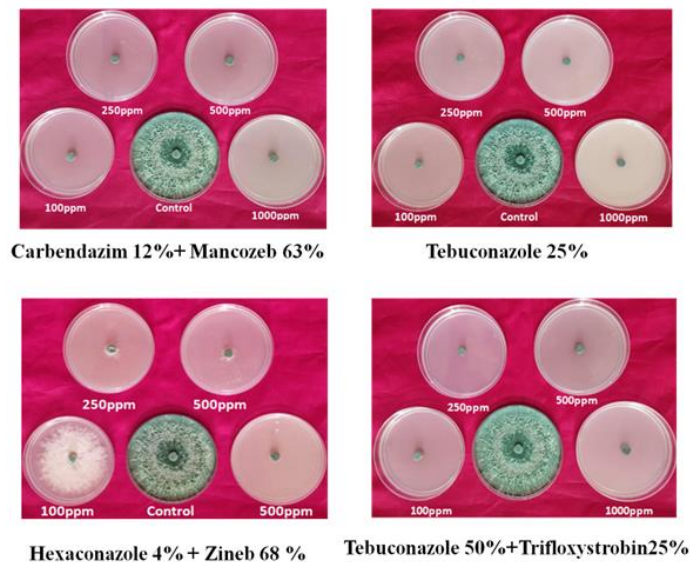


Plate 2. Effect of fungicides on the mycelial growth of *Trichoderma asperellum*

4. CONCLUSION

Among the fungicides, Hexaconazole 4% + Zineb 68% WG exhibited complete inhibition of the pathogen at all concentrations. Carbendazim 12% + Mancozeb 63% WP and Tebuconazole 50% + Trifloxystrobin 25% WG showed complete inhibition at higher concentrations (1000 ppm), and Tebuconazole 25% WG also displayed complete inhibition at 250 ppm, 500 ppm, and 1000 ppm. The results indicate that all tested fungicides were effective in suppressing *R. bataticola* mycelial growth, with efficacy varying with concentration. The compatibility of various fungicides with the bioagent *T. asperellum* was tested at different concentrations to determine their inhibitory effects on mycelial growth. With Hexaconazole 4% + Zineb 68%, highest compatibility (88.89 %) was observed at a concentration of 100 ppm, resulting in 11.11% mycelial growth inhibition. At higher concentrations (250 ppm and 500 ppm), mycelial growth was significantly reduced with inhibition percentages of 70.37% and 90.74% respectively, indicating lower compatibility but stronger inhibitory effects. However, Carbendazim 12% + Mancozeb 63%, Tebuconazole 25%, and Tebuconazole 50% + Trifloxystrobin 25% showed complete inhibition (100%) of mycelial growth at all concentrations tested, indicating no compatibility between the bioagent and the fungicides. These results suggest that Hexaconazole 4% + Zineb 68% WG showed varying degrees of compatibility and inhibitory effects at different concentrations, while the other

fungicides exhibited no compatibility with *T. asperellum* and completely inhibited its mycelial growth. The compatibility of *T. asperellum* with Hexaconazole 4% + Zineb 68% WG offers the possibility of integrating biocontrol strategies with chemical fungicides for improved disease management and better outcomes in groundnut cultivation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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