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In vitro Assessment of Bio-agents and their Culture Filtrate for Sustainable Management of *Rhizoctonia solani*

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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

Rice is a vital cereal crop that forms the staple diet for more than half of the global population. It plays a critical role in food security and nutrition, particularly in Asia, where countries such as China, India, Indonesia and Bangladesh lead the way in cultivation and consumption but severely affected

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by *Rhizoctonia solani* causing heavy yield loss. This study investigated the *in vitro* efficacy of several bio-agents and their culture filtrates in suppressing the mycelial growth of *Rhizoctonia solani*. In the first experiment, direct applications of *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* significantly reduced fungal radial growth, with *T. harzianum* (68.37% inhibition) and *T. viride* (59.18% inhibition) showing the most potent antagonistic activity compared to the untreated control. In the second experiment, culture filtrates from various bio-agents (*Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis*) were evaluated. Results revealed that T4 and T9 produced the highest percent inhibition on the seventh day (82.95% and 78.18%, respectively), while other treatments showed moderate to low efficacy (13.37–59.45% inhibition). Statistical analysis (CD and SE) confirmed the significance of these differences among treatments in both experiments. The collective results indicate that both living bio-agents and their culture filtrates, particularly those derived from effective strains such as *Trichoderma spp*., offer strong potential for the biological control of *R. solani*, contributing to sustainable plant disease management strategies.

Keywords: Culture filtrates, bio-agents; Rhizoctonia solani; Pseudomonas fluorescens;

1. INTRODUCTION

“Rice (*Oryza sativa*) is a staple food crop for more than half of the global population, but its production is severely threatened by several diseases, among which rice sheath blight (ShB) caused by the soil-borne fungus *Rhizoctonia solani* Kühn is one of the most destructive worldwide. In recent surveys across key rice-growing regions such as Tamil Nadu and Telangana in India, disease incidence rates as high as 74.23% have been reported, indicating the increasing prevalence and severity of the disease” (Shiva Jyothi *et al.,* 2025). “The pathogen is highly adaptable, possessing significant genetic variability and a wide host range, which complicates disease management efforts. ShB can lead to yield losses up to 50% or more by affecting the productivity and quality of rice plants through the wilting of leaves and sheaths as well as reduced seed setting” (Chen *et al.,* 2023; Kasniya *et al.,* 2025). “The epidemic is aggravated by intensive agricultural practices, including the use of high-yielding varieties, dense planting, and extensive nitrogen fertilization, which create favorable conditions for the pathogen’s proliferation.

“Conventional control strategies rely heavily on chemical fungicides; however, these pose environmental and health risks and the pathogen's ability to survive in soil via sclerotia demands more sustainable approaches. Biological control, which harnesses antagonistic microorganisms such as *Trichoderma spp*., *Bacillus subtilis*, and *Pseudomonas fluorescens*, has emerged as an effective eco-friendly strategy. These bioagents not only inhibit fungal growth directly but also induce systemic resistance in rice plants, providing a sustainable disease management alternative” (Reedoy *et al.,* 2025; Kalboush *et al.,* 2024). “Despite progress, developing rice varieties with stable and effective resistance remains challenging due to the complex quantitative nature of ShB resistance, controlled by multiple genes” (Chen *et al.,* 2023). This study aims to evaluate the potential of various bioagents and their culture filtrates in managing *Rhizoctonia solani* and offers insights into integrated disease management strategies for improved rice production under current environmental conditions.

Nagendran et al., (2019) tested sixty rhizosphere bacteria against *R. solani* in the lab and found that strains *Pseudomonas fluorescens* Tu (Pf)19 and Kk (Pf) 24, along with *Bacillus subtilis* Tn (Bs)7 and Kk (Bs)19, strongly inhibited the fungus's growth and sclerotia germination. El-Nagdi (2008) demonstrated in lab tests that culture filtrates from *B. subtilis, P. fluorescens, Trichoderma harzianum* and *T. viride* reduced *R. solani* mycelial growth. Singh *et al.,* (2021) showed that *Trichoderma harzianum, Bacillus subtilis*, and *Pseudomonas fluorescens* inhibited the growth of *Fusarium oxysporum* and *R. solani* in dual culture tests.

Chaudhary (2020) found that a 50% culture filtrate of *T. harzianum* (SVPRT-THLi6) reduced *R. solani* growth by 95.9%, while volatile compounds from isolate SVPP-8 caused 72.5% inhibition. Jangir *et al.,* (2021) reported that culture filtrates from B. subtilis and *T. harzianum* significantly inhibited the pathogen. Analysis (GC-MS) identified active compounds like fatty acids, alkanes, phenols, benzene, pyran derivatives, with key antifungal components

being morpholine derivatives for *T. harzianum* and piperidine derivatives for *B. subtilis*. Krishnamurthy *et al.,* (1998) screened thirty-five *Trichoderma viride* and *T. harzianum* strains against *R. solani*, finding enhanced chitinase activity (and thus better inhibition) when grown on chitin instead of glucose. Abo-Zaid GA *et al*. (2024) reported that bio-friendly formulations of actinobacteria plays an active role in managing soil-borne diseases.

2. MATERIALS AND METHODS

2.1 Experimental Site

The present investigation was carried out during 2023-24 in the laboratory of the Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, 208002 (Uttar Pradesh).

2.2 Isolation and Purification of Pathogen (*Rhizoctonia solani*)

Rhizoctonia solani was isolated from infected plant tissue by cutting small lesion samples, surface-sterilizing them with 1% sodium hypochlorite, rinsing thoroughly three times in distilled water to remove chemical residues and transferring the pieces to isolation media, Potato Dextrose Agar (PDA) and incubate at 28 ± 2ºC to promote fungal growth. For purification, mycelial discs (5–8 mm) from initial cultures were placed on PDA or alkaline water agar plates containing 300 ppm streptomycin to suppress bacterial contamination, followed by incubation and subculturing to obtain pure fungal cultures.

2.3 Source of Bioagents

The bioagents were used in this study obtained from the biocontrol laboratory, department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, 208002 (Uttar Pradesh).

2.4 Preparation of Culture Filtrates of Different Bioagents

The biocontrol efficacy of non-volatile metabolites from Trichoderma spp. was estimated following the method of Jariwala et al., (1991) with slight modification. Briefly, 1 ml of spore suspension (1 x 10^5 cfu ml^{-1}) was inoculated in Potato Dextrose Broth (PDB) and incubated at 28±2°C without shaking for 10 days. After incubation, the fungal mycelial mat and spores were removed by filtration through a double layer of Whatman filter paper No. 1 and sterilized by passing through a 0.22 µm pore size syringe filter (Millipore). The filtrates were used for antifungal activity.

In case of bacterial bio agent culture filtrate preparation, we need a 250ml conical flask holding 100ml of nutrient broth was inoculated with different Pseudomonas isolates. Flasks were incubated at 26 ± 2 °C for 96 hours using an orbital shaker operating at 100 rpm. To obtain the cell-free filtrate, the culture was run through Whatman filter paper (no.1) followed by biological membrane filter with 0.2µm pore size. The obtained culture filtrate was of 100% concentration. The final filtrate, conc. of 5% and 10% was prepared and was combined with melted PDA media. In 90mm Petri plates, about 20ml of the media with both filtrate concentrations of different isolates were poured. An individual equal disc (5 mm) of pathogen culture (3-4 days old) used to inoculate the test pathogen. Petri plates devoid of filtrate serve as the control (Nandakumar *et al.,* 2002).

2.5 Evaluation of the Efficacy of Different Bio-Agents against *Rhizoctonia solani* in *in-vitro* Condition

2.5.1 Dual culture technique

The antagonism between fungal antagonists and the pathogen (*R. solani*) was studied following dual culture technique (Dhingra and Sinclair 2000).

Twenty ml of sterilized PDA was poured into each 90 mm diameter sterilized Petri plates under aseptic conditions and were allowed to solidify. Mycelial discs of 5 mm diameter were cut from actively growing mycelium of four days old cultures of antagonist and the test pathogen with sterilized cork borer and inoculated 7 cm apart, leaving 1 cm space from the periphery of Petri plates. In control plates, only *R. solani* was inoculated at one side of Petri plate. Three replicates were maintained for each bioagent and control. Then both the dual cultures and control plates were incubated at 28±1°C.

In case of dual culture with bacterial bio agent the fungal disc was kept at the center of a fresh PDA plate and the selected bacterial bioagent was streaked (co-inoculate) on both sides of the Petri plates equidistant from the disc and incubated at 28 ± 2°C for 48 h. Three replicates were maintained for each bioagent. Observations on radial growth of mycelium of the test pathogen and per cent inhibition of test pathogen was

calculated by using the formula as proposed by Vincent (1947).

$$I = (C-T)/C \times 100$$

Where, I = Per cent inhibition of mycelium
C = Growth of pathogen in control (mm)
T = Growth of pathogen in treatment (mm)

2.6 Evaluation of the Efficacy of Culture Filtrate of Different Bio-Agents against *Rhizoctonia solani* in *in-vitro* Condition

2.6.1 Poison food technique

The effect of non-volatile compounds present in culture filtrates of fungal and bacterial antagonists on the radial growth of *R. solani* was studied by following the method of Dennis and Webster (1971).

For the Food Poison technique, the culture filtrate of bioagent was added to sterilized molten PDA (at 40°C) to obtain final concentrations of culture filtrate as @ 5 % and 10% for all different treatments except control and a standard check which is carbendazim 50% WP @ 0.1 %. Then the supplemented medium was poured into Petri plates at the rate of 20 ml/ plate. After solidification, the Petri plates were centrally inoculated with 5 mm discs of *R. solani* cut from 4 days old culture. PDA plates not amended with culture filtrate and inoculated with the test pathogen were maintained as check. Plates were then incubated in an incubator at 28±1°C. Three replications of each treatment were maintained. Observations on radial growth of mycelium on treated and control plates were recorded up to 7 days. Per cent inhibition of the mycelial growth as a result of addition of culture filtrate was calculated by using Vincent (1947), formula.

$$I = (C-T)/C \times 100$$

Where, I = Per cent inhibition of mycelium

C = Colony diameter (mm) in control
T = Colony diameter (mm) in treatment

3. RESULTS AND DISCUSSION

3.1 Evaluation of the Efficacy of Different Bio-Agents against *Rhizoctonia solani* in *in-vitro* Condition

The data presented in Table -1 showed that among all the treatments *trichoderma harzianum* was the strongest inhibiting bioagent with radial growth of 28.46 mm on day 7, corresponding to 68.37% inhibition compared to control (90.00 mm). *Trichoderma viride* followed, reducing growth to 36.73 mm (59.18% inhibition). *Pseudomonas fluorescens* exhibited moderate antifungal activity with 31.92% inhibition at day 7 (61.27 mm growth), while *Bacillus subtilis* showed the least inhibition (14.47%) with mycelial growth of 76.97 mm on the same day. Ray *et al., (*2007) also tested the in vitro efficacy of three bioagents against *R. solani*, and found *T. harzianum* to be most effective followed by *T. viride* and *P. fluorescens* respectively. Singh *et al.,* (2021) also reported that *T. harzianum* caused maximum growth inhibition against *F. oxysporum* (66%) and *R. solani* (99%) followed by *P. fluorescens* (40 and 83%) and B. subtilis (41 and 48%) respectively.

3.2 Evaluation of the Efficacy of Different Bio-Agents against *Rhizoctonia solani* in *in-vitro* Condition

From Table 2 among the treatments, T4 demonstrated the most pronounced effect in reducing fungal growth, with radial growth measurements of 8.38 mm on the 3rd day, 12.39 mm on the 5th day and 15.34 mm on the 7th day, reflecting an 82.95% inhibition over the control by the 7th day. Similarly, T9 showed substantial inhibition, with radial growth ranging from 10.81 mm to 19.63 mm over the 3rd to 7th day, resulting in 78.18% inhibition by the 7th day. Other treatments such as T2 and T3 also exhibited considerable antifungal effects, demonstrating 59.45% and 49.10% inhibition, respectively at the final observation. The radial growth values for T2 ranged from 16.97 mm on the 3rd day to 36.49 mm on the 7th day, whereas T3 ranged from 21.44 mm to 45.81 mm over the same period. Moderate inhibition was observed with treatments T1 (45.60%), T8 (37.42%), and T7 (29.06%) by the 7th day, while T5 and T6 exhibited minimal antifungal activity with 13.37% and 22.46% inhibition, respectively. Their radial growth values remained relatively high, with T5 and T6 showing 77.96 mm and 69.78 mm mycelial extension on the 7th day, respectively. Chaudhary (2020) also showed that the culture filtrate of *T. harzianum* (SVPRT- THLi6) at 50% was highly effective in reducing *R. solani* mycelial growth up to 95.9%. Kucuk and Kivanc (2003) studied the effect of culture filtrates of T. harzianum isolates on R. solani and found T. harzianum (T-19) to be the most effective strain providing a cent percent inhibition rate. El-Nagdi (2008) showed in vitro, that culture filtrates of *B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride*

Table 1. *In vitro* evaluation of different bio agents on the inhibition of mycelial growth of Rhizoctonia solani.

	Radial mycelial growth (mm)			
Treatments	**3rd Day**	**5th Day**	**7th Day**	**Per cent inhibition over control**
Trichoderma viride	18.83	29.45	36.73	59.18
Trichoderma harzianum	15.79	24.36	28.46	68.37
Pseudomonas fluorescens	27.82	44.75	61.27	31.92
Bacillus subtilis	31.56	63.84	76.97	14.47
Control	49.92	72.34	90.00	0
C.D.	1.633	2.988	3.336	
SE(m)	0.511	0.936	1.045	

Table 2. Efficacy of culture filtrate of different bio-agents against Rhizoctonia solani in in-vitro condition.

Treatment	**Radial mycelial growth (mm)**			**Per cent inhibition over control (untreated)**
	3rd Day	**5th Day**	**7th Day**	
T1= culture filtrate of *Trichoderma viride* @ 5 %	22.56	39.36	48.96	45.60
T2= culture filtrate of *Trichoderma viride* @ 10 %	16.97	27.61	36.49	59.45
T3= culture filtrate of *Trichoderma harzianum* @ 5 %	21.44	34.94	45.81	49.10
T4= culture filtrate of *Trichoderma harzianum* @ 10 %	8.38	12.39	15.34	82.95
T5= culture filtrate of *Bacillus subtilis* @ 5 %	47.35	68.81	77.96	13.37
T6 =culture filtrate of *Bacillus subtilis* @ 10 %	43.61	62.92	69.78	22.46
T7= culture filtrate of *Pseudomonas fluroscence* @ 5 %	36.49	57.73	63.84	29.06
T8= culture filtrate of *Pseudomonas fluroscence* @ 5 %	29.33	48.18	56.32	37.42
T9= Carbendazim 50% WP @ 0.1 %	10.81	15.42	19.63	78.18
T10=control	49.56	72.68	90.00	0
CD (5%)	1.236	2.629	2.776	
SE(m)	0.416	0.885	0.935	

at 10% concentration caused nematode mortalities of 100, 99, 98 and 96%, respectively, after 72 hours exposure to the filtrates. Also, *T. harzianum* greatly reduced mycelial growth of *R. solani*, followed by *T. viride*, *B. subtilis* and *P. fluorescens*. Chethan *et al*. (2025) reported that *T. harzianum* (Th-02) showed 92.59% pathogen inhibition, while tea tree, garlic, lemongrass, turmeric, and tulsi essential oils demonstrated complete (100%) growth inhibition, offering promising eco-friendly disease management alternatives.

4. CONCLUSION

Among all treatments, *Trichoderma harzianum* was the most effective bioagent, inhibiting *Rhizoctonia solani* mycelial growth by 68.37% on day 7, followed by *Trichoderma viride* with 59.18% inhibition. *Pseudomonas fluorescens* showed moderate inhibition (31.92%), while *Bacillus subtilis* exhibited the least effect (14.47%). Among culture filtrate treatments, T4 and T9 demonstrated the highest antifungal activity, achieving over 78% inhibition by day 7. Other treatments showed varying moderate to low inhibition. These findings highlight the strong potential of Trichoderma species and certain bio-agent filtrates as effective, eco-friendly alternatives for controlling rice sheath blight caused by *R. solani.*

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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