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Evaluation of Bio-Agents against *Rhizoctonia solani* of Rice under *in vitro* conditions

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ABSTRACT: Numerous biotic and abiotic factors have an impact on rice production and productivity, resulting in yield losses of up to 20–30%. considered In all regions of the world where rice is farmed, fungi infections are the most common biotic limitations. One of the main biotic restrictions on rice production in India is sheath blight, which is also the most economically significant disease of rice in the world. *R. solani* Kuhn, a fungal pathogen of both rice and soybeans, is the disease's cause (teleomorph: *Thanatophorus cucumeris* (Frank) Donk). When growing in a certain kind of naturally suppressive soil, a fungus called *Trichoderma* spp. protects plants from infectious diseases spread by pathogens in the soil. One of these soil-borne pathogens that severely damages economically significant crops is the fungus *Rhizoctonia solani*. In agriculture, using bio-control agents like *Trichoderma* spp. to fight infections and provide disease control is a viable option. The challenges regarding the bio-control of *Rhizoctonia solani* is scarely known to the farmers. Bio-control agents significantly suppress the growth of plant pathogenic microorganisms and regulate the rate of plant growth. The results of the present study suggest that *Tricoderma harzianum* has a highly antagonistic potential against the test pathogen. The experiment conducted to examine the responses of different isolates towards four bio control *viz., T. viride, T. harzianum, Bacillus* spp, *Pseudomonas* spp, by dual culture method. The zone inhibition of *R. solani* isolates in all four bio-agents was assessed. The findings clearly show that *Tricoderma viride* and *Bacillus* spp were the most efficient at inhibiting *Rhizoctonia solani* isolates. *Trichoderma viride* inhibited the growth of RS-10 by 77.1 percent. *Bacillus* spp. inhibited isolate no RS-5's growth the most.

Keywords: *Rhizoctonia solani*, *Tricoderma* spp, *Bacillus* spp.

INTRODUCTION

Rice is one of the most significant staple cereal crops raised globally (*Oryza sativa* L.). Numerous nematodes, bacteria, viruses, fungi, and other organisms attack rice, resulting in significant monetary losses. Numerous biotic and abiotic stresses interfere with the growth of economically important crops. Plant pathogenic fungi are the main production barrier when it comes to biotic stresses. In India, sheath blight is one of the major biotic restrictions of rice production, which is also the most economically effecting disease of rice across the world (Lee and Rush 1983; Webster and Gunnell 1992). According to Srivastava *et al*. (2016), the major soil-borne plant pathogenic fungus *Rhizoctonia solani* Kuhn (teleomorph: *Thanetophorus cucumeris*) is responsible for annual crop yield losses of 20–40% throughout the world. An aggressive *basidiomycete necrotrophic* plant pathogen with a wide host range is *Rhizoctonia solani* (*R. solani*). It creates structures that are highly resistant and is a significant source of the infection known as sclerotia that enables *R. solani* can endure harsh environmental conditions. Sheath blight management relies heavily on combining traditional methods with chemical fungicides. Despite being extremely expensive and harmful to agro-ecosystems, chemical control is still regarded as one of the most effective control methods. Another crucial strategy for ensuring the sustainability of the ecosystem is the use of resistant cultivars, but breakdown of resistance has remained a concern (Fry, 2008). *R. solani* imposes severe diseases on a variety of plants, including ornamental plants, forest trees, solanaceous crops, cereals, vegetables (bean, sugar beet, lettuce), cotton, and melon (Ghosh *et al*., 2017).

Prior research has reported on the selective isolation of bio-control agents as well as their in vitro pathogenicity and bio-efficacy against various plant parasites (Sarma *et al.*, 2015). Despite providing established primary ecosystem services like nutrient cycling and decomposition, the role of the soil microbial community in ecosystem functioning is widely ignored (Singh *et al*., 2013). Consequently, there has been a lot of interest in finding microbial bio-agents that can specifically counter a specific plant pathogen in combination or individually under field conditions (Harman *et al.*, 2004). There are numerous *Trichoderma*, *Bacillus*, and *Pseudomonas* species in soil. Since (Weindling, 1932) identified the antagonistic activity of *Trichoderma* species against plant

pathogens, primarily as a mycoparasite, several species of this fungus have undergone extensive research to determine their potential as biological control agents against a variety of fungal pathogens (Harman *et al*., 2004; Shoresh *et al.,* 2010). The ability of bio-agents to colonise plant systems could be extremely advantageous when used for biological control against *R. solani*, as this pathogen generally attacks stems and leaf sheath (Harman *et al.,* 2004). Because of concerns about the safety and environmental effects of chemicals, there is a growing demand for plant pathogen control methods other than chemical ones. Biological controls against *R. solani* using *T. viride, T. harzianum*, *Bacillus* spp., and *Pseudomonus* spp. have shown promising results. The current study used various isolates of *T. harzianum*, *T. viride*, *Bacillus* spp., and *Pseudomonus* spp., to find an efficient and competitive bio-agent strain that can be used against *R. solani* in rice and across the plant-pathosystems in the future

MATERIALS AND METHODS

A. In vitro screening against bio agents

By activating one or more organisms, either naturally or artificially, by changing the environment, the host, or the antagonist, or by mass introduction of one or more antagonists, biological control is a tool for reducing the inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state. By suppressing or eradicating plant pathogens without disturbing the ecosystem and without causing problems with pollution, pathogen resistance development, or the emergence of new biotypes, antagonists like fungi and bacteria can act as effective tools for increasing crop yield. As a result, *in-vitro* research was done on the biological control of predominant *R. solani* through antagonists.

B. Studies of Biological agent's adverse effect on R. solani

Four known antagonists namely *Trichoderma viride, T. harzianum, Bacillus* spp, *Pseudomonus* spp were tested *in vitro* to evaluate their antagonistic properties against *R. solani* by adopting dual culture method. Dual culture method (Morton and Strouble 1995).

The test organisms and the pathogen were grown on PDA medium separately and from 7 days old cultures, 5 mm diameter disc of the test organism (antagonist) and pathogen were taken into consideration. Using 5 mm diameter culture blocks spaced 70 mm apart, *R. solani* and test organisms were aseptically inoculated into the 90 mm Petri plates. Five repetitions of each treatment were kept and the petri plates with only pathogen served as control. All the plates were incubated at temperature (28 ± 20ºC) and the radial growth of the test organism and pathogen was measured after 7 days. The formula provided by Vincent (1947) was used to calculate the percent growth inhibition (PGI).

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

Where as

I = Per cent growth inhibition.

C = Average diameter of mycelial colony of control set.

T = Average diameter of mycelial colony of treated set.

RESULTS AND DISCUSSION

A. Bio-control of rice sheath blight

R. solani is a dangerous pathogen that causes sheath blight of rice (*Oryza sativa*). Given that half of the world's population relies on rice as a food source, managing this disease is necessary (Yang, 2009). In addition to Asia, South America, the United States, and Africa, the rice sheath blight disease has also severely affected rice in countries like China, India, Pakistan, Bangladesh, and Sri Lanka (Rabindran and Vidhyasekaran 1996). This is because *R. solani*, which lives in the soil, causes mycelia to spread externally and sclerotia to form when it is moist. By developing lesions on the tillers, the fungus slowly spreads to the upper leaves. The disease is initially soil-borne, but as mycelia spreads, it finally becomes foliar (Rabindran and Vidhyasekaran 1996). By using the bacterium *Bacillus* spp, rice sheath blight has been controlled (Yang *et al*., 2009). This bacterium is widely distributed in nature and is not harmful to people, animals, or plants (Acea *et al.,* 1988). The bacterium secretes a few antifungal substances, including zwittermicin-A and kanosamine, lipopeptides, and a unique protein called bacisubin (Bais *et al*., 2004; Liu *et al.,* 2007). The zone of inhibition around *R. solani* in the laboratory experiments suggested the presence of an active metabolite (antibiosis), which may have diffused in the PDA media. That active metabolite is what causes the morphological changes in the *R. solani* mycelium.

B. Effect of some bio-agents on growth of different isolates of R. solani in vitro

The experiment conducted to examine the responses of different isolates towards four bio-control *viz., Tricoderma viride*, *Tricoderma harzianum*, *Bacillus* spp, *Pseudomonas* spp, by dual culture method. The zone inhibition of the different isolates of *R. solani* in all four bio-agents measured and presented in Tables 1-10.

Table 1: *In vitro* evaluation of bio control against RS-1.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition (%)
T1	*Trichoderma viride*	69.20	23.11
T2	*Trichoderma harzianum*	72.00	20.00
T3	*Bacilius* spp	43.40	51.77
T4	*Pseudomonas* spp	50.80	43.50
T5	Control	90.00	
	C.D at 5%	0.71	

Table 2: *In vitro* evaluation of bio control against RS-2.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	49.40	45.10
T2	*Trichoderma harzianum*	57.00	36.70
T3	*Bacilius* spp	90.00	0.00
T4	*Pseudomonas* spp	90.00	0.00
T5	Control	90.00	
	C.D at 5%	0.70	

Table 3: *In vitro* evaluation of bio control against RS-3.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	20.60	77.10
T2	*Trichoderma harzianum*	38.60	57.10
T3	*Bacilius* spp	42.80	52.40
T4	*Pseudomonas* spp	64.20	28.70
T5	Control	90.00	
	C.D at 5%	0.49	

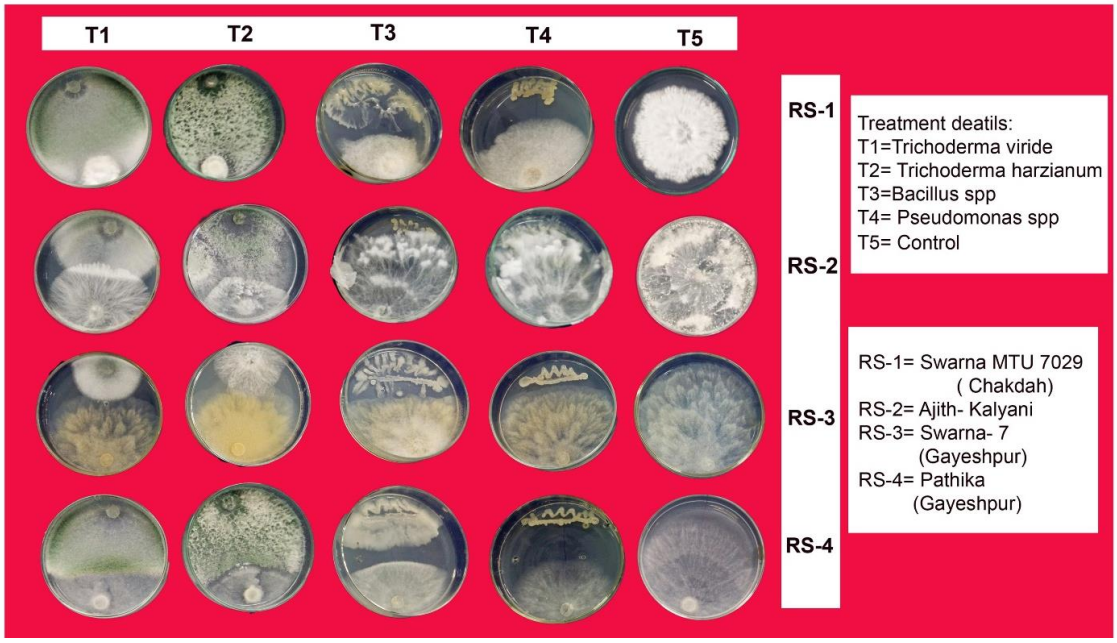

Plate 1. *In vitro* effect of antagonists against *R. solani.*

Table 4: *In vitro* evaluation of bio control against RS-4.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	56.60	29.30
T2	*Trichoderma harzianum*	59.60	25.50
T3	*Bacilius* spp	37.80	52.80
T4	*Pseudomonas* spp	46.00	42.50
T5	Control	90.00	
	C.D at 5%	0.13	

Table 5: *In vitro* evaluation of bio control RS-5.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	59.80	33.60
T2	*Trichoderma harzianum*	69.60	29.30
T3	*Bacilius* spp	28.00	68.90
T4	*Pseudomonas* spp	40.00	55.60
T5	Control	90.00	
	C.D at 5%	0.62	

Table 6: *In vitro* evaluation of bio control against RS-6.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	58.60	21.90
T2	*Trichoderma harzianum*	58.60	21.90
T3	*Bacilius* spp	40.40	46.10
T4	*Pseudomonas* spp	53.00	29.30
T5	Control	90.00	
	C.D at 5%	0.13	

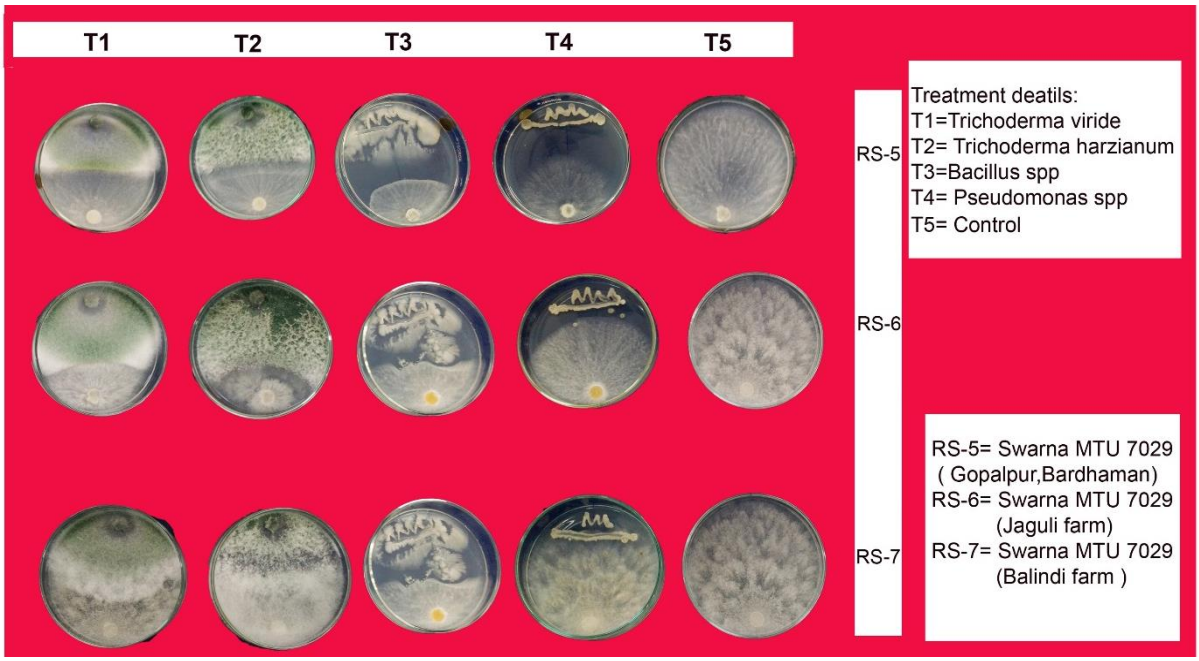

Plate 2. *In vitro* effect of antagonists against *R. solani.*

Table 7: *In vitro* evaluation of bio control against RS-7.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	49.60	44.90
T2	*Trichoderma harzianum*	49.60	44.90
T3	*Bacilius* spp	50.00	44.40
T4	*Pseudomonas* spp	90.00	0.00
T5	Control	90.00	
	C.D at 5%	0.52	

Table 8: *In vitro* evaluation of bio control against RS-8.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition (%)
T1	*Trichoderma viride*	49.8	44.7
T2	*Trichoderma harzianum*	59.6	33.8
T3	*Bacilius* spp	41.2	54.2
T4	*Pseudomonas* spp	64.4	28.4
T5	Control	90	
	C.D at 5%	0.60	

Table 9: *In vitro* evaluation of bio control against RS-9.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	49.00	24.60
T2	*Trichoderma harzianum*	55.80	14.20
T3	*Bacilius* spp	51.40	20.90
T4	*Pseudomonas* spp	62.00	4.60
T5	Control	90.00	
	C.D at 5%	0.72	

Table 10: *In vitro* evaluation of bio control against RS-10.

Treatment No.	Name of bio Control	Av. colony diameter of pathogen(mm)	Growth inhibition(%)
T1	*Trichoderma viride*	46.00	48.90
T2	*Trichoderma harzianum*	53.80	40.20
T3	*Bacilius* spp	30.40	66.20
T4	*Pseudomonas* spp	41.60	53.80
T5	Control	90.00	
	C.D at 5%	0.70	

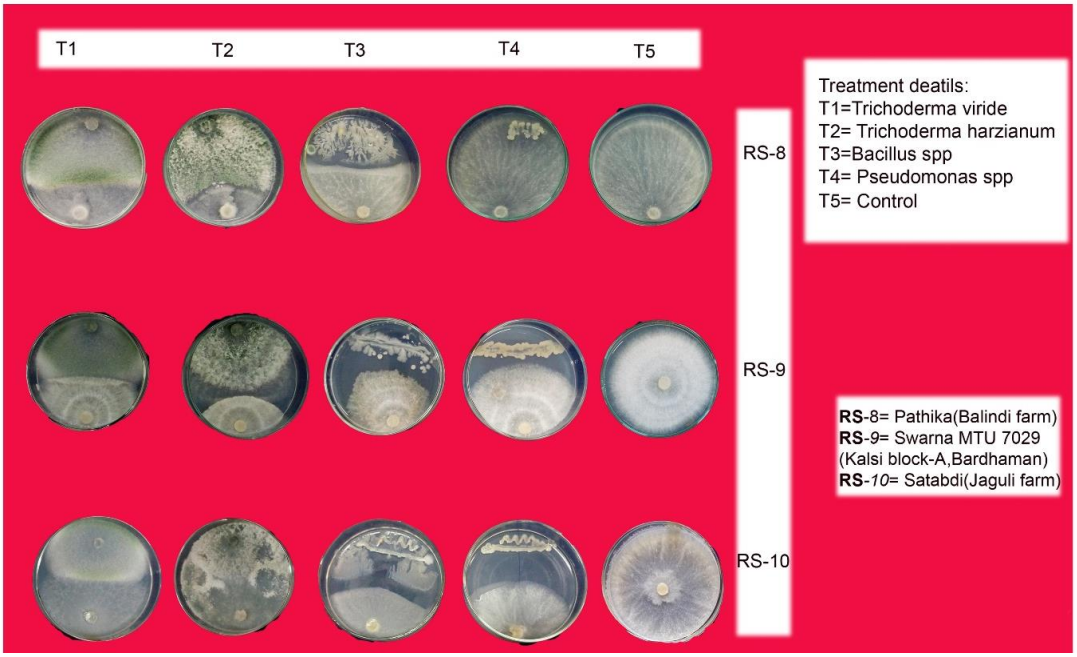

Plate 3. *In vitro* effect of antagonists against *R. solani.*

It is clear from the data that *Tricoderma viride* and *Bacillus* spp proved to be most effective to inhibit the isolates of *R. solani* (Plate 1-3). *Trichoderma viride* gave 77.1% growth inhibition against RS-10. *Bacillus* spp gave maximum growth inhibition against isolate no RS-5.

The current study confirmed the findings of Kishan *et al.* (2019); Seema *et al.* (2011); Lenka *et al.* (2012); Srinivas *et al.* (2014), who reported that *T. viride*, followed by *T. harzianum*, showed the highest growth inhibition of *R. solani* (70%). Shiva *et al.* (2019) observed that *Bacillus* spp, followed by *Pseudomonas* spp, exhibited the most significant growth inhibition of *R. solani.*

CONCLUSIONS

The pathogen *R. solani* significantly reduces the yield of important crops for the economy. *T.viride*, and *Bacillus* species, is extremely helpful in controlling diseases brought on by *R. solani*. Significant progress has been made as a result of the active marketing of *T. viride* and members of the *Bacillus* spp. family as bio-control agents. Because of the negative effects that pesticide residues have on the environment and the health of organisms, the market demand for bio-control agents and PGBR has increased recently. *Bacillus* species and *R. solani*'s effects on the microbial community and the plant rhizosphere are being studied using metagenomic sequencing. A thorough understanding of the mechanism at work needs to be investigated despite the abundance of reports on the genetic understandings of the interactions between *Bacillus* species and *R. solani* and plants. In conclusion, *Bacillus* spp. and *T. viride* has multifaceted beneficial features that may be ideal for its integrated use in disease control.

Various isolates of *R. solani* were successfully inhibited from growing in dual culture studies using the bioagents *T. viride* and *Bacillus* spp. The highest growth inhibition was observed against RS-5 in *Bacillus* species (68.9%), while the highest growth inhibition was observed against RS-3 in *T. viride* (77.10%).

FUTURE SCOPE

Future research should validate bio-agents' effectiveness against *Rhizoctonia solani* in controlled field settings, ensuring practical applicability. Exploring combined bio-agent effects and refining application techniques could improve sustainable rice disease management. Molecular approaches to comprehend bio-agent-pathogen interactions would aid in developing precise and potent bio-control remedies.

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Conflict of Interest. None.

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