

Original Research Article

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**Invitro Evaluation of Native Rice Specific Isolates of *Trichoderma* against Rice Sheath Blight caused by *Rhizoctonia solani***

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*Trichoderma* is a free living fungi which are highly interactive in root, soil and foliar environments as well. *Trichoderma* can be used as a biological control agent due to its ability such as mycoparasitism, production of antibiotic and/or hydrolytic enzymes, competition for nutrients, as well as induced plant resistance; production of numerous secondary metabolites inhibitory to the growth of several plant pathogens. In this study, the antagonistic potential of some native rice specific *Trichoderma* isolates were evaluated against sheath blight disease of rice caused by *Rhizoctonia solani*. It revealed that the inhibition percentages of *R. solani* by the native rice specific *Trichoderma* isolated from various soil samples of Manipur ranges from 62.50% to 87.50% with highest per cent inhibition by WAI-D, *T. harzianum* (MH257323), and lowest by LAM-B, *T. brevicompactum* (MH257322) of 87.50% and 62.50% respectively. Bell's scale study showed class III category by *T. brevicompactum* (MH257322) and class II showed by *T. harzianum* (MH257323) against *Rhizoctonia solani*. Among the native rice specific *Trichoderma* isolates, WAI-D, *T. harzianum* (MH257323) is found to be the most effective in reducing the rapid growth of pathogen. Further, all native *Trichoderma* isolates significantly inhibited the mycelial growth of the pathogen.

**Introduction**

Rice (*Oryza sativa* L.) is a pre-eminent crop of India as it is the staple food for most of the people of the country. It is one of the major food crops of India. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live (Mahajan *et al.*, 2017). China produces the largest amount of rice (142.3 million tonnes)

followed by India (110.4 million tonnes) (According to FAO: Rice Market Monitor 2018). Rice is the staple food crop of Manipur. It is widely cultivated in both hill and valley areas of Manipur occupying nearly 1.80 lakh ha of the total cropped area in the state (Goud *et al.*, 2018). Rice is found to suffer from many fungal and bacterial diseases which result in heavy grain yield losses. Its productivity is affected by several

pathogens that often place major constraints on production, among which, *Rhizoctonia solani*, the causal organism of sheath blight, is responsible for yield loss up to 45% (Margani and Widadi 2018). Rice sheath blight is a fungal disease caused by an agaricomycete, *Rhizoctonia solani* found to be prevalent in intensively cultivated rice fields. Sheath blight is widely distributed in many rice-growing countries and has often caused serious damage to rice in both the temperate and tropical regions. (Hashioka and Makino, 1969; Ou and Jennings, 1969). The pathogen *Rhizoctonia solani* Kunh (anamorph), *Thanatephorus cucumeris* (Frank) Donk (teleomorph) is a soil-dwelling saprotroph and facultative parasite. The pathogen causes lesions on the sheath affecting grain filling and yield in rice (Wu *et al.*, 2012). Sheath blight in rice was first reported in Japan in 1910. Sheath blight in rice subsequently spread across the region, particularly where rice was grown under intense cultivation (Srinivasachary Willocquet and Savary 2011). In order to tackle problems of sheath blight, there is heavy dependence on agrochemicals.

A prevalent misconception present among the modern farmers that chemical pesticide application is the only way out of the problem has led to indiscriminate use of agrochemicals causing numerous deleterious side effects. This incorrect practice has resulted in more damages than amelioration of the problems. Another pressing problem that arises in the larger picture is accumulation of pesticide residues in environment which affects the food web and the food chain, thereby leading to ecological imbalances as well as polluting the soil and water resources. So, keeping in view the ever increasing demand of food safety and security without harming the environment, a search for alternatives to agrochemicals has shown the pivotal role of application of the biocontrol agents.

One such biocontrol agent which has been explored since years is *Trichoderma*. The genus *Trichoderma* houses a variety of free living fungi that are common in soil and root ecosystems. It is a secondary fast growing opportunistic invasive, which produces large numbers of spores, enzymes able to degrade the fungal cell wall (chitinases, glucanases, and proteases) and compounds with antimicrobial activity. They are found to be very promising against phytopathogenic fungi. Many *Trichoderma* species are also well known as biocontrol agents (BCA) of important phytopathogenic fungi. The primary mechanisms of biocontrol used by *Trichoderma* in direct confrontation with pathogenic fungi are the mycoparasitism (Papavizas, 1985), antibiosis, and competition for nutrients with the pathogen (Harman and Kubicek, 1998). The present investigation were carried out to understand the effect of native rice specific isolates of *Trichoderma* on the growth of *Rhizoctonia solani* *in-vitro*.

## Materials and Methods

Native rice specific *Trichoderma* spp. were isolated by soil dilution plate technique (Dhingra and Sinclair, 1995) using *Trichoderma* specific medium (TSM) (Elad and Chet, 1983). Different dilutions ranging from  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  of the soil samples collected from four valley districts of Manipur were used. The native *Trichoderma* isolates were identified by molecular techniques carried out by amplification of the ITS region of fungal isolates. Genomic DNA was isolated from the fungal isolates using a HiPurA™ fungal DNA isolation Kit (Hi media, India) as per the manufacturer's protocol, polymerase chain reaction (PCR) amplification of the target nucleotide sequences were carried out with the genomic DNA as the template for fungal isolates. Universal primers coding for the ITS region viz., ITS1 5'- TCCGTAGGTGAACCTGCG

G - 3' & ITS4 5'- TCCTCCGCTTATTGAT ATGC - 3' (Vilgalys, R., *et al.*, 1994) were used as the forward and reverse primers for the amplification of the target nucleotide. Nucleotide sequencing of the amplified DNA for the ITS region of the fungal isolates were carried out by automated sequencing service rendered by Xcelris Genomics, Ahmedabad, India and sequences were submitted to NCBI GenBank and accession numbers were obtained accordingly.

The infected rice plant showing typical symptoms of sheath blight were collected and examined under microscope in Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal laboratory. Later the collected samples were lacerated to small pieces (<1.0 cm) and were washed under tap water twice to remove soil particles and other debris. Surface sterilization was done by dipping the cut pieces in 1% Sodium hypo chloride (NaOCl) solution and through a series of sterile distilled water at 3 times for one minute intervals respectively. The treated sample pieces were blot dried and then transferred to petri plates containing sterilized potato dextrose agar medium with four pieces per plate using sterile forceps. The isolated fungus was identified as *Rhizoctonia solani* (MT584664). All plates were kept at  $25 \pm 2^\circ\text{C}$  for 3-4 days and from these plates pure cultures of *R. solani* isolates were maintained. The fungus was then sub cultured whenever needed during the present study.

In-vitro evaluation of bio control agents against growth of *Rhizoctonia solani* by Dual culture method

In vitro antagonistic activity of the native rice specific isolates of *Trichoderma* against *Rhizoctonia solani* was studied in dual culture technique by following the method by Kucuk and Kivanc (2003). Antagonistic potentials of the rice specific native isolates of

*Trichoderma* against *Rhizoctonia solani* were evaluated from the dual culture technique using formula given by Bell (1982). The petri dishes containing sterile PDA were inoculated with 5mm diameter plug of 4-day old pure culture of antagonistic fungi and the pathogen. One mycelial disc of each of the fungus was placed on the opposite poles of PDA plates using sterile cork borer and sterile needle and incubated at  $25^\circ\text{C}$  in BOD incubator and radial growths of the pathogen were recorded at 24hrs interval. A petridish without the antagonist served as the control. Each treatment were replicated thrice. The per cent inhibition of the mycelial growth of *Rhizoctonia solani* over the control were calculated using the formula suggested by Dennis and Webster (1971).

Per cent Inhibition of Radial Growth (% IRG) =  $100 [(C-T) / C]$ , where C- linear growth of the fungus in control,

T- Linear growth of the fungus in treatment

Bell's scale with slight modification

Class I: The antagonist completely overgrew the test pathogen (100 % overgrowth).

Class II: The antagonist overgrew at least 2/3rd of the test pathogen surface (75% over growth).

Class III: The antagonist colonized on half of the growth of the test pathogen surface (50% over growth).

Class IV: The test pathogen and the antagonist locked at the point of contact.

Class V: The test pathogen overgrew the antagonist.

Class VI: The test pathogen and antagonist form inhibition zone.

## Results and Discussion

The study demonstrated the differential biocontrol ability of the fourteen (14) isolates of native rice specific *Trichoderma* spp. (given in Table1) by dual culture technique against *R. solani* causing sheath blight of rice which were recorded and percent inhibition tabulated as given in Table2., and Graph.1. Among the fourteen (14) native rice specific *Trichoderma* spp. used WAI-D, *T. harzianum* (MH257323), resulted in best mycelial growth inhibition by (87.50%). However all the species showed a considerable mycelial growth inhibition i.e., TAN-A, *T. koningii* (MH257321) by 80.50%, LAM-B *T. brevicompactum* (MH257322) by 62.50%, LIL-E, *T. harzianum* (MH257324) by 62.75%, KWS-F, *T. harzianum* (MH257325) by 78.50%, NAM-G *T. harzianum* (MH257326) by 70.00%, TKS-H *T. asperellum* (MH257327) by 78.50%, CHK-I *T. viride* (MH257328) by 77.50%, THML-J, *T. asperellum* (MH257329) by 69.00%, WAN-K, *T. harzianum* (MH257330) by 75.00%, WANJ-L, *T. harzianum* (MH257331) by 72.50%, NAR-M, *T. harzianum* (MH257332) by 85.00%, KSS-O, *T. harzianum* (MH257333) by 72.50% and SAI-C, *T. koningiopsis* (MN080228) by 72.50%. The highest percent of inhibition 87.50% was shown by WAI-D, *T. harzianum* (MH257323) and the least percent inhibition of 62.50% was shown by LAM-B, *T. brevicompactum* ((MH257322). *T. harzianum* giving the best inhibition were also reported in findings of (Seema and Devaki, 2012). *Trichoderma* spp. produces substantial and diversified secondary metabolites like pyrones, koniginins, viridins, nitrogen heterocyclic compounds, azaphilones, butenolides and hydroxy-lactones, isocyno metabolites, diketopiperazines, peptaibols, etc., (Francesco Vinale *et al.*, 2014). These heterogenic

secondary metabolites yielded by *Trichoderma* triggers the activities like mycoparasitism, competition for nutrition (carbon, nitrogen and also free space) and rapid colonization. Baker and Cook (1979) have reported that enzymes may be produced by *Trichoderma* that digest the mycelial walls and septal walls or antibiotics may be formed that inhibit growth or cause endolysis.

Dennis and Webster (1971) have reported that *Trichoderma* spp. are known to produce a number of antibiotics such as trichodermin, trichodermol, harzianum A and harzianolide as well as some cell wall degrading enzymes such as chitinases, glucanases that break down polysaccharide, chitins and  $\beta$ -glucans, thereby destroying cell wall integrity (Elad, 2000; Devaki *et al.*, 1992). These may also play a major role in mycoparasitism because of changes in cell wall integrity. All these distinguished features of *Trichoderma* accomplish it as a bio control agent against *R. solani*.

The Bell's scale classified the antagonistic nature of WAI-D, *T. harzianum* (MH257323), TAN-A, *T. koningii* (MH257321), KWS-F, *T. harzianum* (MH257325), TKS-H *T. asperellum* (MH257327), CHK-I *T. viride* (MH257328), NAR-M, *T. harzianum* (MH257332), WAN-K, *T. harzianum* (MH257330), to class II where the antagonist overgrew at least two thirds of the pathogen surface and the rest other antagonists, LAM-B *T. brevicompactum* (MH257322), LIL-E, *T. harzianum* (MH257324), NAM-G *T. harzianum* (MH257326), THML-J, *T. asperellum* (MH257329), WANJ-L, *T. harzianum* (MH257331), KSS-O, *T. harzianum* (MH257333), SAI-C, *T. koningiopsis* (MN080228) to Class III where the antagonist which colonized only half of the growth of the pathogen.

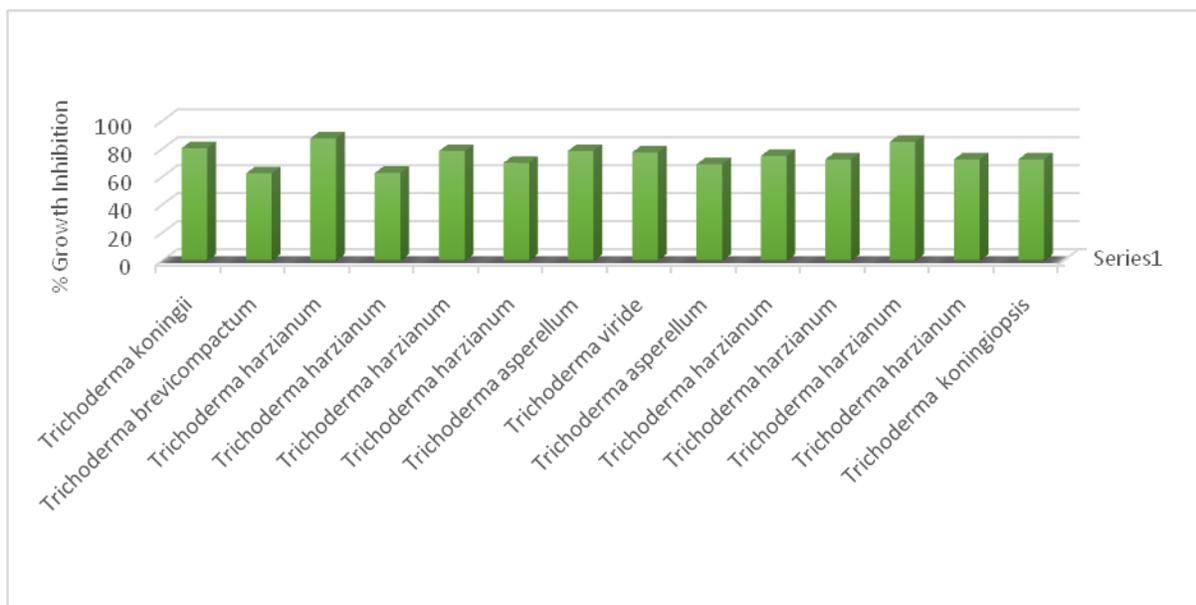
**Table.1** The list of native *Trichoderma* isolates used is listed with Isolate code and Accession number

Sl.No.	Isolate code ( <i>Trichoderma</i> isolates)	Accession number
1.	TAN-A ( <i>T. koningii</i> )	MH257321
2	LAM-B ( <i>T. brevicompactum</i> )	MH257322
3	WAI-D ( <i>T. harzianum</i> )	MH257323
4	LIL-E ( <i>T. harzianum</i> )	MH257324
5	KWS-F ( <i>T. harzianum</i> )	MH257325
6	NAM-G ( <i>T. harzianum</i> )	MH257326
7	TKS-H ( <i>T. asperellum</i> )	MH257327
8	CHK-I ( <i>T. viride</i> )	MH257328
9	THML-J ( <i>T. asperellum</i> )	MH257329
10	WAN-K ( <i>T. harzianum</i> )	MH257330
11	WANJ-L ( <i>T. harzianum</i> )	MH257331
12	NAR-M ( <i>T. harzianum</i> )	MH257332
13	KSS-O ( <i>T. harzianum</i> )	MH257333
14	SAI-C ( <i>T. koningiopsis</i> )	MN080228

**Table.2** *In vitro* evaluation of biocontrol activity by Dual culture of *Trichoderma* isolates against *Rhizoctonia solani*

Sl.no	Bio control agents	Bell's Scale	Inhibition %*
1	TAN-A ( <i>T. koningii</i> )	Class II	80.50
2	LAM-B ( <i>T. brevicompactum</i> )	Class III	62.50
3	WAI-D ( <i>T. harzianum</i> )	Class II	87.50
4	LIL-E ( <i>T. harzianum</i> )	Class III	62.75
5	KWS-F ( <i>T. harzianum</i> )	Class II	78.50
6	NAM-G ( <i>T. harzianum</i> )	Class III	70.00
7	TKS-H ( <i>T. asperellum</i> )	Class II	78.50
8	CHK-I ( <i>T. viride</i> )	Class II	77.50
9	THML-J ( <i>T. asperellum</i> )	Class III	69.00
10	WAN-K ( <i>T. harzianum</i> )	Class II	75.00
11	WANJ-L ( <i>T. harzianum</i> )	Class III	72.50
12	NAR-M ( <i>T. harzianum</i> )	Class II	85.00
13	KSS-O ( <i>T. harzianum</i> )	Class III	72.50
14	SAI-C ( <i>T. koningiopsis</i> )	Class III	72.50

**Graph.1** Percent inhibition of mycelial growth of *R. solani* by rice specific native *Trichoderma* isolates



In conclusion the present study showed that the native *Trichoderma* isolates reduced the growth of rice sheath causal organism *R. solani* significantly by suppressing its mycelial growth. These findings showed that rice specific native isolates of *Trichoderma* can be used as bio control agent for management of *R. solani*, however, the study is *in vivo*, solely conducted under laboratory conditions. The degree of antagonism varied between and within species of *Trichoderma* against the pathogens. Hence, further investigation of these biocontrol agents with proper field studies can lead to incorporation of such native biocontrol agents in the integrated disease management of many soil borne plant pathogens for sustainable crop production.

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