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Biocontrol and growth enhancement potential of *Trichoderma* spp. against *Rhizoctonia solani* causing sheath blight disease in rice

S. Chaudhary[1], S. Sagar[1], M. Lal[3], A. Tomar[2], V. Kumar[4] and M. Kumar[1]*

[1]Department of Agricultural Biotechnology, College of Agriculture, SVP University of Agriculture and Technology, Meerut-250 110, India
[2]Department of Recombination Techniques, College of Biotechnology, SVP University of Agriculture and Technology, Meerut-250 110, India
[3]Division of Plant Protection, ICAR-Central Potato Research Institute, Regional Station, Meerut-250 110, India
[4]ICAR-National Institute of Biotic Stress Management, Raipur - 493 225, India

***Corresponding Author Email :** mukeshbt06@gmail.com

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Abstract

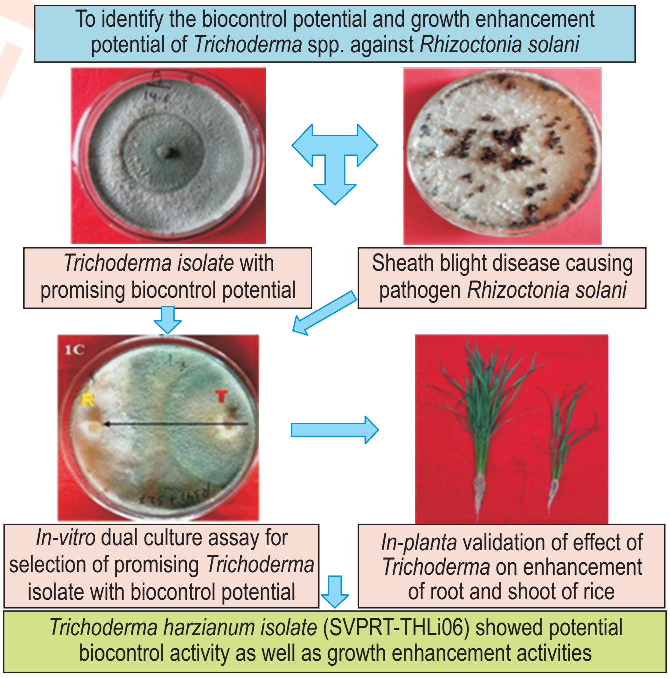
Aim: The present study aimed to investigate the biocontrol and growth enhancement potentials of *Trichoderma* spp., against *R. solani.*

Methodology: A total of 31 *Trichoderma* spp. were examined for their antagonistic potentials against *R. solani* in dual culture, effect of volatile and non-volatile metabolites on the growth inhibition of test pathogen under *in-vitro* condition. The efficacy of *Trichoderma* spp. were further evaluated for controlling sheath blight and promoting rice growth under *in-vivo* conditions with different mode of applications.

Results: The results of *in-vitro* confrontation assay revealed that among the different *Trichoderma* spp. tested, *T. harzianum* (SVPRT-THLi06) and *T. atroviride* (SVPP-4) exhibited excellent biocontrol efficacy with 90.9% and 72.0% mycelial growth inhibition, respectively. The culture filtrate of *T. harzianum* (SVPRT- THLi6) at 50% was highly effective in reducing *R. solani* mycelial growth up to 95.9% and in case of volatile metabolites, maximum inhibition (72.5%) in mycelial growth of *R. solani was recorded* with *T. harzianum* isolate SVPP-8. A combined mode of application (soil treatment+ root dipping+ foliar spray) with *T. harzianum* (SVPRT-THLi06) was found most effective under greenhouse condition, which showed the least disease severity (12.4% vs control 49.6%) and disease incidence (27.1% vs control 96.4%).

Interpretation: The present study revealed that *T. harzianum* (SVPRT-THLi06) has potential to control sheath blight disease as well as improve growth of rice crop. Therefore, in future it may be used as bio-based formulation for plant growth enhancement and managing sheath blight disease in rice cultivation and further may be applied in organic rice cultivation.

Key words: Necrotrophic fungus, *Rhizoctonia solani*, Rice, Sheath blight, *Trichoderma* spp.



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Introduction

Rice (*Oryza sativa* L.) is one of the major cereal food crops and form staple diet of about half of the world's population. Globally, the production of rice is estimated 769.6 million tons and rice cultivation area is estimated to be around 167.2 million ha (Chaudhary *et al*., 2019). India would require about 156 mt of rice by the year 2030 at an annual increment of 3 million tons in the current rice production (Dass *et al.,* 2016, 2017), which should come from yield enhancements as there almost no scope for increasing rice area. However, rice crop is subjected to more than forty diseases, which are one of the biotic factors affecting rice production and productivity in the world including India. Sheath blight caused by soil borne necrotrophic fungal pathogen *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (A.B. Frank)], is an economically important disease in rice. It can reduce yield up to 25-50% in Philippines, 20-50% in Japan and 5.2-50% in India (Kumar *et al*., 2013; Richa *et al*., 2016). Introduction of new, susceptible, high-yielding compact semi-dwarf varieties, excessive use of nitrogen fertilizers and changes in agricultural practices associated with these varieties contribute to increase in sheath blight disease incidence and severity in rice-growing areas in the world (Groth and Bond, 2007). Furthermore, changes in climatic conditions also favour the spread of disease. *R. solani* survives in soil within diseased plant debris as mycelium or sclerotia during unfavourable environmental conditions for several years, which serve as source of primary inoculum. Control of rice sheath blight pathogen is difficult due to high genetic variability, extremely broad host range and its ability to survive in soil for a long time under various environmental conditions (Taheri and Tarighi, 2011). So far, absolute resistance to *R. solani* is not available in any of the rice germplasms, although partial genetic resistance has been reported (Yadav *et al.*, 2015; Laha *et al.*, 2017). Owing to lack of desired level of host resistance, the disease is controlled by extreme use of synthetic fungicides, which have negative effect on the soil microflora, pollute the atmosphere, and are environmentally hazardous. Moreover, it has been reported that some effective fungicides are highly phytotoxic to rice and in low severe condition; these fungicides may reduce yields (Groth *et al*., 1993). Therefore, biological control through antagonistic microorganisms may be an effective approach to minimize the incidence of sheath blight.

Trichoderma is a fungal genus, that includes anamorphic fungi, isolated primarily from soil and decomposing organic matter (Harman *et al*., 2004). Among the antagonistic microorganisms, the genus *Trichoderma* is especially known for its biocontrol activities against several phytopathogens, including *Rhizoctonia solani* (Harman, 2006; Kotasthane *et al*., 2015; Swain *et al*., 2018). *Trichoderma* spp. shows competitive nature for rice plant residue by exhausting the available nutrients for the pathogen, ultimately reducing the survival of sheath blight pathogen (Mostafa and Shahjahan, 1995). In addition, *Trichoderm* spp. has been reported to enhance plant growth in maize, wheat, tomatoes, gray mangroves and rice (Raman, 2012; Saravanakumar *et al*., 2013; Doni *et al*., 2014; Mahato *et al*., 2018). In view of the above, the present study aimed to evaluate the potential of different native *Trichoderma* isolates in controlling rice sheath blight pathogen *R. solani* both *in-vitro* and *in-vivo* conditions and to investigate the growth promotion potential of *Trichoderma* spp. in rice cultivation.

Materials and Methods

Identification and maintenance of fungal isolates : A total of 31 *Trichoderma* strains, isolated and characterized in the previous study were used for experiments (Table 1). Pathogenic isolate of *Rhizoctonia solani* (SVPRS06) was recovered from naturally infected rice plants showing typical symptoms of sheath blight disease in rice fields. To confirm pathogenecity, artificial inoculations were done in greenhouse on rice cv. PB-1 by inserting a 5-mm mycelial disc of *R. solani* (SVPRS06) isolate between the joint of basal leaf sheath and stem above the water line at maximum tillering stage. Re-isolation of *R. solani* was done from artificial inoculated PB-1 rice plants showing distinctive lesions of sheath blight. Anastomosis group (AG) and subgroup of *R. solani* isolate was confirmed by anastomosis reaction with tester AG1-IA isolate (ITCC 7650) procured from ICAR-Indian Agricultural Research Institute (IARI), Pusa Campus, New Delhi, and rDNA sequencing using ITS1 and ITS4 primers (Accession no.- KU215869). *R. solani* and *Trichoderma* isolates were grown on potato dextrose agar (PDA) at 28°C for 5 days and stored at 4°C for further use.

In-vitro plate confrontation assays for biocontrol

Dual culture assay : *In-vitro* antifungal efficacy of *Trichoderma* spp. against *R. solani* was tested by dual culture technique. Mycelial disc (~5 in mm dia) of *R. solani* and *Trichoderma* spp. were cut from advancing periphery of 5-day-old PDA culture, and separately positioned opposite to each other ~1.5 cm away from the edge of 90 mm Petri plates containing PDA medium. Plates inoculated with *R. solani* (SVPRS06) alone served as control. Each pair was replicated three times and incubated for seven days at 28±2°C. The reduction in mycelial growth of *R. solani* was recorded and the percentage of inhibition over control for each treatment was calculated according to Hajieghrari (2010).

The degree of antagonism was scored using the rating system described by Bell *et al.* (1982) on a scale of 1-5: Class 1: *Trichoderma* completely overgrew *R. solani* and covered the entire medium surface; Class 2: *Trichoderma* overgrew at least two third of the medium surface; Class 3: *Trichodema* and *R. solani* each colonized 50 % of the medium surface and neither organism appeared to dominate the other; Class 4: *R. solani* colonized at least two-third of the medium surface and appeared to withstand encroachment by *Trichoderma*, and Class 5: *R.*

Table 1 : Isolate codes and GenBank accession no. for *Trichoderma* spp. used in the study

Species	Isolate code	GenBank accession numbers
Trichoderma harzianum	SVPRT-THLi6, SVPRT-THLi7, SVPRT-THLi8, SVPRT-THLi9, SVPRT-TH1, SVPP-8, SVPP-9, SVPP-10, SVPP-12, SVPP-14, SVPP-16, SVPP-18, SVPRT-THL01, SVPRT-THL02, SVPRT-THL03, SVPRT-THL04, SVPRT-THL05, SVPRT-THL08, SVPRT-THL09, SVPP-6	KC582837, KC582838, KC582839 , Kc582840, KX139409, KU215926, KU215920, Ku215921, KU215922, KU215923, KU215924, Ku215925, JX232593, JX232594, JX232595, Jx232596, JX232597, KX139407KX139408, Ku215917
Trichoderma longibrachiatum	SVPRT-TL10, SVPRT-LB02, SVPRT-LB06, SVPRT-LB07, SVPP-7	KC582841, JX908722, JX908726, JX908727, Ku215918
Trichoderma asperellum	SVPP-2, SVPP-3, SVPP-5, SVPP-11	KU215913, KU215915, KU215914, Ku215912
Trichoderma atroviride	SVPP-4	Ku215916
Trichoderma erinaceum	SVPUAT-TE-01	Kc121065

solani completely overgrew the entire medium surface. *Trichoderma* isolates were considered antagonistic to *R. solani*, if the mean score for a given comparison was class ≤ 2, but not highly antagonistic if the number was class ≥ 3.

For mycoparasitism test, clean glass slide assay was used. Molten water agar (2%) was poured and evenly spread over the sterile glass slide to make a thin agar film. The mycelium disks of *R. solani* and *Trichoderma* isolates were placed on the slide 1 cm apart from each other and incubate at 28±2°C for 3 days. At the end of the incubation period, the contact/inhibition zone mounted under lectophenol-cotton-blue and observations were made under a light microscope for the presence of coiling structures and wall disintegration in the hyphae of *Trichoderma* and *R. solani* respectively. The frequencies of coiling or wall disintegration were recorded by observing the coils or areas of disintegration in five different microscopic fields (Sivakumar *et al.*, 2000).

Estimation of volatile metabolites from *Trichoderma* spp. : The effect of volatile metabolites from *Trichoderma* spp. against *R. solani* was investigated using paired plate technique (Dennis and Webster, 1971). *Trichoderma* spp. was centrally inoculated in Petri dish containing PDA medium with 5 mm mycelial disc taken from 3-day-old actively growing culture. The lids of each *Trichoderma* inoculated Petri dishes were replaced with bottom of PDA containing Petri dishes inoculated centrally with 5 mm mycelial disc of *R. solani* and sealed by three layers of parafilm to prevent the loss of volatile metabolites. Petri-plate without *Trichoderma* spp. served as control. The plates were incubated at 28±2°C with a photoperiod of 12 hr. The average diameter of treatments was measured as the time at which the pathogen completely covered the control plate and was further used to calculate the percentage of growth inhibition. Each test was performed in triplicate. The percent inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = (C1 - C2)/C1 \times 100$$

where, C1 means growth of *R. solani* in the presence of *Trichoderma* volatiles and C2 means growth of control (*R. solani* alone).

Estimation of antifungal non-volatile metabolites from *Trichoderma* spp. : 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 (1×10^5 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 double layer of Whattman 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. *Trichoderma* culture filtrates were supplemented with molten PDA medium to obtain 5, 10, 25 and 50 % (v/v) concentration and poured in sterile Petri plates. Mycelial disc of 5 mm diameter were removed from the edge of 3-day-old actively growing *R. solani* culture and placed at the centre of culture medium and incubated at 28±2°C for 6-7 days. PDA plates without *Trichoderma* culture filtrate served as control. The average radial growth of the fungal mycelium was recorded and the percentage inhibition was calculated using the formula described earlier. Each assay was performed in triplicate.

In-vivo evaluation of *Trichoderma* spp. for their biocontrol and plant growth promotion potential under green house

Rice seedling and rice plant preparation : Rice seeds (cv. PB-1, high yielding and sheath blight susceptible) from Experimental Research Centre of SVP University of Agriculture and Technology, Meerut, were sown in plastic tray. Pots (40 cm dia. x 30 cm height) were filled with 10.0 kg of rice field soil and fertilized by soil application of NPK (100:60:40 kg ha^{-1}). Twenty-one day old rice seedlings were transplanted in each pot (one plant per pot) under wet conditions and watered regularly to keep them always submerged. The pots were arranged in a completely randomized design (CRD) replicated three times for each treatment and kept under green house at 25 to 34°C temperature and 85 to 90% relative humidity.

Table 2 : *Trichoderma* species and treatments used for *in-vivo* biocontrol and plant growth promotion under pot experiment

S. No.	Treatments	*Trichoderma* isolate used	Species
T1	Seed + foliar spray	SVPRT-THLi03	*Trichoderma harzianum*
T2	Soil + foliar spray		
T3	Seedling root dip + foliar spray		
T4	Seedling root dip + soil + foliar spray		
T5	Seed + foliar spray	SVPRT-THL04	*Trichoderma harzianum*
T6	Soil + foliar spray		
T7	Seedling root dip + foliar spray		
T8	Seedling root dip + soil + foliar spray		
T9	Seed + foliar spray	SVPRT-THLi06	*Trichoderma harzianum*
T10	Soil + foliar spray		
T11	Seedling root dip + foliar spray		
T12	Seedling root dip + soil + foliar spray		
T13	Seed + foliar spray	SVPRT-TL10	*Trichoderma longibrachiatum*
T14	Soil + foliar spray		
T15	Seedling root dip + foliar spray		
T16	Seedling root dip + soil + foliar spray		
T17	Seed + foliar spray	SVPP-4	*Trichoderma atroviride*
T18	Soil + foliar spray		
T19	Seedling root dip + foliar spray		
T20	Seedling root dip + soil + foliar spray		

Pathogen inoculation and application of *Trichoderma* spp.: Rice plants were inoculated by placing the mycelium disc of *R. solani* (SVPRS06) at the centre of hill above the water level, 30 days after transplanting (Sudhakar *et al.*, 1998). Based on their *in-vitro* performance, five potent *Trichoderma* isolates were selected for antagonistic performance and plant growth promotion activity under *in-vivo* greenhouse conditions. The selected isolates *T. harzianum, T. longibrachiatum* and *T. atroviride.* Detailed treatment procedure for application of *Trichoderma* spp. are given in Table 2.

Spore suspensions of *Trichoderma* isolates were prepared by harvesting the spore from 10-day-old culture grown on PDA by adding sterile water to the plates and scraping the culture with a scalpel. These suspensions were filtered through a triple layer Whattman filter paper No. 1 to separate mycelium from conidia. The concentration of spores was determined by haemocytometer and adjusted to 10^7 conidia ml^{-1}. Tween 20 @ 0.1% was supplemented to the suspensions for better adhering of conidia to plant surfaces. Inoculated rice plants were sprayed with spore suspensions of *Trichoderma* spp. (50 ml per replicate) at two stages: 24 hr after inoculation and 15 days after first spray. Two sprays of Propiconazole 25 EC @ 0.1% were kept as standard chemical control for comparison. Plants inoculated with *R. solani* only served as control. Seed treatment was done by seed smearing before sowing (15 g talc-based formulation per 1 kg seed). Talc-based formulation of *Trichoderma* spp. was prepared by following the method of Jeyarajan *et al.* (1994). Soil treatment was done at the time transplanting @ 15g kg^{-1} of filling and for seedling treatment; 21-day-old rice seedlings were uprooted and dipped in *Trichoderma* spore suspensions.

Assessment of disease and rice growth components: Disease severity on rice plants was recorded according to (0-4) scale representing severity levels of 0, 1, 5, 15, and 50% (Willocquet *et al.*, 2010). Disease severity and disease incidence were recorded on tiller basis. Disease incidence, disease severity and disease reduction in treated and control plants were calculated by the standard formulae (Helfish *et al.*, 2017).

Statistical analysis : Disease incidence and disease severity values were subjected to transformation for statistical analysis. All data were statistically analysed by one-way analysis of variance (ANOVA). The significance of the effect of the treatment was determined using F-test and to determine the significance of the difference between the means of the treatments, least significant difference (LSD) was calculated at 5% probability level.

Results and Discussion

Pathogenecity test in greenhouse indicated that *R. solani* (SVPRS06) was a strong virulent pathogen on rice and showed typical symptoms of sheath blight. The BLAST analysis revealed that SVPRS06 showed highest similarity to *R. solani* AG1-IA isolates with reference GenBank accession numbers: KX674520, KX674519, KJ577141, KP978016 (E-value = 0.0, max. identity = 99%), available at NCBI database.

The data of dual culture assay revealed that all 31 *Trichoderma* spp. strains tested significantly differed from the control in relation to growth diameter of *R. solani*. However, most *T. harzianum* isolates showed higher growth inhibition of *R. solani* compared to the other isolates. Among the *T. harzianum* isolates, percent growth inhibition was highest in SVPRT- THLi6 (90.9%)

followed by SVPP-8 (81.3%), SVPRT- THLi8 (77.2%), SVPRT-THL07 (74.9%) and SVPRT-THL01 (72.4%) whereas the minimum growth inhibition (41.9%) was observed in *T. asperellum* isolates SVPP-5 at 7 DAI (Table 3). *T. harzianum* isolate SVPRT-THLi6 showed initial faster growth, overgrew on *R. solani*, destroyed it completely and sporulated after 5 days of inoculation. No inhibition halo zone was observed between SVPRT-THLi6 and *R. solani*, indicated that the antagonistic effect was due to competition for space and nutrient and not on a classic antibiosis or chemical aggressiveness (Fig.1A-1D). *T. erinaceum* isolate SVPUAT-TE-1 co-culture with *R. solani* also showed faster growth and overgrew on the pathogen (Fig. 1E). A clear zone of interaction between the isolate SVPP-4 and SVPRT-THL01 with pathogen was observed, where both isolates inhibited the growth of *R. solani* after physical contact (Fig. 1 F and G). All *T. harzianum* and *T. atroviride* isolates showed Bell's rating of 1 or 2, revealing the fact that these isolates had superior antagonistic potential against *R. solani in-vitro* as compared to other isolates of *Trichoderma* spp. The overgrowth of antagonist is achieved when a fungus exhibits higher growth rate, tolerance against metabolites produced by the pathogens (Mathivanan *et al.,* 2000) and capacity to produce antibiotics. Abo-Elyousr *et al.* (2014) noted that mycoparasitism in *Trichoderma* was associated with its ability to produce extra cellular chitinase enzymes. Previous studies have reported inhibitiary action of *T. harzianum* isolates against various phytopathogens like *R. solani* (Yadav *et al.*, 2018), *Sclerotinia sclerotiorum* (Inbar *et al.*, 1996), *Fusarium oxysporum* (Prasad *et al.*, 2016) etc., in confrontation assay.

Microscopic analysis showed that hyphae of *T. harzianum* readily interacted with hyphae of *R. solani* and exhibited successful mycoparasitism. *Trichoderma* mycelium was distinguished from *R. solani* by hyphal diameter; *T. harzianum* hyphae were thinner as compared to *R. solani* hyphae. *T. harzianum* was capable of degrading *R. solani* hyphae by coiling around the hyphae with apressoria and hook- like structure (Fig. 2C, D). Apressoria-like structures allowed the hyphae of *Trichoderma* spp. to adhere to the surface of *R. solani* hyphae. However, some biocontrol agents applied diverse mechanism against *R. solani* as just touched the hyphae without coiling (Fig. 2E and F). As a consequence of mycoparasitic activity, the hyphae of *R. solani* were pierced from the septaum side, causing coagulation of protoplast, resulting in lysis of the host hyphae (Fig. 2G, H). Although, *T. atroviride* spores accumulated around *R. solani* hyphae, but were not attached to hyphae (Fig. 2I). Similar observations were noted against *R. solani* by Khan and Sinha (2007) and Asad *et al.* (2014). Elad *et al.* (1983) reported *R. solani* hyphal coiling and penetration by *Trichoderma harzianum* and *T. hamatum* and appressorium-like structures and hooks. However, *T. atroviride* (SVPP-4) did not coil around the hyphae of *R. solani,* hence, the main mechanism of biocontrol for this strain is antibiosis or production of hydrohytic enzymes (Harman *et al.*, 2004).

The culture filtrates of *Trichoderma* spp. inhibited mycelium growth of *R. solani* and inhibition percentage increased proportionally with the filtrate concentration (Fig. 3). *T. harzianum*

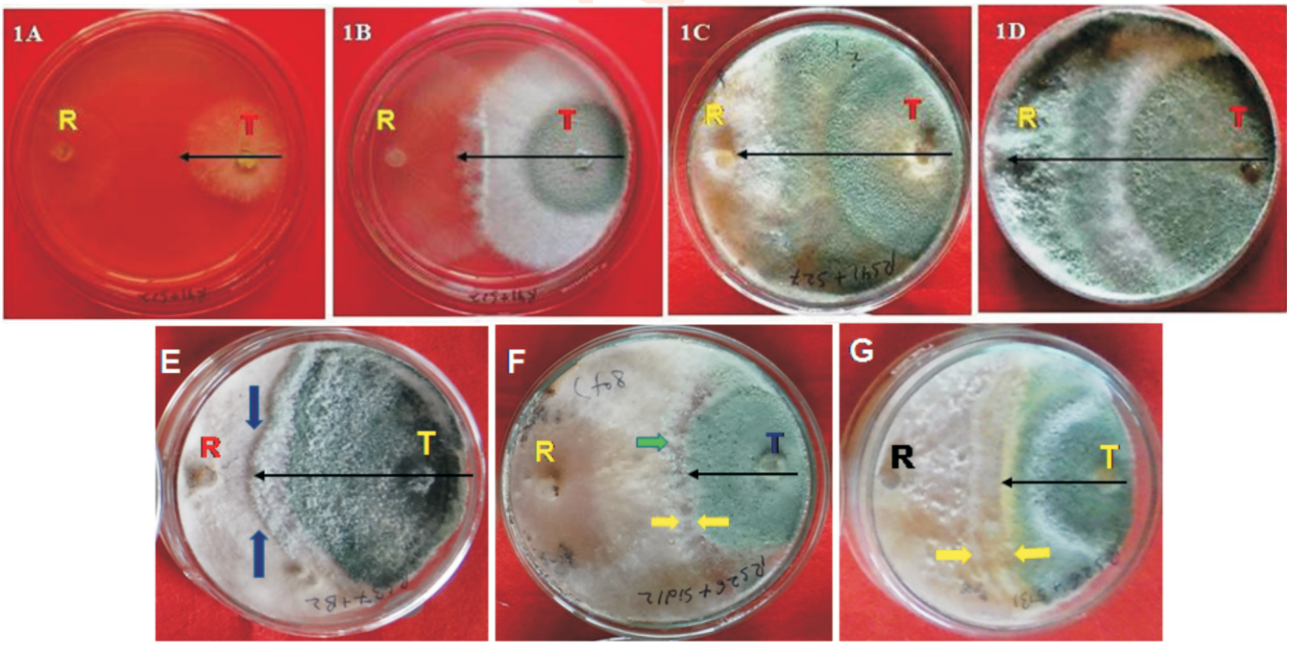

Fig. 1 : Dual plate assay for antagonistic activity of *Trichoderma* with *Rhizoctonia solani*: (1A-1D) *T. harzianum* (SVPRT-THLi06) growth and inhibition of *R. solani* growth after 24, 48, 96 and 120 hr, respectively (arrow marks show: *Trichoderma* growth), (E) *T. erinaceum* (SVPUAT-TE-1) growth and inhibition of *R. solani* (blue arrow show: *Trichoderma* overgrowth), (F and G) *R. solani* growth inhibition by *T. harzianum* (SVPRT-THL01) and *T. atroviride* (SVPP-4) respectively (yellow arrow show inhibition zone between them, green arrow show initial sporulation).

Table 3 : *In-vitro* screening of *Trichoderma* isolates against *Rhizoctonia solani* in confrontation assay

***Trichoderma* isolates**	**Dual culture**				**Volatile compounds**		**Non-volatile compounds**					
	R. solani		***Trichoderma* over growth (mm)***	**Rating#**	***R. solani***		**10%**		**25%**		**50%**	
	Growth (mm)*	**Inhibition over control (%)$^{\$}$**			**Growth (mm)***	**Inhibition over control (%)$^{\$}$**	**Growth (mm)***	**Inhibition over control (%)$^{\$}$**	**Growth (mm)***	**Inhibition over control (%)$^{\$}$**	**Growth (mm)***	**Inhibition over control (%)$^{\$}$**
SVPRT-THL01	24.9 ± 1.1	72.4 (58.3)	35.3 ± 0.7	(1)	43.8 ± 3.1	51.4 (45.8)	81.7 ± 3.2	9.2 (17.6)	62.6 ± 1.7	30.6 (33.6)	30.4 ± 3.1	66.2 (54.5)
SVPRT-THL02	32.2 ± 1.8	64.2 (53.2)	27.7 ± 0.9	(2)	53.9 ± 1.4	40.0 (39.2)	72.9 ± 2.0	18.9 (25.8)	62.9 ± 1.6	30.1 (33.2)	31.5 ± 3.8	64.9 (53.7)
SVPRT-THL03	28.1 ± 1.2	68.7 (55.9)	20.3 ± 0.8	(2)	66.4 ± 1.4	26.2 (30.8)	49.5 ± 4.3	45.0 (30.4)	45.6 ± 2.6	49.4 (44.5)	30.6 ± 1.8	66.0 (50.1)
SVPRT-THL04	28.6 ± 1.5	68.2 (55.7)	25.3 ± 0.4	(1)	57.4 ± 1.5	36.6 (37.2)	41.5 ± 4.6	53.9 (52.6)	27.9 ± 1.4	68.9 (56.1)	16.8 ± 1.6	81.4 (64.4)
SVPRT- THL07	26.2 ± 1.7	74.9 (59.9)	24.1 ± 0.8	(1)	34.8 ± 2.3	61.3 (51.5)	29.4 ± 4.7	67.3 (55.1)	25.5 ± 2.5	71.7 (57.9)	16.6 ± 2.3	81.5 (64.6)
SVPRT- THL08	22.6 ± 0.8	70.8 (57.3)	28.2 ± 0.7	(1)	43.9 ± 3.2	51.2 (45.7)	77.7 ± 4.6	13.6 (21.7)	75.6 ± 2.6	16.0 (23.6)	40.7 ± 1.6	54.8 (47.8)
SVPRT- THL09	26.5 ± 0.9	70.6 (57.2)	21.3 ± 0.5	(2)	37.3 ± 1.5	58.5 (49.9)	77.4 ± 2.4	13.9 (21.9)	66.6 ± 3.8	26.0 (30.7)	64.1 ± 3.4	28.8 (32.4)
SVPP-6	39.7 ± 1.7	55.9 (48.4)	18.3 ±0.7	(2)	86.7 ± 1.9	3.6 (10.9)	79.1 ± 3.9	12.1 (20.4)	75.5 ± 4.4	16.1 (23.7)	61.1 ± 2.0	32.1 (34.5)
SVPP-8	16.8 ± 1.5	81.3 (64.4)	31.7 ± 0.8	(1)	30.3 ± 2.9	72.5 (58.3)	15.4 ± 1.0	82.8 (65.5)	10.7 ± 0.6	88.1 (69.8)	5.4 ± 0.9	94.0 (75.9)
SVPP-9	25.5 ± 1.0	71.6 (57.8)	34.3 ± 0.7	(1)	34.7 ± 2.2	61.4 (51.6)	68.3 ± 2.9	24.1 (33.3)	64.0 ± 3.9	28.9 (32.5)	51.7 ± 1.2	42.5 (47.9)
SVPP-10	26.6 ± 1.9	63.4 (52.8)	24.3 ± 1.2	(2)	75.5 ± 0.8	16.1 (23.7)	55.3 ± 4.8	38.6 (38.4)	51.8 ± 1.1	42.5 (40.7)	39.1 ± 2.9	56.5 (48.7)
SVPP-12	28.3 ± 0.7	68.5 (55.9)	33.1 ± 1.4	(2)	58.9 ± 1.3	34.6 (36.0)	61.4 ± 0.7	31.8 (34.3)	53.3 ± 2.1	40.8 (39.7)	32.7 ± 2.7	63.6 (52.9)
SVPP-14	29.4 ± 1.6	60.6 (51.1)	18.3 ± 1.2	(3)	76.5 ± 3.2	15.0 (22.8)	59.6 ± 5.8	33.7 (35.5)	51.0 ± 2.9	43.3 (41.1)	35.8 ± 3.8	60.2 (50.9)
SVPP-18	30.9 ± 1.2	59.1 (50.2)	21.2 ± 0.9	(2)	62.2 ± 3.7	30.9 (33.7)	73.9 ± 3.9	17.9 (25.0)	71.1 ± 1.1	21.0 (27.3)	56.5 ± 3.1	37.1 (37.5)
SVPRT- THLi6	8.1 ± 0.7	90.9 (72.5)	38.4 ± 0.7	(1)	24.8 ± 1.2	66.4 (54.6)	11.6 ± 0.8	87.2 (42.1)	9.5 ± 0.9	89.5 (71.1)	3.7 ± 0.4	95.9 (54.4)
SVPRT- THLi7	31.8 ± 1.5	64.6 (53.5)	11.45 ± 0.76	(3)	55.3 ± 2.7	38.6 (38.4)	45.0 ± 1.6	50.0 (45.0)	31.5 ± 1.2	65.0 (53.7)	23.3 ± 1.9	74.0 (59.4)
SVPRT- THLi8	20.5 ± 1.8	77.2 (61.5)	24.3 ± 0.9	(2)	32.3 ± 2.5	64.0 (53.2)	22.6 ± 0.8	74.9 (59.9)	18.4 ± 1.9	79.6 (63.1)	5.7 ± 1.4	93.6 (75.4)
SVPRT- THLi9	37.3 ± 1.6	58.5 (49.9)	26.9 ± 0.6	(1)	53.5 ± 5.2	40.5 (39.5)	17.5 ± 0.9	80.5 (63.8)	13.6 ± 0.5	84.8 (67.1)	6.8 ± 1.4	92.5 (74.1)
SVPRT- TH1	27.9 ± 1.1	68.9 (56.1)	24.1 ± 1.1	(2)	78.9 ± 6.3	12.3 (20.5)	48.9 ± 1.4	45.7 (42.5)	39.0 ± 1.5	56.6 (48.8)	18.4 ± 1.9	79.5 (63.1)
SVPRT- THL01	33.4 ± 1.8	62.8 (52.4)	25.3 ± 1.3	(1)	67.6 ± 3.8	24.9 (29.9)	53.9 ± 2.3	40.1 (39.3)	51.4 ± 0.8	42.8 (40.9)	28.2 ± 2.0	68.6 (55.9)
SVPRT-LB02	36.2 ± 1.6	59.7 (50.6)	22.7 ± 1.1	(2)	64.6 ± 3.5	28.2 (32.1)	57.6 ± 0.8	36.0 (36.9)	53.4 ± 2.7	40.6 (39.6)	32.7 ± 2.1	63.7 (52.9)
SVPRT- LB06	38.9 ± 1.2	56.7 (48.8)	7.5 ± 1.1	(4)	78.7 ± 1.3	12.6 (20.8)	68.7 ± 5.5	23.6 (29.1)	62.4 ± 1.9	30.6 (33.6)	45.7 ± 3.2	49.2 (44.5)
SVPRT- LB07	32.4 ± 1.5	63.9 (53.1)	19.6 ± 0.2	(2)	63.9 ± 5.8	28.9 (32.5)	62.9 ± 2.2	30.1 (40.0)	58.3 ± 3.3	35.3 (36.4)	40.4 ± 2.0	55.1 (49.1)
SVPRT- TL10	27.9 ± 1.1	68.9 (56.1)	16.2 ± 1.1	(3)	62.2 ± 1.1	30.8 (33.7)	52.8 ± 2.0	41.3 (69.0)	48.9 ± 2.6	45.6 (42.5)	38.5 ± 1.9	57.2 (78.3)
SVPP-7	39.3 ± 1.5	56.3 (48.6)	8.4 ± 0.7	(4)	61.9 ± 1.9	31.1 (33.9)	66.7 ± 3.9	25.9 (30.6)	57.3 ± 3.0	36.3 (37.0)	41.5 ± 1.3	53.9 (47.2)
SVPP-2	36.8 ± 1.1	59.1 (50.2)	16.2 ± 0.9	(2)	57.8 ± 3.2	35.8 (36.7)	74.6 ± 3.5	17.1 (24.4)	68.1 ± 5.5	24.3 (29.5)	50.9 ± 3.4	43.5 (41.3)
SVPP-3	34.1 ± 1.2	62.1 (52.0)	19.9 ± 1.1	(3)	64.8 ± 3.9	28.0 (31.9)	66.9 ± 2.6	25.6 (47.2)	55.2 ± 1.2	38.7 (38.5)	36.9 ± 2.6	58.9 (66.4)
SVPP-5	52.2 ± 1.3	41.9 (40.4)	9.4 ± 0.7	(4)	49.5 ± 2.0	44.9 (42.1)	74.7 ± 0.7	16.9 (24.3)	52.9 ± 2.3	41.2 (39.9)	42.0 ± 2.1	53.3 (46.9)
SVPP-11	42.9 ± 1.5	52.3 (46.3)	8.6 ± 1.1	(4)	64.3 ± 3.6	28.6 (32.3)	76.2 ± 3.0	15.3 (23.1)	65.7 ± 1.7	27.0 (31.3)	53.5 ± 2.9	40.6 (39.6)
SVPP-4	25.2 ± 1.0	72.3 (58.1)	23.7 ± 0.7	(1)	31.9 ± 3.4	64.5 (53.4)	33.2 ± 1.1	63.0 (52.6)	30.3 ± 2.1	66.3 (54.5)	14.3 ± 1.8	84.0 (64.4)
SVPUAT-TE-1	29.3 ± 1.1	67.5 (55.2)	18.6 ± 1.1	(2)	46.7 ± 3.5	48.1 (43.9)	82.6 ± 3.0	8.2 (16.6)	73.7 ± 3.3	18.1 (25.2)	41.0 ± 2.9	54.4 (47.5)
Control	90.0	—	—	—	90.0	—	90.0	—	90.0	—	90.0	—
P value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
LSD (P=0.05)	2.7		1.2		5.9		5.9		4.0		3.8	

*Mean ± SD (Standard deviation); $^{\$}$Values are the mean percentage inhibition (angular transformed data) of three replicates, # Bell's rating; ^{a}LSD is the mean significant difference at 5% (P = 0.05) isolate SVPRT- THLi6 showed maximum inhibition at 50% concentration with an inhibition potential of 95.9%, followed by SVPP-8 with 94.0% inhibition potential and minimum inhibition potential (28.8%) recorded by SVPRT-THL09 (Table 3). However, even at 10% concentration, the growth of *R. solani* was significantly inhibited by the culture filtrate compared to the control, suggesting that the SVPRT- THLi6 culture filtrate had a strong inhibitory potential against *R. solani.*

The mycelial growth of *R. solani* was significantly inhibited by volatile compounds produced by *T. harzianum* isolate. *T. harzianum* isolate (SVPP-8) was effective with highest inhibition rate (72.5%), followed by *T. harzianum* SVPRT-THLi06 and *T. atroviride* SVPP-4 (66.4% and 64.5%), respectively (Table 3). Production of volatile and non-volatile compounds has been associated with the ability of *Trichoderma* to control phytopathogens (Harman, 2006). Dennis and Webster (1971)

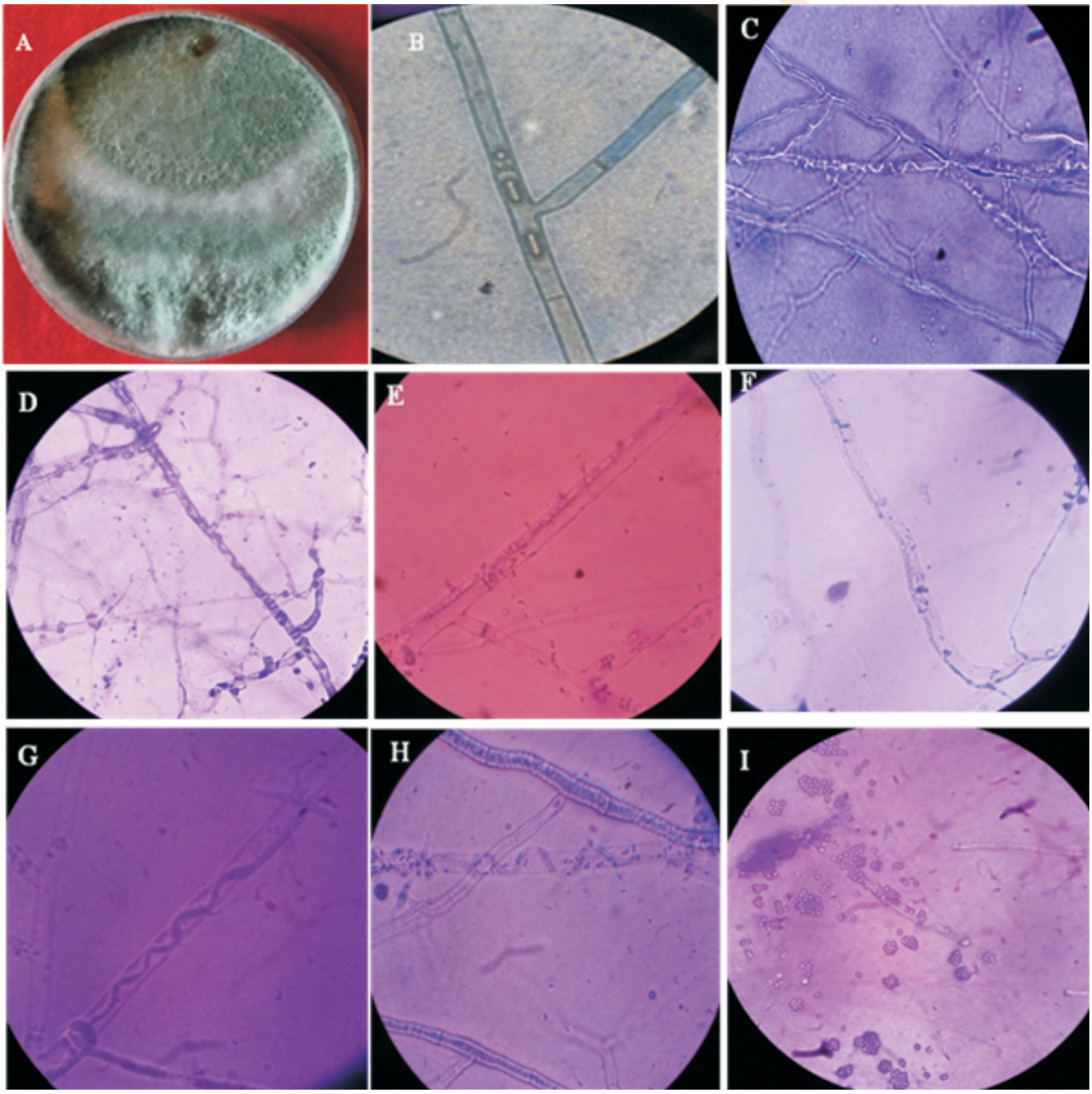

Fig. 2: Photomicrographs showing hyphal interaction between *T. harzianum* and *R. solani;* (A) *T. harzianum* and *R. solani* interaction in dual culture; (B) Mycelium of *R. solani;* (C, D) *T. harzianum* hyphae coiling to hyphae of *R. solani* ; (E, F) *T. harzianum* hyphae running parallel to hyphae of *R. solani;* (G, H) Lysis of *R. solani* hyphae; (I) Spores of *T. harzianum* attached on hyphae of *R. solani.*

observed that *Trichoderma* spp. produce a volatile antibiotic that inhibited mycelial growth of *R. solani* and other test fungi. The volatile and non-volatile metabolites of *Trichoderma* spp. have also been reported to reduce mycelial growth and sclerotial germination in sclerotial forming fungus by several workers (Srinivasa *et al.,* 2014; Kumar *et al.,* 2017).

The antagonistic potential of five *Trichoderma* isolates selected by *in-vitro* screening experiments were further evaluated for their biocontrol of sheath blight disease *in-vivo*. The selected isolates belonged to three species, *i.e., T. harzianum*, *T. longibrachiatum* and *T. atroviride*. Applications of spore suspension and talk based formulation of *T. harzianum* significantly reduced the disease severity and infected tillers/hills (disease incidence) over control and other *Trichoderma* spp., irrespective of its mode of application. Significant disease reduction was recorded when all the tested *Trichoderma* spp. were applied using a mixed mode comprising all three delivery systems together, *i.e.* soil treatment + seedling root dip + foliar spray followed by dual inoculation of bio-agent using seedling root dip + foliar spray. However, among the *T. harzianum* treatments, bio-agents SVPRT-THLi06 and SVPP-8 recorded highest value of disease reduction from soil treatment + seedling root dip + foliar spray (75.1% and 69.8%) as compared to fungicide propiconazole 25EC @ 0.1% which showed disease reduction of 83.9% (Table 4). Moreover, *T. atroviride* isolate (SVPRT-TVir01) showed disease reduction over disease severity (63.0%), when applied with soil treatment and foliar spray. No significant difference in disease severity was recorded between soil treatment + foliar spray and seedling root dip + foliar spray with all the treatments. However, among different treatments, maximum and significant higher disease severity (34.5%) and incidence (59.4%) were recorded with soil treatment + foliar spray of the bioagent SVPRT-TL10. Tewari and Singh (2005) reported that maximum rice sheath blight disease control could be achieved when the bioagents were applied through soil, seedling root dip and foliar spray. Furthermore, Verma *et al.* (2007) reported that *Trichoderma* spp. can be efficiently used as spores or conidia, which are more lenient to adverse environmental conditions, in contrast to their mycelial and chlamydospore forms as microbial propagules.

The observations of growth parameters revealed that *Trichoderma* spp. treated rice plants significantly enhanced the rice plant growth performance (Table 4). A significant increase in plant height was observed in all the *Trichoderma* spp. treatments. The plant heights ranged from 80.4 – 124.7 cm while in propiconazole 25 EC (0.1%) treated plants and control were

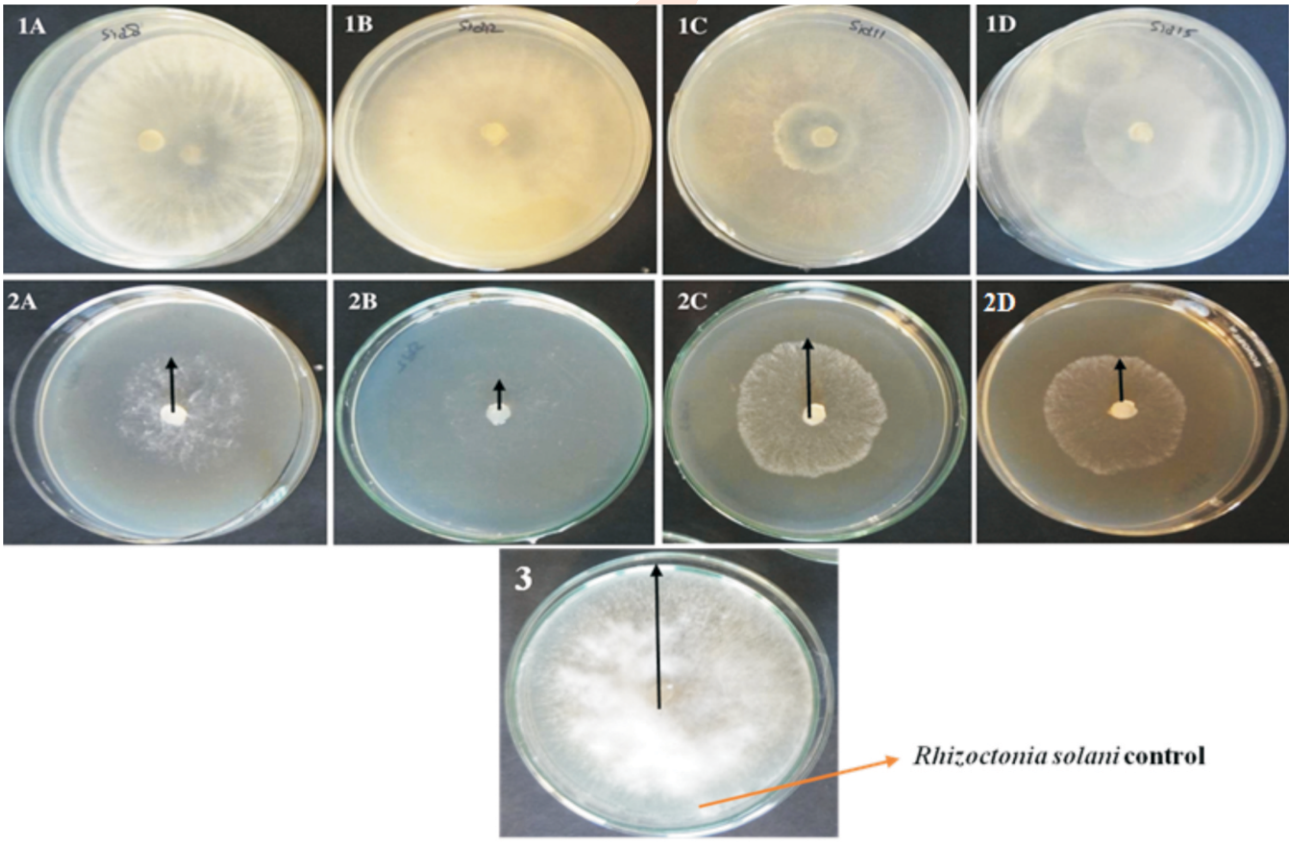

Fig. 3 : *In-vitro* effect of volatile compounds released by *Trichoderma* isolates on *R. solani*. 1A-1D: Inoculation of *R. solani* upper side and *Trichoderma* at lower side. 2A-2D: Growth inhibition of *R. solani* growth after 7DOI. 3: *R. solani* control. Arrow indicate growth of *R. solani*

Table 4 : Effect of *Trichoderma* spp. inoculation on disease incidence, disease severity of sheath blight and growth enhancement parameters of rice

Treatment	Disease incidence (%)$^{\$}$	Disease severity (%)$^{\$}$	Disease reduction over severity(%)$^{\$}$	Plant height (cm)*	Tiller number*	Root length (cm)*	Root dry weight (g)*	Root fresh weight (g)*	Grains/ panicle*	1000-grain weight (g)*
T1	40.4 (39.5)	22.5 (28.3)	54.7 (47.7)	101.7± 3.1	11± 1.5	20.9±1.3	2.5±1.1	12.3±0.7	176.7±11.9	23.8±1.6
T2	36.6 (37.3)	18.2 (25.3)	53.7 (47.1)	116.6± 2.1	10± 2.5	21.2±1.3	3.2±1.0	12.9±1.4	135.7±5.9	24.0±1.3
T3	45.2 (42.3)	22.4 (28.2)	54.9 (47.8)	102.5± 2.2	11± 1.5	19.6±2.3	2.6±1.2	10.7±1.4	169.0±10.6	25.9±2.2
T4	38.1 (38.1)	14.9 (22.7)	69.8 (56.7)	114.5± 2.2	11± 1.5	21.5±1.1	3.7±1.4	12.6±1.0	135.0±13.0	25.3±0.8
T5	55.7 (48.3)	30.9 (33.7)	37.8 (37.9)	103.2± 6.1	12± 1.1	21.6±0.9	2.8±0.3	12.5±0.9	176.7±12.5	26.9±1.5
T6	45.4 (42.4)	30.3 (33.4)	38.8 (38.5)	102.9± 4.2	11± 0.0	19.9±1.3	3.7±2.2	11.4±1.5	151.0±16.1	25.9±2.1
T7	58.8 (50.1)	29.8 (33.1)	39.6 (38.9)	94.6± 2.5	10± 0.6	17.9±0.4	2.7±1.5	10.9±0.6	164.3±4.5	26.4±1.4
T8	52.5 (46.4)	28.5 (32.3)	42.6 (40.7)	90.5± 2.1	10± 0.6	20.9±0.3	2.6±2.3	9.9±1.3	154.7±21.5	25.0±0.8
T9	45.1 (42.2)	22.2 (28.1)	54.9 (47.8)	96.2± 3.9	11± 1.0	21.9±1.1	3.3±0.9	10.4±1.2	135.0±9.6	25.1±3.2
T10	41.4 (40.4)	20.2 (26.7)	59.3 (50.3)	106.3± 3.1	12± 1.0	22.4±1.0	3.1±1.8	10.4±1.9	162.0±16.8	26.1±0.8
T11	41.5 (40.1)	18.2 (25.2)	63.4 (52.8)	107.7± 3.2	9± 1.5	20.1±0.5	3.2±0.9	11.3±2.1	164.7±10.5	25.9±1.3
T12	27.1 (31.4)	12.4 (20.6)	75.1 (60.1)	124.7± 2.7	13± 1.5	23.4±1.9	4.2±2.6	14.2±0.9	184.7±5.0	27.4±2.2
T13	59.4 (50.4)	30.9 (33.8)	37.3 (37.6)	107.9± 6.8	11± 2.1	21.1±1.0	3.2±1.7	12.8±1.5	172.3±16.6	25.2±0.7
T14	57.7 (49.4)	34.5 (35.9)	30.4 (33.4)	97.3± 4.6	9± 1.5	18.6±1.1	1.9±0.8	10.6±1.9	179.7±7.0	24.3±1.4
T15	57.1 (49.1)	28.5 (32.2)	42.6 (40.7)	102.3± 2.6	10± 2.1	21.7±0.5	3.2±1.1	10.8±1.8	154.7±19.0	24.7±2.9
T16	43.6 (41.3)	25.8 (30.5)	47.9 (43.8)	80.4± 1.2	9± 2.0	20.3±1.8	1.8±1.3	9.7±1.9	165.3±15.6	27.3±2.4
T17	54.8 (47.8)	31.0 (33.9)	37.1 (37.5)	97.1± 1.5	8± 1.5	17.8±1.4	1.9±0.9	10.4±1.9	147.3±9.9	23.3±1.7
T18	37.2 (37.6)	18.2 (26.3)	63.0 (52.5)	80.6± 1.6	8± 1.0	18.9±1.4	1.9±1.6	9.5±2.1	144.3±13.6	24.0±2.8
T19	57.6 (49.4)	27.1 (31.4)	45.4 (42.4)	91.5± 1.7	9± 1.5	16.9±1.3	1.3±1.1	8.3±0.7	155.7±6.0	22.5±2.2
T20	43.9 (41.5)	24.6 (29.7)	50.1 (45.1)	99.2± 2.2	10± 1.0	15.7±0.7	1.8±1.6	9.6±0.8	110.0±6.1	21.9±1.6
Propiconazole 25 EC (0.1%)	20.7 (27.1)	7.9 (16.4)	83.9 (66.4)	108.5± 1.3	10± 1.0	16.9±1.7	1.5±0.8	10.7±1.2	109.0±8.2	19.4±9.4
Control	96.4 (79.1)	49.6 (44.8)	-	75.6± 4.3	7± 1.0	14.1±1.1	1.7±0.9	8.4±1.1	109.7±11.5	18.1±1.9
LSD (p=0.05)	7.4	3.1	-	7.5	3.9	2.9	ns	3.1	34.0	8.4

*Values are mean of sixteen replicates ± SD, $^{\$}$Values in parentheses are angular transformed data; ^{a}LSD is the mean significant difference at 5% (P = 0.05) recorded at 108.5 cm and 75.6 cm, respectively. However, isolate SVPRT-THLi06 recorded highest increase in plant height (124.7 cm) with the treatment soil + seedling root dip + foliar spray followed by isolate SVPRT-THLi04 (114.5 cm) with soil and foliar spray treatment. Significantly more number of tillers was also recorded for *Trichoderma* spp. treated rice plants. The highest number of tillers (13) was recorded for isolate SVPP-4 (soil + seedling root dip + foliar spray) treated plants and the lowest number of tillers (8) for SVPRT-TL10 (soil treatment+ foliar spray) treated plants. On the other hand, the propiconazole 25 EC (0.1%) treatment and control recorded a tiller number of 10 and 7, respectively. Root length was found to be significantly increased for SVPRT-THLi06 (soil + seedling root dip + foliar spray) treated plants with 23.4 cm, while the propiconazole (0.1%) and control recorded root length of 16.9 cm and 14.1 cm, respectively. *T. harzianum* isolate SVPRT-THLi06 (soil + seedling root dip + foliar spray) treated rice plants recorded the highest value of root fresh weight (14.2 g), while for propiconazole 25 EC (0.1%) treated plants and control were 10.7 g and 8.4 g, respectively. Significantly higher no. of grains/panicle was also observed for *Trichoderma* spp. treated rice plants compared to propiconazole 25 EC (0.1%) treated plants and control. Isolate SVPRT-THLi06 (soil + seedling root dip + foliar spray) treated rice plants registered the highest no. of grains/panicle which was 179.7, while for propiconazole 25 EC (0.1%) treated plants and control were 109.0 and 109.7, respectively. Significant increase in weight of 1000 grains was observed for all the *Trichoderma* spp. treatments compared to propiconazole 25 EC (0.1%) treated rice plants and control. The highest no. weight of 1000 grains was recorded with SVPRT-THLi06 (soil + seedling root dip + foliar spray) treated rice plants of 27.4 g while for propiconazole 25EC (0.1%) treated plants and control were 19.4 g and 18.1 g, respectively. Among the *Trichoderma* spp. SVPRT-THLi06 (soil + seedling root dip + foliar spray) treated rice plants showed the highest boost in plant height, root length, root fresh weight, no. of grains/panicle and weight of 1000 grains, while *Trichoderma* spp. SVPP-4 (soil + seedling root dip + foliar spray) had the greatest effect in enhancing the tiller numbers. However, no significant improvement in root dry weight was seen for the *Trichoderma* spp. treated rice plants.

Rice plants treated with *Trichoderma* spp. significantly increased rice growth parameters. Higher plant height was observed in *Trichoderma* spp. inoculated rice plants as compared to control. Saba *et al.* (2012) suggested that *Trichoderma* treated rice plants have better nutrient ability that enhance the physiological processes within the rice plants, leading to excellent growth performance. Significantly higher tiller number was recorded in *Trichoderma* spp. treated rice plants compared to control. Doni *et al.* (2014) also found that *Trichoderma* sp. SL2 inoculation could increase tiller number in rice. Furthermore, Shukla *et al.* (2012) reported that *T. harzianum* significantly increased rice plant ability to tolerate drought stress and increase water holding capacity, believed to be important factors that led to higher tiller number. IAA produced by *T. virens* and *T. atroviridae* were found to stimulate the growth of lateral roots of *Arabidopsis* (Contreras-Cornejo *et al.*, 2009). In this study, *Trichoderma* sp. treated rice plants recorded impressive increase in root length and root fresh weight compared to non-inoculated rice plants. *Trichoderma* isolates induced fresh and dry root weight of inoculated rice plants. Swain *et al.* (2018) observed that *Trichoderma* spp. treated rice plants showed better growth performance as compare to control.

Trichoderma spp. may be used as an eco-friendly and sustainable tool to enhance yield of different crop plants, including rice (Hossain *et al.*, 2017). Application of *T. harzianum* and *T. viride* significantly improved millable canes over control plants (Srivastava *et al.,* 2006). After inoculating with *T. koningii* or *T. hamatum*, crop productivity increase up to 300% in the field (Sanjeev *et al.,* 2014). Recently, Doni *et al.* (2018) reported that *Trichoderma*-based biofertilizer increased rice yield under system of rice intensification (SRI) conditions by 30% at very low cost ha^{-1}. However, the accurate reason for enhanced yields seems to be unclear yet, but in most cases, it is probably due to greater supply of nutrients by *Trichoderma* spp. to rice plants (Hossain *et al.,* 2017). Yedidia *et al.* (2001) suggested that presence of plant growth promoting fungi in the rhizosphere increases root surface area allowing the roots to explore larger volume of soil; thus, more nutrients are available to the plants, especially under nutrient-stressed soil environments. Some *Trichoderma* spp. strains show abilities to improve photosynthetic efficiency (Doni *et al.,* 2014). All of these capabilities singly or in combination contribute to improve crop yield.

It may be concluded that *Trichoderma* spp. have the potential to biocontrol the sheath blight disease caused by *R. solani* AG1-IA and enhance rice plant growth parameters. Among the *T. harzianum* strains, SVPRT-THLi06 was the most effective strain having great potential to control sheath blight and rice plant growth promotion compared to other strains tested. Therefore, this strain can be used for production and development of *T. harzianum* based biofertilizers-cum biopesticide to *manage Rhizoctonia* disease and growth regulator for plants. Furthermore, studies are needed on this promising *T. harzianum* strain to identify potential metabolites and evaluate possible mode of actions related to biocontrol and plant growth.

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