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In vitro evaluation of antagonistic potential of native *Trichoderma spp*. against *Rhizoctonia solani* causing sheath blight of rice in Manipur

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Abstract
The antagonistic potentialities of six species of native Trichoderma viz., *T. asperellum* (KU933475), *T. koningiopsis* (KU904460), *Hypocrea lixii* (KX0113223), *T. harzianum* (KU904458), *T. ovalisporum* (KU904456) and *T. atroviradae* (KU933472) were evaluated *in vitro* against *Rhizoctonia solani* causing sheath blight of rice. The current study of dual culture assay, revealed the percentages of mycelial growth inhibition of *R. solani* by *T. asperellum* (KU933475), *T. koningiopsis* (KU904460), *Hypocrea lixii* (KX0113223), *T. harzianum* (KU904458), *T. ovalisporum* (KU904456) and *T. atroviradae (*KU933472*)* were 56.44%, 68.44%, 75.5%, 66.6%, 63.1%, and 54.2% respectively. All the species considerably inhibited the growth of *Rhizoctonia solani* pathogen. The outcomes direct that the extent of inhibition by all the six species of *Trichoderma* provides use of excellent potential antagonists capable of reducing the growth of *R. solani* on sheath blight of rice.

Keywords: Sheath blight, Rice, *Rhizoctonia solani, Trichoderma spp*. dual culture, antagonism

Introduction
Rice (*Oryza sativa*) is the India's pre-eminent cereal crop. Most of the world's human population consumes rice as staple food, especially Asia. Moreover, India is one of the leading producers of the crop. On 27 February 2018, the Government of India stated the production of rice during the 2017 season, pegging total Indian output at an all-time high of 166.5 million tonnes (111.0 million tonnes, milled basis). This level would stand 1.2 percent above the final estimate for the 2016 season (FAO, 2018) [5]. In addition to this India is forecast to rise by an additional 1.8 percent in 2018, to reach an all-time high of 169.5 million tonnes (113.0 million tonnes, milled basis). Being most widely cultivated crop around the country and world, many fungal pathogens show their aggressiveness in attempt to inflict various diseases to the crop. Among them Sheath Blight, a soil borne disease caused by *Rhizoctonia solani* (Kuhn) stands at peak to incur most economically significant losses. Management of sheath blight disease is difficult because the pathogen produces sclerotia which remain viable for long time. Synthetic chemicals used for the management practice show drastic effects i.e., development of resistance in pathogen, residual toxicity, environmental pollution, high cost and results in more carcinogenic risk than other pesticides which might give rise to avert biological effects on humans and animals etc., (Brent and Hollomon, 1998; Schillberg *et.al*., 2001) [2, 10].
Organic farming and bio pesticides etc., are gaining more importance due to inherent hazardous ramifications involved by the use of chemicals in management. The suppression of growth and infection/ reproduction of one organism by other organism are Biological control (Cook, 1993) [3]. Biological control is pioneer at engaging of exotic or existing species in the eco system as natural enemies of pest and pathogen to manage their population and their ill effects. By following such sustainable methods to manage or control the disease and disease causing organisms in absence of resistant/ tolerant varieties results in eco friendly environment.
Biological agent: The anamorphic fungal genus *Trichoderma spp*. contains cosmopolitan soil-inhabiting fungi that are a major component of the mycoflora in soils of various ecosystems (Harman *et al*., 2004) [7]. *Trichoderma* has various inhibiting mechanisms (hyper parasitism, enzymes (Lorito *et al*., 1993) [9] competition and induced systemic resistance (De Mayer *et al*., 1998) [4]. *Trichoderma* spp. also has characteristic feature of produces various volatile and

non-volatile antibiotics which have antagonistic nature of other mycoflora. Different species of *Trichoderma* have different scales in production of chemicals and their mechanism of action against the pathogens. Therefore native *Trichoderma* of species were evaluated for their antagonistic potentialities against *Rhizoctonia solani* causing sheath blight of rice.

Materials and Methodologies

Isolation of fungus

The plant showing typical symptoms of sheath blight were collected and were examined under microscope. Later the infected samples were lacerated to small pieces (about 0.5 to 1.0 cm) and were rinsed under running tap water twice. Surface sterilization was done by dipping the pieces in 1% Sodium hypo chloride solution and through a series of sterile distilled water at 3 intervals one minute each respectively. The pieces were dried using a blotting paper. Finally the pieces were then aseptically placed on sterilized potato dextrose agar (PDA) petridishes using sterile forceps. The inoculated petridishes were then incubated in BOD incubator at 25± 1° C for two days and observed for growth of the fungus. Purified cultures of the fungus ware obtained from hyphal tip culture methods. Identified was done according to the key of Kubick and Harman (1998) [8].

In vitro Evaluation of Antagonistic Potential of bio control agents against growth *Rhizoctonia solani*

Antagonistic potentials of *Trichoderma* spp. against *Rhizoctonia solani* were studied by performing dual culture technique given by Bell (1982) [1]. The dual culture technique was performed on PDA by placing mycelial disc of 5mm diameter of *R. solani* at one end of the petridish using sterile cork borer and sterile needle and mycelial disc of 5mm diameter of *Trichoderma* spp. was placed quite opposite i.e., 180° at the other end of the same petridish. A petridish without antagonist served as control. The plates were then incubated in BOD incubator at 25± 1 °C. The extent of antagonistic activity by *Trichoderma* spp. i.e., growth after contact with *R. solani* was recorded after incubation period by measuring growth of fungal plant pathogen in dual culture plate and in control plate. Each treatment was replicated thrice. The list of Bio control agents used is listed in (Table.1). The bio control agents (different native *Trichoderma* spp.) used in this study was collected from department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal. The per cent inhibition of mycelial growth of test fungus (*R. solani*) over control was calculated by using the formula suggested by Vincent (1927) [12].

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

Where
I = Percent inhibition,
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 Discussions

The study demonstrated the differential ability of six native *Trichoderma* spp. which was studied by dual culture technique against *R. solani* causing sheath blight of rice is tabulated and percent inhibition were tabulated and recorded in Table.2, Plate.1, and Graph.1. Among six *Trichoderma* spp. used *Hypocrea lixii (KX0113223)* resulted in best mycelial growth inhibition by (75.5%). However all the species showed a considerable mycelial growth inhibition i.e., *T. koningiopsis (*KU904460*)* by *(*68.44%), *T. harzianum (*KU904458) by (66.66%), *T. ovalisporum (*KU904456*)* by (63.1%), *T. asperellum (*KU933475*)* by *(*56.44%) and *T. atroviradae (*KU933472*)* by *(*54.22%) respectively. The highest percent of inhibition 75.5% was shown by *Hypocrea lixii (KX0113223)* and the least percent inhibition of 54.22% was shown b*y T. atroviradae (*KU933472). Similar findings were recorded by (M. Seema and N.S. Devaki, 2012) [11]. *Trichoderma* spp. produces substantial and diversified secondary metabolites like Pyrones, Koninginins, Viridins, Nitrogen Heterocyclic Compounds, Azaphilones, Butenolides and Hydroxy-Lactones, Isocyano metabolites, Diketopiperazines, Peptaibols, etc., (Francesco Vinale *et. al*., 2014) [6].. These heterogenic secondary metabolites yielded by *Trichoderma* triggers the activities like myco parasitism, competition for nutrition (carbon, nitrogen and also free space) and rapid colonization. All these distinguished features of *Trichoderma* accomplish it as a bio control agent against *R. solani*.
The Bell's scale classified the antagonism nature of *Hypocrea lixii (KX0113223)* to class II where the antagonist over grew at least two thirds of the pathogen surface. And the rest other antagonists *T. koningiopsis (*KU904460*)*, *T. harzianum (*KU904458)*, T. ovalisporum (*KU904456*)*, *T. asperellum (*KU933475, and *T. atroviradae (*KU933472 to Class III where the antagonist colonized on half of the growth of the pathogen.

Table 1: List of bio control agents used

Sl. No.	Isolate code	Bio control agent	Accession number
1	CAUNCIPM-7	*T. asperellum*	KU933475
2	CAUNCIPM-18	*T. koningiopsis*	KU904460
3	CAUNCIPM-48	*Hypocrea lixii*	KX0113223
4	CAUNCIPM-78	*T. harzianum*	KU904458
5	CAUNCIPM-96	*T. ovalisporum*	KU904456
6	CAUNCIPM-118	*T. atroviradae*	KU933472

Table 2: *In vitro* evaluation of bio control agents against growth of *R. solani*

Sl. No.	Bio control agent	Bell's scale	Inhibition (%)*
1	*T. asperellum (*KU933475*)*	Class III	56.44 (3.23)**
2	*T .koningiopsis (*KU904460*)*	Class III	68.44 (2.35)
3	*Hypocrea lixii (*KX0113223*)*	Class II	75.56(1.83)
4	*T. harzianum (*KU904458*)*	Class III	66.66 (2.5)
5	*T. ovalisporum (*KU904456*)*	Class III	63.1 (2.76)
6	*T. atroviradae (*KU933472*)*	Class III	54.16 (3.43)
SE (d)			1.451
$CD_{(P=0.05)}$			3.049

*Mean of three replications
**figures in parent thesis in cm

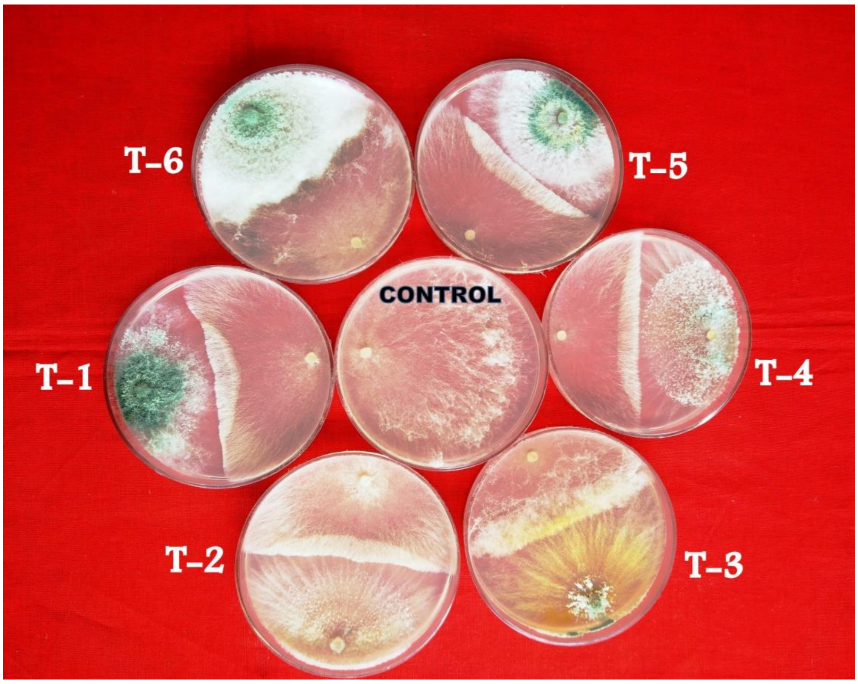
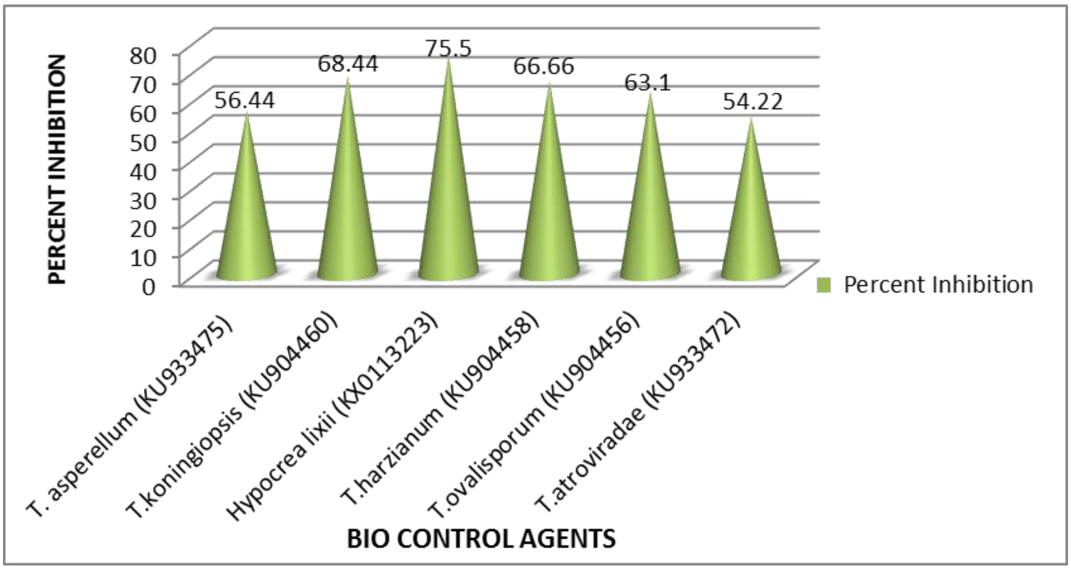


Plate 1: *In vitro* evaluation of bio control agents against growth of *R. solani*

C. Control,
1. *T. asperellum* (KU933475), 2. *T. koningiopsis* (KU904460),
3. *Hypocrea lixii* (KX0113223), 4. *T. harzianum* (KU904458),
5. *T. ovalisporum* (KU904456) 6. *T. atroviradae (*KU933472*)*



Graph 1: Percent inhibition of mycelial growth of *R. solani* by *Trichoderma spp.*

Conclusion

It is evident that all the *Trichoderma* spp. used in this investigation exhibited antagonism in suppressing the mycelial growth of *R. solani*. These findings showed that for management of *R. solani*, *Trichoderma spp.* can be used as bio control agent. Hence, further investigation with these potential bioagents and their bioactive compounds effective against R. solani can be exploited for future plant disease management to control sheath blight of rice with proper field studies.

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