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Eco-safe Management of Groundnut Stem and Pod Rot Pathogen through Bio-Agents and Fungicides under *in vitro* Conditions

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ABSTRACT: Stem and pod rot caused by *Sclerotium rolfsii* is a major constraint in groundnut production, particularly under irrigated conditions, where chemical dependence and pathogen persistence in soil pose serious management challenges. The present investigation was undertaken to evaluate eco-safe bio-agents and fungicides against *S. rolfsii* under *in vitro* conditions. Five bio-control agents were tested using the dual culture technique, among which *Trichoderma harzianum* exhibited maximum inhibition of mycelial growth (72.30%), followed by *T. viride* (51.10%). Ten fungicides were evaluated using the poisoned food technique at 100, 250 and 500 ppm concentrations. Azoxystrobin 23% SC, azoxystrobin 11% + tebuconazole 18.3% SC, tebuconazole 25.9% EC and hexaconazole 5% SC completely inhibited the pathogen at all tested concentrations. A positive correlation was observed between fungicide concentration and growth inhibition of *S. rolfsii*. The study highlights the potential of integrating bio-agents with eco-safe fungicides for sustainable management of groundnut stem and pod rot.

Contribution of the study: This study provides comparative evidence on eco-friendly biological and chemical options for managing *S. rolfsii*, contributing toward reduced chemical load and sustainable disease management strategies in groundnut.

Keywords: *Sclerotium rolfsii*, groundnut, bio-agents, *Trichoderma* spp., fungicides, poisoned food technique; growth inhibition.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops in India, occupying a prominent position in terms of cultivated area and production. Gujarat ranks first in area, production and productivity of groundnut in the country. Despite its economic importance, productivity is severely constrained by several soil-borne diseases such as collar rot, stem rot, pod rot, root rot and seedling blight, among which stem and pod rot caused by *Sclerotium rolfsii* is considered one of the most destructive diseases (Rani, 2017).

The pathogen survives in soil through persistent sclerotia and infects plants under warm and humid conditions, resulting in severe yield losses ranging from 45 to 80 per cent (Maddu and Ravuri 2015; Ganesan *et al.*, 2007). The widespread use of chemical fungicides for disease management has led to serious concerns related to environmental pollution, pesticide residues and the development of resistance in plant pathogens (Perez-Moreno *et al.*, 2009). Consequently, the emphasis has shifted toward eco-safe and sustainable disease management strategies involving biological control agents and reduced-risk fungicides.Biological control using antagonistic microorganisms, particularly *Trichoderma* spp. and plant growth-promoting rhizobacteria, has been recognized as an effective alternative for managing soil-borne pathogens (Gowdra and Nagaraja 2015; Wavare *et al.*, 2017). Recent studies have further demonstrated the efficacy of *Trichoderma* spp. in suppressing *S. rolfsii* through mechanisms such as mycoparasitism, antibiosis and competition (Choudhary *et al.*, 2019; Patel *et al.*, 2018; Sharma *et al.*, 2021). Similarly, integrated disease management approaches combining bioagents and fungicides have shown promising results in groundnut (Meena *et al.*, 2022).

In recent years, several new fungicide molecules with broad-spectrum activity and reduced environmental impact have been introduced for the management of soil-borne diseases. Studies conducted during 2019–2023 reported high efficacy of strobilurin- and triazole-based fungicides against *S. rolfsii* under *in vitro* conditions (Kumar *et al.*, 2020; Raghavendra and Srinivas 2020; Verma *et al.*, 2023). However, comparative information on the efficacy of newer fungicides along with promising bioagents against *S. rolfsii* under Indian agro-climatic conditions remains limited.

Therefore, the present investigation was undertaken to evaluate the antagonistic potential of selected bioagents and the efficacy of commonly used and newer fungicides for eco-safe management of groundnut stem and pod rot caused by *Sclerotium rolfsii* under *in vitro* conditions.

MATERIALS AND METHODS

The antagonistic potential of bioagents viz., *Trichoderma viride*, *T. harzianum*, *T. asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *Sclerotium rolfsii* was evaluated under *in vitro* conditions using the dual culture technique. Fungal antagonists were maintained on potato dextrose agar (PDA), whereas bacterial antagonists were maintained on nutrient agar medium.

For fungal bioagents, 5 mm mycelial discs of the pathogen and antagonist were placed on PDA plates at a distance of 6 cm. In the case of bacterial antagonists, a 5 mm mycelial disc of *S. rolfsii* was placed on one side of the PDA plate and a loopful of bacterial culture was streaked 3 cm away. Plates inoculated with the pathogen alone served as control. Each treatment was replicated three times and incubated at 28 ± 1 °C.

The efficacy of ten fungicides, namely carbendazim 50% WP, carbendazim 12% + mancozeb 63%, pyraclostrobin 20% WG, metalaxyl 8% + mancozeb 64%, azoxystrobin 23% SC, azoxystrobin 11% + tebuconazole 18.3% SC, propiconazole 25% EC, copper oxychloride 50% WP, tebuconazole 25.9% EC and hexaconazole 5% SC, was evaluated at 100, 250 and 500 ppm concentrations using the poisoned food technique (Nene and Thapliyal 1993). The required quantity of fungicide was mixed thoroughly with molten PDA and 20 ml of the medium was poured into sterile 90 mm Petri plates. PDA without fungicide served as control. A 5 mm mycelial disc from the actively growing margin of a 5-day-old culture of *S. rolfsii* was placed at the centre of each plate. Each treatment was replicated twice and incubated at 28 ± 1 °C.

Radial mycelial growth (mm) was recorded at 24 h intervals until complete growth of the pathogen in control plates. Per cent inhibition of mycelial growth over control was calculated using Vincent's formula (1947):

$$PGI = \frac{DC - DT}{DC} \times 100$$

where DC represents the mean colony diameter in control and DT represents the mean colony diameter in treated plates.

The experimental data were subjected to analysis of variance (ANOVA) using OPSTAT statistical software, and treatment means were compared at the 5 per cent level of significance.

RESULT AND DISCUSSION

All the tested bioagents significantly inhibited the mycelial growth of *Sclerotium rolfsii* compared to the untreated control. Among the bioagents, *Trichoderma harzianum* recorded the highest per cent inhibition of mycelial growth (72.30%), followed by *T. viride* (51.10%). The superior antagonistic activity of *T. harzianum* may be attributed to its rapid colonization, mycoparasitic ability and production of antifungal metabolites. Similar observations were reported earlier by Gowdra and Nagaraja (2015); Wavare *et al.* (2017); Choudhary *et al.* (2019), who also recorded maximum inhibition of *S. rolfsii* by *Trichoderma* spp. under laboratory conditions. The present findings further corroborate the reports of Sharma *et al.* (2021); Patel *et al.* (2018), highlighting the potential of *Trichoderma* spp. as effective biocontrol agents against soil-borne pathogens.

Table 1: Effect of different bio-agents against *S. rolfsii* under *in-vitro* conditions.

Tr. No.	Treatments	Mycelial growth (mm)	Growth inhibition (%)
T_1	*Trichodermaviride* (2×10^8)	44.01	51.10
T_2	*Trichodermaharzianum*(2×10^8)	24.93	72.30
T_3	*Trichodermaasperellum*(2×10^8)	47.01	47.77
T_4	*Pseudomonasfluorescens* (1×10^8)	60.39	32.91
T_5	*Bacillussubtilis* (1×10^8)	64.45	28.39
T_6	Control(Test pathogenonly)	90.00	-
S.Em. ±		1.47	-
C.D. at 5%		4.37	-
C.V. (%)		5.33	-

To achieve effective management of stem and pod rot of groundnut, the antifungal efficacy of different fungicides was evaluated against *Sclerotium rolfsii* at 100, 250 and 500 ppm concentrations using the poisoned food technique. The results are presented in Table 2.

At 100 ppm concentration, complete inhibition (100%) of mycelial growth of *S. rolfsii* was recorded with azoxystrobin 23% SC, azoxystrobin 11% + tebuconazole 18.3% SC, tebuconazole 25.9% EC and hexaconazole 5% SC, as evidenced by zero mycelial growth. Pyraclostrobin 20% WG was the next most effective fungicide, recording 71.11 per cent growth inhibition with a mycelial growth of 26.00 mm. Moderate inhibition was observed with propiconazole 25% EC (58.46%), metalaxyl 8% + mancozeb 64% WP (52.77%) and carbendazim 50% WP (48.89%). Carbendazim 12% + mancozeb 63% WP showed comparatively lower inhibition (32.22%), whereas copper oxychloride 50% WP was the least effective,

recording only 17.22 per cent inhibition with maximum mycelial growth (74.50 mm).

At 250 ppm concentration, azoxystrobin 23% SC, azoxystrobin 11% + tebuconazole 18.3% SC, tebuconazole 25.9% EC and hexaconazole 5% SC again exhibited complete inhibition of *S. rolfsii*. Pyraclostrobin 20% WG showed 78.89 per cent inhibition with 19.00 mm mycelial growth. Carbendazim 12% + mancozeb 63% WP, metalaxyl 8% + mancozeb 64% WP and propiconazole 25% EC recorded moderate inhibition ranging from 64.16 to 65.00 per cent and were statistically at par with each other. Carbendazim 50% WP resulted in 57.78 per cent inhibition, while copper oxychloride 50% WP remained the least effective with only 20.56 per cent inhibition.

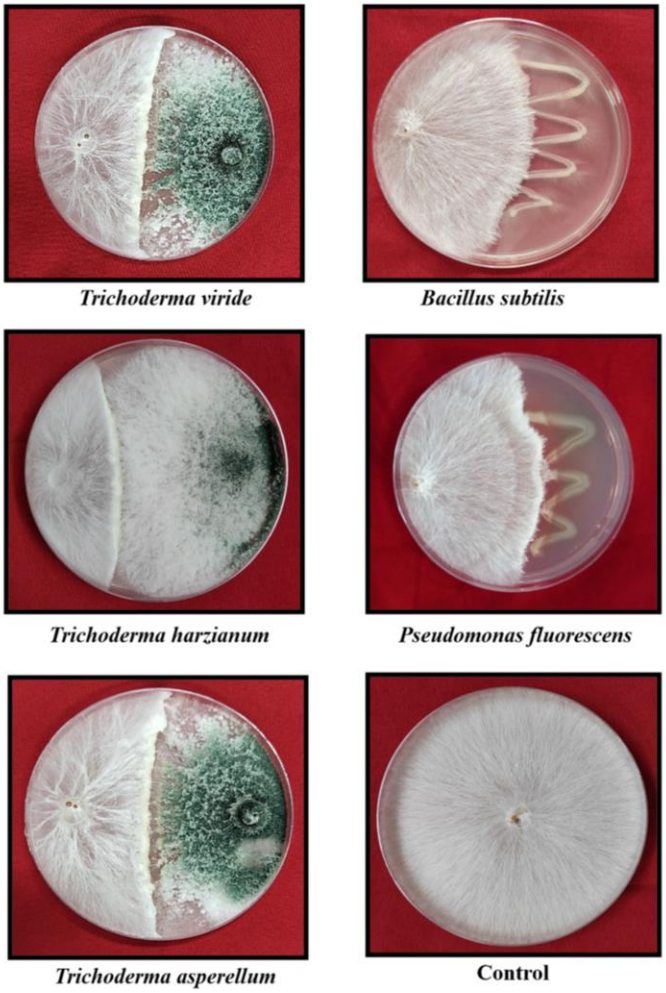

Fig. 1. Efficacy of different bio-agents against *S. rolfsii* under in-vitro conditions.

At 500 ppm concentration, cent per cent inhibition of mycelial growth was consistently observed with azoxystrobin 23% SC, azoxystrobin 11% + tebuconazole 18.3% SC, tebuconazole 25.9% EC and hexaconazole 5% SC. Pyraclostrobin 20% WG recorded 87.07 per cent inhibition, followed by carbendazim 12% + mancozeb 63% WP (80.55%) and metalaxyl 8% + mancozeb 64% WP (79.84%), which were statistically at par. Carbendazim 50% WP and propiconazole 25% EC showed moderate inhibition, whereas copper oxychloride 50% WP recorded the lowest inhibition (33.89%) even at higher concentration.

The results clearly indicated a dose-dependent increase in mycelial growth inhibition with increasing fungicide concentration. Similar findings were reported by Raghavendra and Srinivas (2020), who observed complete inhibition of *S. rolfsii* by azoxystrobin, tebuconazole and hexaconazole at higher concentrations. Sekhar *et al.* (2020) also reported significant inhibition of *S. rolfsii* by hexaconazole under in vitro conditions. The present findings are in conformity with earlier studies by Perez-Moreno *et al.* (2009); Kumar *et al.* (2020), who reported higher sensitivity of *S. rolfsii* to triazole and strobilurinbased fungicides. Recent reports published further support the superior efficacy of newer fungicide molecules against soil-borne pathogens (Meena *et al.*, 2022; Verma *et al.*, 2023).

Overall, the present study confirms the effectiveness of newer fungicides, particularly azoxystrobin- and triazole-based formulations, and supports their integration with bioagents for sustainable management of groundnut stem and pod rot.

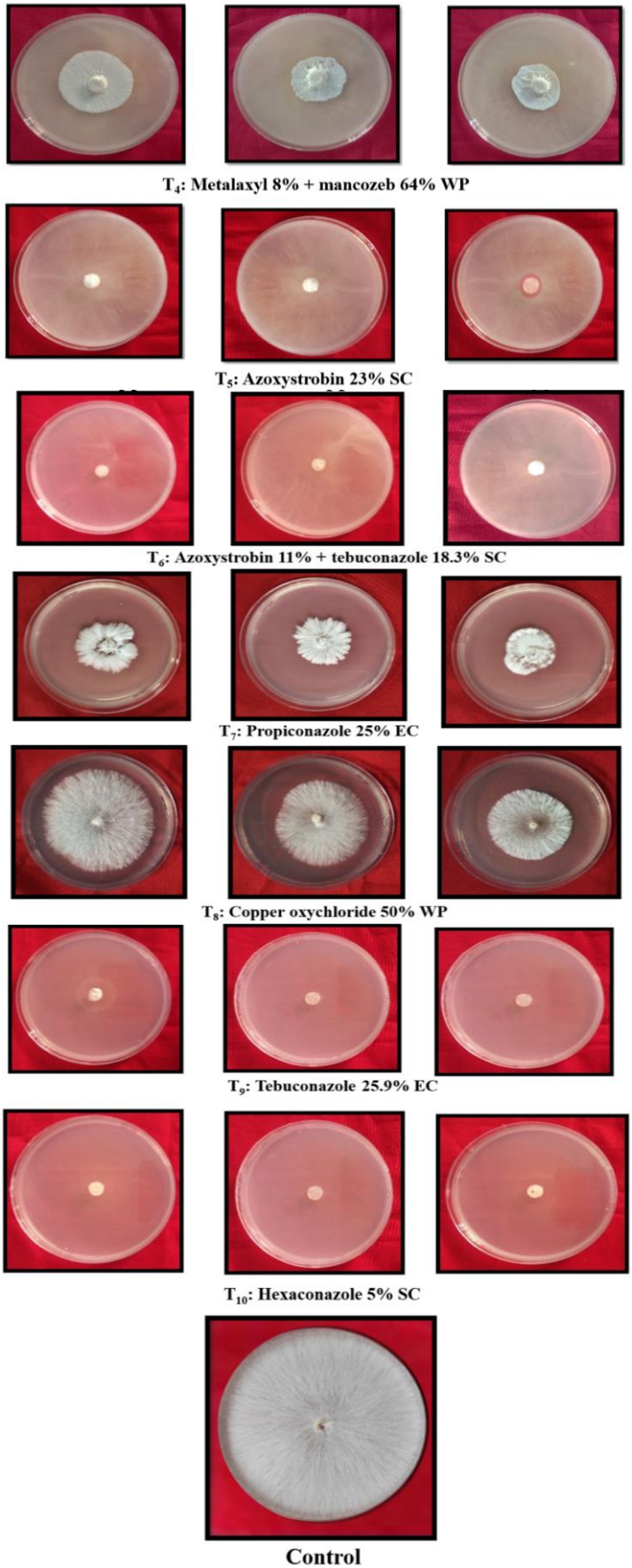

Fig. 2. Efficacy of different fungicides against *S. rolfsii* under in-vitro conditions.

Table 2: EEffect of different fungicide against *S. rolfsii*on mycelial growth under *in-vitro* conditions.

Tr. No.	Treatments	Mycelial growth of pathogen (mm)			Growth inhibition (%)		
		100 ppm	250 ppm	500 ppm	100 ppm	250 ppm	500 ppm
T_1	Carbendazim 50% WP	46.00^{d}	38.00^{c}	21.25^{d}	48.89	57.78	76.38
T_2	Carbendazim 12% + mancozeb 63% WP	61.00^{c}	32.00^{d}	17.50^{e}	32.22	64.44	80. 55
T_3	Pyraclostrobin 20% WG	26.00^{g}	19.00^{e}	11.63^{f}	71.11	78.89	87.07
T_4	Metalaxyl 8% + mancozeb 64% WP	42.50^{e}	31.50^{d}	18.13^{e}	52.77	65.00	79.84
T_5	Azoxystrobin 23% SC	0.00^{h}	0.00^{f}	0.00^{g}	100	100	100
T_6	Azoxystrobin 11% + tebuconazole 18.3% SC	0.00^{h}	0.00^{f}	0.00^{g}	100	100	100
T_7	Propiconazole 25% EC	37.38^{f}	32.25^{d}	26.00^{c}	58.46	64.16	71.11
T_8	Copper oxychloride 50% WP	74.50^{b}	71.50^{b}	59.50^{b}	17.22	20.56	33.89
T_9	Tebuconazole 25.9% EC	0.00^{h}	0.00^{f}	0.00^{g}	100	100	100
T_{10}	Hexaconazole 5% SC	0.00^{h}	0.00^{f}	0.00^{g}	100	100	100
T_{11}	Control (Test pathogen)	90.00^{a}	90.00^{a}	90.00^{a}	-	-	-
S.Em. ±		0.93	0.71	0.61	-	-	-
C.D. at 5%		Sig.	Sig.	Sig.	-	-	-
C.V. (%)		3.83	3.52	3.89	-	-	-

Note: Treatment means with the letter/letters in common are not significant by Duncan's new multiple range test (DNMRT) at a 5% level of significance

CONCLUSIONS

It can be concluded from the present experiment that among all the tested bioagents and various eco safe fungicides, groundnut stem and rot pathogen effectively controlled by *T. harzianum*, *T. viride*, azoxystrobin 23% SC, azoxystrobin 11% + tebuconazole 18.3% SC, tebuconazole 25.9% EC and hexaconazole 5% SC and pyraclostrobin 20% WG.

FUTURE SCOPE

—Validation of effective bioagents and fungicides under field conditions

—Development of integrated disease management modules

—Exploration of bioformulations and reduced-dose fungicide combinations

—Molecular studies on antagonistic mechanisms of *Trichoderma* spp.

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Conflict of Interest. The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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