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Assessing the *In vitro* Antagonistic Effects of *Trichoderma* and *Pseudomonas* Bioagents on Rice Sheath Blight Pathogen

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ABSTRACT

Sheath blight, caused by the fungus *Rhizoctonia solani*, is a devastating disease that poses a significant threat to rice production worldwide. In recent years, biocontrol agents such as *Trichoderma* and *Pseudomonas* have gained attention for their potential to manage sheath blight while minimizing the environmental impact associated with chemical pesticides. The present investigation assessed the effect of *Trichoderma harzianum* and *Pseudomonas fluorescens* on *Rhizoctonia solani in vitro* after collection and mass multiplication from different locations. The dual culture assay revealed complex interactions between *R. solani* and biocontrol agents such as *T. harzianum* and *Pseudomonas fluorescens*. *T. harzianum* exhibited significant antagonistic activity, as evidenced by the inhibition of *R. solani* growth, hyphal coiling around *R. solani* mycelia, and direct mycoparasitic interactions. *Pseudomonas fluorescens*, on the other hand, demonstrated an indirect antagonistic effect by producing siderophores and antibiotics that hindered the growth of *Rhizoctonia solani*. *Trichoderma harzianum* isolate (IRRI-1) was found to be most inhibitory for *Rhizoctonia solani in vitro* followed by *Trichoderma harzianum* (SV-3) and *Pseudomonas fluorescens* (PF-2). These observations provide insights into the mechanisms underlying the biocontrol potential of *T. harzianum* and *Pseudomonas fluorescens* against *R. solani*. In conclusion, the dual culture assay proved to be an effective method for studying the interactions between *R. solani* and biocontrol agents. The findings contribute to our understanding of the underlying mechanisms of biocontrol agents and offer valuable information for the development of sustainable agricultural strategies aimed at managing plant diseases caused by *R. solani*.

Key words: *Bioagents, In-vitro, Pseudomonas, Rhizoctonia and Trichoderma,*

Introduction

Rice (*Oryza sativa* L.) one of the three primary cereals, is the primary dietary staple for 60% of the global population. It holds this role across various regions of Africa, Asia, South America, and, to a lesser extent, the United States. To enhance rice production, numerous countries have introduced high-yielding cultivars. Sheath blight, induced by the pathogen *Rhizoctonia solani*, is an important and severe ailment affecting rice. According to Savary *et al.* (2000), the lossesby this pathogen in paddy fields vary from 8 to 50%. Gopalakrishnan *et al.* (2003) evaluated the antagonistic properties of twelve *Tri-*

choderma isolates against *R. solani* through *in vitro* testing. Among these isolates, *T. viride* displayed the most rapid growth rate and strong antagonism, resulting in a significant 56.2% inhibition of *R. solani* growth in the dual culture setup. Pandey *et al*. (2005) examined the interaction between *Trichoderma spp*. and *R. solani* hyphae. Their investigation revealed the formation of loops and coiling around the pathogen's hyphae by the antagonist. This interaction led to the formation of a dense, rope-like structure that subsequently ruptured, resulting in the release of hyphal protoplasm, air bubbling within the cytoplasm, cytoplasmic discontinuity, and severe vacuolation. Khan and Sinha (2007) screened various *Trichoderma* spp. isolates in both *in vitro* and *in vivo* conditions to determine their ability to suppress *R. solani* growth. All tested isolates demonstrated a reduction in disease severity and incidence of rice sheath blight. Notably, *T. harzianum* exhibited remarkable effectiveness. The culture filtrates of *T. harzianum* and *T.virens* at a concentration of 50% displayed 76.3% and 70% inhibition, respectively, in *R. solani* mycelial growth. Fausto *et al.* (2007) conducted mycoparasitism studies using *T. harzianum* strains against R. solani, employing light and transmission electron microscopy. The study indicated that growth inhibition of *R. solani* resulted upon contact with the antagonist, and all tested *T. harzianum* isolates demonstrated coiling around *R. solani* hyphae. Pan and Jash (2009) evaluated the antagonistic potential of three *Trichoderma* isolates against *R. solani*. The isolate *T. viride* exhibited the highest mycelial growth inhibition of *R. solani*, attributed to the production of volatile compounds. *T. roseum* and *T. harzianum* followed with slightly lower inhibition rates. In a study by Singh *et al.* (2016), multiple strains of *P. fluorescens*, *T. viride* and *T. harzianum* were screened for their biocontrol efficacy. Six strains exhibiting substantial inhibition of fungal mycelium in dual plate assays were selected. These strains were further evaluated for their influence on the accumulation of natural antioxidants and defense-related biomolecules, resulting in enhanced plant growth and protection from pathogenic stress conditions in rice. The strains *P. fluorescens* PF-08 and *T. harzianum* UBSTH-501 showed the most promise, both individually and in combination, by increasing defense-related biomolecules and enzymes and demonstrating biocontrol potential against *R. solani*.

Materials and Methods

Source and maintenance of *Trichoderma harzianum* culture

Trichoderma harzianum was isolated from field soil through a process involving the collection of soil samples from crop fields located within the Crop Research Centers (CRC) of the SVPUAT, University Meerut. To initiate the isolation procedure, 10g of the soil sample was introduced into a 250 ml conical flask along with 90 ml of sterilized distilled water (SDW) and then thoroughly mixed. By diluting the stock solution to a magnitude of 10^-6, various dilutions of functional samples were generated. For the actual isolation process, one milliliter of the final serial dilution (10^-6) was applied to a medium of Potato Dextrose Agar. This medium was used as the growth substrate to facilitate the isolation of *Trichoderma harzianum*. The Petri dishes containing the medium were placed within an incubator set at a temperature of 28±2 °C for 7 days. Following this incubation period, a pure culture of the *Trichoderma harzianum* was successfully grown.

Source and maintenance of *P. fluorescens* culture

To isolate the bacterial biocontrol agent known as *Pseudomonas fluorescens* from soil, soil samples were collected from the Crop Research Centre (CRC) at the University. For the purpose of isolation, 10 grams of soil were put into a 250 ml conical flask containing 90 ml of sterilized distilled water (SDW), and this mixture was thoroughly blended. Various dilutions of these prepared samples were made by diluting the initial solution down to a 1 in 10^8 concentration. Subsequently, one milliliter of the final serial dilution (1 in 10^8) was spread onto a selective King's B medium specific to *Pseudomonas fluorescens*, aiming to isolate the bacterium. The petri dishes were then left to incubate for a span of 2 days at a temperature of 37±2 °C. After the incubation period, a pure culture of the bacterium had grown. The identification of bacterial colonies was conducted based on the fluorescent pigment generated by the bacterial culture on the culture dish. Initially, the fluorescent pigment appeared yellow, but over time, it transitioned into a yellow-green color as the pigmentation intensified.

Mass multiplication of *P. fluorescens* and *T. harzianum* bioagents

Each selected strain of *P. fluorescens* and *T. harzianum*

were grown in Petri plates and were inoculated in 8 different plates. Sorghum seeds, weighing 100 g, underwent a boiling process for 20 to 25 minutes to soften them; subsequently, excess water was drained, and the seeds were isolated for cooling to reduce moisture content. Once adequately cooled and excess moisture was eliminated, the seeds were placed into 250 ml flasks. These flask-contained seeds were sterilized in an autoclave at a temperature of 121 °C for a duration of 20 minutes. After cooling, the sorghum seeds were introduced into aseptic flasks and inoculated with 2-5 days old, actively growing culture of either *P. fluorescens* or *T. harzianum* (2-3 pieces measuring 5 mm each), all conducted under sterile conditions within a laminar airflow chamber. The flasks containing sorghum seeds and the chosen microorganism were then moved to a BOD incubator set at 28°C, where they were maintained for a span of 15 days. Once per day, the flasks were shaken.

In vitro evaluation of bioagents against *Rhizoctonia solani* by dual culture assay

A total of 8 different bioagents were tested for their antagonistic activity against *R. solani in vitro*. Fungal biocontrol agents were screened for their antagonistic potential against *R. solani* by using a dual culture technique as described by Dennis and Webster (1971). 20 ml of sterilized melted PDA medium for testing fungal bio-agents and was aseptically poured into the sterilized 9 cm diameter Petri plates and allowed to solidify. Five mm mycelial disc of *R. solani* and test biocontrol agents cut with the help of sterilized cork borer from the edge of four days actively growing culture plates were placed on solidified PDA in such a manner that they lie just opposite to each other (approximately 6 cm apart from each other). Inoculated petri plates were incubated at 28±10 ^{0}C in BOD. Bacterial bioagents were also tested for their ability to inhibit the mycelial growth of *R. solani in vitro.* KB+PDA (3:1) media was poured aseptically in sterilized 90 mm diameter petri plates and allowed to solidify. Five mm mycelial discs of *R. solani* were cut with the help of a sterilized cork borer from the edge of four days culture plates, and placed on solidified KB+PDA (3:1) towards the edge of the medium in the Petri plates at an equal distance and the other side of the same plate was streaked with *Pseudomonas sp.* Control was maintained by inoculating *R. solani* only at one side of the periphery of the petri plates. The petri plates were incubated at 28±10 ^{0}C in a BOD incubator. Different isolates of *Trichoderma harzianum* and *fluorescent Pseudomonads* were tested by the Dual culture technique for their antagonistic activity against a causal pathogen i.e., *Rhizoctonia solani*. Colony size in each treatment was recorded and percent inhibition was calculated using the formula as proposed by Vincent (1947). 5<=56"5G565K 100

Where:

I = Inhibition of mycelial growth

C = Growth of pathogen in control

T = Growth of pathogen in treatment

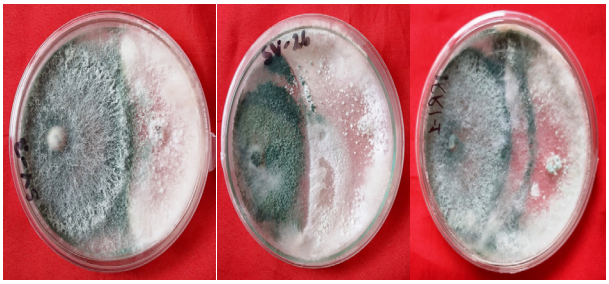

Fig. 2. Effect of different isolates of *T.harzianum* (SV-3, SV-26, and IRRI-1) on the growth of *Rhizoctonia solani in-vitro*

Results

It is evident from Table 1, that the isolate of *T. harzianum* IRRI-1 resulted in the highest inhibition of *R. solani* through dual culture technique with 1.50 cm radial growth and 68.75% reduction in colony diameter followed by the colony diameter noticed in the treatment T2, T4, T7, and T8 as they resulted in 1.60, 1.68,1.62 and 1.71 cm of colony diameter with a growth reduction of 66.66, 65.00, 66.45 and 64.37 percent respectively. All these values were at par with each other. The remaining three other isolates of *T. harzianum* viz SV-19, SV-26, and SV-27 were also quite effective but comparatively little less effective in minimizing the radial growth of *Rhizoctonia solani in vitro*. After 7 days of incubation, the colony diameter of *Rhizoctonia solani* was monitored under the effect of six isolates of *Trichoderma harzianum* and *Pseudomonas flourescens*. The pattern of growth reduction in *Rhizoctonia solani* by all the respective isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* was exactly similar as it was observed after 3 days of incubation.

Discussion

The successful demonstration of *Trichoderma*'s po-

tential in stimulating growth response and showing antagonistic effects against rice sheath blight disease has been evidenced (Chaube and Sharma, 2002). Sharma *et al.* (2012) reported that certain *Trichoderma* isolates effectively promoted seed germination, and root and shoot length in crops such as paddy, tomato, and mustard. *Trichoderma* exhibits not only parasitic behavior towards plant pathogenic fungi but also synthesizes antibiotics and triggers plant defense mechanisms, including localized and systemic resistance (Ha, 2010; Bisen *et al.*, 2016). The substantial reduction in disease incidence observed in *Trichoderma*-treated plants can be attributed to its robust antagonistic potential, consistent with findings from various studies. The underlying mechanisms involve competition for nutrients, niche displacement, and production of antimicrobial compounds (Jain *et al.*, 2012). Silva *et al.* (2012) isolated 13 *Trichoderma* strains from the Amazon Forest soil and identified four out of these 13 isolates that exhibited over 50% reduction in *Rhizoctonia solani* mycelial growth and sclerotial viability, whether applied as seed treatment, substrate incorporation, or foliar spray. Phenolic compounds are essential for plant pigmentation, growth, reproduction, and resistance to pathogens, as highlighted by Lattanzio *et al.* (2006).

Out of six isolates of *Trichoderma harzianum* tested for antagonistic activity against *R. solani* it was found that all *Trichoderma harzianum* isolates were almost highly antagonistic against *R.solani* because all of them showed a very high level of inhibition in the mycelial growth of *R.solani* and out of these six isolates IRRI-1 isolate was found to be most aggressive. Similarly, two *Pseudomonas fluorescens* isolates in this study were also highly inhibitory to *Rhizoctonia solani.* The present investigation was conducted to assess the effect of *Trichoderma harzianum* and *Pseudomonas fluorescens on Rhizoctonia solani in -vitro*. *Trichoderma harzianum* isolate (IRRI-1) was found to be most inhibitory for *Rhizoctonia solani in-vitro* followed by *Trichoderma harzianum* (SV-3) and *Pseudomonas fluorescens* (PF-2). The antagonistic capacity of *Trichoderma harzianum* against the majority of the fungal pathogen has been well documented. The *R. solani* being grown in a dual culture may be deprived of the nutrients and space for growth. This may be another reason for inhibition of the growth of *Rhizoctonia solani* because of the tough competition posed by *Trichoderma harzianum* in dual culture. Production of volatile and non-volatile secondary metabolites by *Trichoderma harzianum* is another reason for the inhibitory effect against *Rhizoctonia solani*. These volatile and non-volatile secondary metabolites by virtue of their toxicity to fungal organisms may result in the inhibition of fungal growth of pathogenic fungi. Meena *et al.,* (2003) tested three *Trichoderma isolates* for the production of volatiles and found that *T.harzianum* was effective in suppressing the growth by 80% and sclerotia formation by 33.5 % of *R. solani f. sp. sasakii in vitro*. Sharma *et al.* (2001) reported that *T. harzianum* and *T. viride*, when tested in dual culture against *R. solani* in vitro started inhibiting the growth of *R. solani* after seven days and covered the entire petri dish within 10 days of inoculation. Thus, the finding of the present

Table 1. Effect of different bioagents of *P. fluorescens* and *T. harzianum* on the growth of *Rhizoctonia solani in-vitro*

Treatments	Treatments details	Fungal colony (cm) in dual culture technique			
		Avg. diam. of fungal colony (cm) at 3 days	Percent inhibition over control	Avg. diam. of fungal colony (cm) at 7 days	Percent inhibition over control
T_1	*Trichoderma harzianum* (IRRI-1)	1.50	68.75	2.12	77.44
T_2	*Trichoderma harzianum* (SV-3)	1.60	66.66	2.50	73.40
T_3	*Trichoderma harzianum* (SV-19)	2.89	60.62	3.00	68.05
T_4	*Trichoderma harzianum* (SV-21)	1.68	65.00	2.50	73.40
T_5	*Trichoderma harzianum* (SV-26)	1.90	60.41	2.45	73.40
T_6	*Trichoderma harzianum* (SV-27)	1.89	60.62	2.40	74.46
T_7	*Pseudomonas fluorescens* (PF-2)	1.62	66.45	3.10	67.02
T_8	*Pseudomonas fluorescens* (PF-4)	1.71	64.37	2.90	69.14
T_9	Control	4.80	9.40		
SE(m)±			0.072		0.159
CD (*p*=0.05)			0.217		0.476

study regarding growth inhibition by *Trichoderma harzianum* is in conformity with the report of earlier workers mentioned above. *Pseudomonas fluorescens* is a bacterial bioagent that acts by production of siderophores (Fe chelating compounds). Since chelated Iron (Fe) cannot be utilized by the fungal organism they become Fe deficient and their growth as well as virulence and aggressiveness get inhibited. This activity is evident in the present finding also as two isolates of *Pseudomonas fluorescens* have been inhibitory against the radial/mycelial growth of *R. solani*.

Conflict of interest

None

References

Bisen, K., Keswani, C., Patel, JS., Sarma, B.K. and Singh, H.B. 2016. *Trichoderma* spp.: efficient inducers of systemic resistance in plants. *Microbial-Mediated Induced Systemic Resistance in Plants*. Springer, Singapore, pp 185-195.

Chaube, H.S. and Sharma, J. 2002. Integration and interaction of solarization and fungal and bacterial bioagents on disease incidence and plant growth response of some horticultural crops. *Plant Dis Res.* 17(1): 201.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma*: I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society.* 57(1): 25-IN3.

Fausto, B.R.A., Fernanda, M.C., Roberto, N.S., Cirano, J.U. and Adilson, L.L. 2007. Mycoparasitism studies of *Trichoderma harzianum* strains against *R.solani:* valuation of coiling and hydrolytic enzyme production. *Biotechnology Letters*. 29: 1189-1193.

Gopalakrishnan, C., Ramanujan, B., Prasad, R.D., Rao, N.S. and Rabindra, R.J. 2003. Screening and selection of potential *Trichoderma* isolates for the control of cotton seed rot and damping off. *Journal of Biological Control*. 17 (2): 161-165.

Ha, T.N. 2010. Using *Trichoderma* species for biological control of plant pathogens in Vietnam. *J ISSAAS*. 16(1): 17–21.

Jain, R.M., Mody, K., Mishra, A. and Jha, B. 2012. Physicochemical characterization of biosurfactant and its potential to remove oil from soil and cotton cloth. *Carbohydr Polym.* 89(4): 1110–1116.

Khan, A.A. and Sinha, A.P. 2007. Screening of *Trichoderma* spp. against *Rhizoctonia solani* the causal agent of rice sheath blight. *Indian Phytopathology*. 60 (4): 450-456.

Lattanzio, V., Lattanzio, V.M. and Cardinali, A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochem Adv Res*. 661(2): 23–67.

Meena, R.L., Rathore, R.S. and Kusum, M. 2003. Efficacy of biocontrol agents against *Rhizoctonia solani* f. sp. sasakii causing banded leaf and sheath blight of maize. *Journal of Mycology and Plant Pathology*. 33 (2): 310-312.

Pan, S. and Jash, S. 2009. Variability in sclerotial antagonism of mycoparasitic *Trichoderma* spp. against *Rhizoctonia solani* and *Sclerotium rolfsii. Journal ofMycology and Plant Pathology*. 39(3): 409-415.

Pandey, K.K., Pandey, P.K. and Upadhyay, J.P. 2005. Mycoparasitism of *Trichoderma* spp. on *Fusarium* and *Rhizoctonia. Journal of Mycology and Plant Pathology*. 35(1): 174-176.

Sarkar, S.C. and Chowdhury, A.K. 2003. Spread of rice sheath blight under field situation. *Environment and Ecology.* 21(3): 632-638.

Savary, S., Willocquet, L., Elazegui, F.A., Castilla, N.P. and Teng, P.S. 2000. Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. *Plant Dis.* 84: 357–369.

Sharma, K.K., Zaidi, N.W. and Singh, U.S. 2012. Efect of biological seed treatment on seed germination and growth promotion of paddy, tomato and mustard. *Vegetos.* 25(2): 375–386.

Sharma, G., Saxena, S.C. and Sharma, G. 2001. Evaluation of biocontrol agents against *Rhizoctonia solani* the causal agent of banded leaf and sheath blight of maize. *Annals of Plant Protection Sciences*. 9 (1): 144-145.

Silva, E.J.N.L., Herrera, D.R., Almeida, J.F.A., Ferraz, C.C.R., Gomes, B.P.F.A. and Zaia, A.A. 2012. Evaluation of cytotoxicity and up-regulation of gelatinases in fibroblast cells by three root repair materials. *Int Endod J.* 45(9): 815-820.

Singh, U.B., Malviya, D., Singh, S., Pradhan, J.K., Singh, B.P., Roy, M., Imram, M., Pathak, N., Baisyal, B.M., Rai, J.P. and Sarma, B.K. 2016. Bio-protective microbial agents from rhizosphere eco-systems trigger plant defense responses provide protection against sheath blight disease in rice (*Oryza sativa* L.). *Microbiological Research.* 19(2): 300-312.

Vincent, J.M. 1947. Distoration of fungal hyphae in the presence of certain inhibitors. *Nature.* 159: 850.