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Antagonistic potential of certain phylloplane fungi against *Rhizoctonia solani* Kühn. - the causal organism of banded blight of Finger millet

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The antagonistic potential of four phylloplane fungi of finger millet, namely *Aspergillus flavus*, *Aspergillus niger, Penicillium oxalicum* and *Trichoderma atroviride* against *Rhizoctonia solani* was evaluated through an *in vitro* experiment using dual culture colony interaction,as well as an *in vivo* trial involving the control of disease induced by directly inoculating the fungal pathogen onto healthy finger millet plants.*T.atroviride* exhibited the highest percentage inhibition (84.79), while *A. flavus* had the lowest (32.13) against the pathogen in the *in vitro* experiment. Similar results were observed in the *in vivo* experiment, where *T. atroviride* exhibited the lowest disease severity (30.67%), achieving the highest disease reduction among the four phylloplane fungi.

Keywords : Disease severity, *in vitro,in vivo,* phylloplane fungi

INTRODUCTION

The leaf surface of a plant is an area exposed to various external abiotic factors,forming a unique habitat that supports a diverse array of microorganisms, including saprophytic and parasitic forms. Various microorganisms that reside on the phylloplane for extended periods parasitise the leaf tissues and interrupt the functions of the leaves. Phylloplane microflora plays an important role in affecting plant-microbe interactions on the leaf surface and thereby significantly contributes to benefit plant growth and disease suppression (Tanti *et al*. 2016). As the control of diseases using chemical means is harmful to the environment, researchers are inclined to explore various biological control methods, including the use of phylloplane fungi as an antagonist. It is a less toxic and safer method that reduces the severity of various crop diseases (Ayaz *et al*. 2023).

Similar views have been expressed by Mazinani*et al*.(2017) in combating phytopathogens through antibacterial and antifungal properties. Phylloplane mycoflora was also reported to have potential for use in the biological control of *Alternaria alternata,* with *in vitro* screening of certain fungal species revealing a significant percentage of inhibition by isolates of *Trichoderma* spp. (Thakur and Harsh, 2014; Chauhan and Navneet, 2015; Thakur, 2019). Biocontrol treatments using isolates of *Trichoderma* spp. were also found to be significantly effective in controlling fungal diseases caused by*Rhizoctonia solani*on different host plants(Kavita *et al*.2025; Phanindra *et al*.2025).

Banded blight disease of finger millet, caused by *Rhizoctonia solani* Kühn, was reported on finger millet in Manipur by Dhiren and Chhetry (2022).*R. solani* is a soil-borne, diverse phytopathogenic species that affects a wide range of crop plants. The complex nature of this species lies in its capability to thrive both as a saprophyte and a parasite. The fungal pathogen is difficult to eradicate solely with chemicals because it survives in soil and in crop field stubbles in a persistent manner as dormant sclerotia (Raju *et al.*2021). When conditions are favourable, the damage inflicted by *R.solani* can be substantial, highlighting the need for an integrated and sustainable management approach. Varying degrees of yield loss in small millets due to banded blight disease were reported by Patro *et al.* (2021). The objective of the experiment was

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to evaluate the antagonistic potential of certain phylloplane fungi against *Rhizoctonia solani* through *in vitro* and *in vivo* experiments.

MATERIALS AND METHODS

The test pathogen,*Rhizoctonia solani* Kühn, was isolated from diseased leaves,while the phylloplane fungi were isolated from fresh healthy leaves,collected in separate sterile polythene bags from a study site on IT Road, 10 Mile Gorkha Village,Kangpokpi District, Manipur, where finger millet was cultivated. Leaf pieces of 5-10 mm were cut from diseased samples containing parts of both diseased and healthy tissues and surface-sterilised with 0.1% sodium hypochlorite for 1 min. Then,they were rinsed three times with sterile distilled water and placed on sterile blotting paper to dry. They were then transferred to Petri plates containing pre-cooled Potato Dextrose Agar media (Riker and Riker, 1936) and incubated at 25 ± 1 °C for seven days. The isolation of phylloplane fungi was conducted using two standard methods, the Serial Dilution Method and the Leaf Impression Method, with sterilised Czapek Dox Yeast Agar (CDYA) medium amended with Rose Bengal.The inoculated plates were incubated at 25 ± 1 °C for 7 days (Aneja, 2003; Chauhan and Jain, 2020; Dahal, 2022; Jagiya, 2022). After seven days of incubation, the fungal pathogen and the phylloplane fungi were identified on the basis of their morphological characteristics observed under the binocular microscope using standard taxonomic keys (Subramanian, 1971; Barnettand Hunter, 1972; Ellis, 1971,1976; Watanabe, 2002).Koch's postulate was performed with *R. solani*on disease-free healthy plants of finger millet,and the identity of the fungal pathogen was confirmed based on the disease symptoms developed after inoculation with the pathogen. Pure cultures of the fungal pathogen, as well as the phylloplane fungi, were then transferred to PDA slants and preserved in the refrigerator at 4! for future use.The percentage of abundance of the phylloplane fungi was calculated from the recorded data by the following formula (Shukla and Sharma, 2016):

$$\text{Percentage of abundance} = \frac{\text{Total no. of colonies of a species in all the observations}}{\text{Total number of colonies of all species}} \times 100$$

Fourfungal species withhigher abundance, namely*Aspergillus flavus* Link, *Aspergillus niger*Tiegh,*Penicillium oxalicum*Currie & Thom.and *Trichoderma atroviride*P. Karst.were selected as antagonists for the *in vitro*and *in vivo* experiments.

In vitro experiment

In vitro colony interaction between the test pathogen, *R.solani*Kühn,andfour antagonist phylloplane fungi was conducted using the dual culture technique. Sterilised potato dextrose agar medium was aseptically poured into sterilised90mm diameter Petri dishes. Culture discs of 5 mm diameter were cut from the edges of 7-day-old actively growing colonies of the pathogen using a sterilised steel cork borer. A disc of the test fungus was placed aseptically 1 cm from the rim of the Petri dish. Then, a 5 mm mycelial disc of the antagonist fungus was inoculated on the opposite side, also 1 cm from the rim of the Petri dish. Three replicates of the dual culture setup were prepared for each of the four antagonistic fungi. The paired cultures were incubated at 25°C ± 1°C for 7 days (Skidmore and Dickinson,1976;Virdiana *et al*.2019). A control culture was established by inoculating a 5 mm diameter culture disc of the test pathogen, *R.solani,* aseptically at 1 cm from the rim of the Petri dish. It served as a control, providing baseline data in the antagonistic experiment. Three replicates were maintained and incubated at 25 ± 1 °C for 7 days. After seven days of incubation, the radial growth of the pathogen in both the dual cultures and single cultures (control) was measured using a digital vernier calliper on the reverse side of the Petri plates, and the data were recorded. The percent growth inhibition of the pathogen was calculated using the following formula (Vincent, 1927) :

$$I\ (\%) = \frac{C - T}{C} \times 100$$

where, I = Percent growth inhibition

C= Radial growth of mycelium in the control culture

T= Radial growth of mycelium in dual culture (treatment).Scoring for the degree of antagonism

was performed on a scale of 1 to 5 classes, with each class level determined by the radial growth patterns of the pathogen and the antagonist fungi in the dual culture plates (Bell *et al.* 1982).

Class 1: the antagonist completely overgrew the pathogen, covering the entire surface of the medium.

Class 2: the antagonist grew at least two-thirds of the medium surface.

Class 3: the antagonist and the pathogen each colonized approximately one-half of the medium surface, and neither organism appeared to dominate the other.

Class 4: the pathogen colonized at least two-thirds of the medium surface and appeared to withstand encroachment by the antagonist.

Class 5: the pathogen completely overgrew the antagonist and occupied the entire surface of the medium.

***In vivo* experiment**

An *in vivo* experiments were conducted using finger millet seeds of a local cultivar (LC1) collected from Toribari Gorkha Village,Kangpokpi District, to evaluate the biocontrol efficacy of four selected phylloplane fungi against *R.solani* in a poly greenhouse (temperature 25-30°C and relative humidity >80%). The seeds were manually cleaned to remove any chaff and then surface-sterilised to eliminate contaminants or pathogens for enhancing seed germination (Akbari *et al.* 2012). Three-week-old healthy seedlings, raised in sterile soil, were transplanted into sterile poly grow bags filled with sterile soil, with two plants per bag.

Only sterilised water was given to the experimental plants. After the plants reached the tillering stage, they were inoculated directly by gently removing the sheath and placing a 5-mm mycelium disc from a 5-day-old pure culture of *R.solani.* Then the inocula were covered with sterile, moistened absorbent cotton (Park *et al*. 2008; Yadav *et al*. 2024).

Spore suspensions of the antagonist fungi were prepared using sterile distilled water, with spores collected from freshly prepared pure culture colonies at a concentration of 10% (Thakur *et al*. 2022). After four days of inoculation,when the disease lesions appeared, each spore suspension was uniformly sprayed on the aerial parts of an inoculated plant (Rashid *et al*. 2020). A positive control treatment was established by spraying the aerial parts of the plant with Carbendazim 50% WP,a broad-spectrum fungicide (Brand name - Dhanustin), diluted to 2 g/L, after four days ofpathogen inoculation. Spraying of spore suspensions and the chemical fungicide was repeated at an interval of ten days. An experimental control was also established by inoculating with *R.solani*,and without using chemical or biological fungicides,allowing the disease to develop. Lastly, a negative control was prepared without the pathogen inoculation and also without the application of chemicals or biocontrol agents. Five replications were maintained for all biocontrol treatments, as well as the three different control treatments, to ensure reliable data for the evaluation. Visual observations of disease development under the different treatments were recorded over three consecutive weeks, starting one week after the initial spraying of spore suspension and the chemical fungicide. Diseasescoring was recorded using a 0 -5rating scale,and disease severity was calculated (Patro*et al*.2020). The scale used was 0- no incidence, 1- lesions up to 20% of the plant height, 2- lesions up to 21- 30% of the plant height, 3- lesions up to 31- 45% of the plant height, 4- lesions up to 46-65% of the plant height, and 5- lesions up to 66-100% of the plant height.Disease severity, Area Under the Disease Progress Curve (AUDPC) and rate of apparent infection (r) were calculated using the formula given below:

$$\text{Disease severity} = \frac{\text{Sum of all disease ratings}}{\text{Total no.of ratings} \times \text{Maximumdisease grade}} \times 100$$

1. $$\text{AUDPC} = \sum_{i=1}^{n} \left[\left(Y_{i+n1} + Y_i \right) / 2 \right] \left[X_{i+1} - X_i \right]$$

(Shaner and Finney, 1977)

Where, Y_i = disease severity at the i^{th}observation X_i = time in the i^{th} observation and n = total number of observations

$$r = \frac{2.3}{t_2 - t_1}\left[\log\frac{x_2}{1-x_2} - \log\frac{x_1}{1-x_1}\right]$$

where, $t_2 - t_1$= time interval of consecutive observations

X_1 and X_2 = disease severity in time t_1 and t_2 respectively

presented in Fig. 1. In the *in vitro* experiment, the radial growth of the fungal pathogen completely covered the entire area of the PDA in the control culture petri plate (90mm). In contrast, when paired with four different antagonistic fungi in dual cultures, the fungal pathogen displayed different patterns of radial growth. It was found that *T. atroviride* achieved the highest percentage of

Table 1: *In vitro* antagonistic activity of selected phylloplane fungi of finger millet against *Rhizoctonia solani*in dual culture

Phylloplane fungi	*Rhizoctonia solani*Kühn		Class of antagonism
	Radial growth of mycelium in dual culture (mm)	Inhibition (%)	
Aspergillus flavus Link	54.3 ± 0.09	32.13 ± 0.5	4
*Aspergillus niger*Tiegh	46.83 ± 0.05	41.46 ± 0.38	3
Penicillium oxalicum Currie & Thom	50.8 ± 0.08	36.5 ± 0.45	4
*Trichoderma atroviride*P. Karst.	12.17 ± 0.07	84.79 ± 0.44	1

± denotes SD

Table 2: Effect of selected phylloplane fungi of finger millet on severity of disease (%) caused by *Rhizoctonia solani*

Treatments	Disease severity (%)	AUDPC	Apparent rate of infection (r)
R.solani+ Aspergillus flavus	53.33	1596	0.27
R.solani+ Aspergillus niger	50.67	1540	0.22
R.solani+Penicillium oxallicum	48.00	1456	0.20
R.solani+ Trichoderma atroviride	30.67	1008	0.07
Positive control (*R. solani* + Carbendazim)	22.67	784	0.02
Experimental control (*R. solani* only)	56.00	1680	0.31
Negative control (no inoculation)	0	0	0
CD at 5%	15.9		

RESULTS AND DISCUSSION

Altogether, twelve fungal species belonging to eight genera were identified from the phylloplane of finger millet. The identified fungal species, along with their percentage of abundance,are

inhibition at 84.79 ± 0.44%. The lowest percentage of inhibition was observed in the dual culture with *A. flavus* at 32.13 ± 0.5%. Radial growth of the test fungi and the inhibition percentage in dual culture involving four different antagonist fungi are shown in Table1. It was revealed that *T. atroviride*

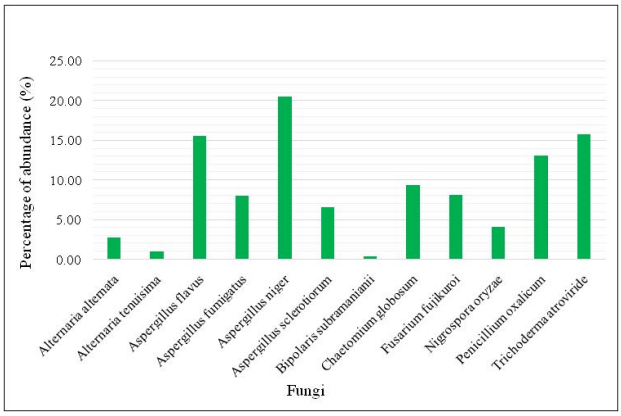

Fig.1: Phylloplane fungi with their respective percentage of abundance

exhibited a strong antagonistic effect and was classified as Class 1. *A. niger* exhibited moderate antagonism and was classified as Class 3. Lastly, *A. flavus* and *P. oxalicum* showed weak antagonism and were classified as Class 4. In the *in vivo* experiment, a mean disease severity of 30.67% was recorded in the biocontrol treatments with *T. atroviride,* which exhibited a significant reduction in disease severity compared to the other three phylloplane fungi. As the experiment was a curative treatment, disease severity progressed almost equally in the first week across all treatments. However, in the second and third weeks, disease progression was significantly reduced in the treatments with *T. atroviride* and the Positive control. This was observed inthe Area Under the Disease Progress Curve (AUDPC) calculated for all treatments. Furthermore, a one-way ANOVA analysis was performed on the recorded data of the treatments, and the Critical Difference was established at 15.9 (Table2).

The results of the *in vitro* dual culture experiment show that the growth of the test fungi was inhibited by the antagonistic activity of the selected leaf surface fungi with varying degrees of inhibition. *R. solani* exhibited the least radial growth (12.17 ± 0.07mm) in the dual culture with *T. atroviride,* demonstrating the strongest antagonistic effect by *T. atroviride* (84.79 ± 0.44%). The other three phylloplane fungi also exhibited antagonism, but to a lesser extent. The findings of this investigation also corroborate the results reported by Madbouly *et al.* (2014) and Chauhan *et al.* (2019). In the *in vivo* experiment, biocontrol treatments with *T. atroviride* also exhibited the lowest disease severity (30.67%), demonstrating a significant percentage of disease reduction (45.23%) from the recorded data of disease severity in the experimental control. Similar findings have been reported for some *Trichoderma* isolates, which exhibited significant disease reduction (Shara *et al*.2023; Mostafa *et al*.2025). Although the other three phylloplane fungi exhibited some degree of antagonism in the *in vitro* dual culture interaction, they did not show a significant reduction of disease in the *in vivo* experiment. Thus, it can be said that *Trichoderma atroviride,* isolated from the phylloplane of finger millet, may be considered a potential biocontrol agent against *Rhizoctonia solani,* subject to variations in environmental factors at the experimental site.

DECLARATION

Conflict of Interest. Authors declareno conflict of interest.

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