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In-vitro evaluation of *Trichoderma* spp. for the management of *Rhizoctonia oryzae-sativae* causing aggregate sheath spot of rice in Manipur

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Abstract
Present research was carried out to understand antagonistic potentiality of six native *Trichoderma* spp. against *Rhizoctonia oryzae-sativae* causing Aggregate sheath spot of rice. Dual culture technique revealed the inhibition percentages of *R. oryzae-sativae* by *T. asperellum* (KU933475), *T. koningiopsis* (KU904460), *Hypocrea lixii* (KX0113223), *T. harzianum* (KU904458), *T. ovalisporum* (KU904456) and *T. atroviridae* (KU933472) of 58.43%, 68.23%, 60.0%, 72.94%, 64.31%, and 70.19% respectively. Bell's scale study showed class III category by *Trichoderma* spp and class II showed by *T. harzianum* (KU904458) against *Rhizoctonia oryzae-sativae.* Among the different biological agents *Trichoderma harzianum* is the most effective one for reducing rapid growth of the pathogen. Further, all *Trichoderma* isolates significantly inhibited the mycelial growth of the pathogen.

Keywords: *rhizoctonia oryzae-sativae,* dual culture, *trichoderma* spp

Introduction
Rice (*Oryza sativa* L.) is the most prominent 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) [13]. Rice is the principal food crop in 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) [8]. It suffers from many fungal and bacterial diseases resulting in heavy losses of grain yield. *Rhizoctonia oryzae-sativae* (Sawada) Mordue causes aggregate sheath spot in rice. *R.oryzae-sativae* was first described in Taiwan as *Sclerotium oryzae-sativae* (Sawada, K., 1922) [19]. Since then, the disease has been reported in Iran (Rahimian, 1989) [16] Chile (Ricardo-Madariaga *et al*. 1999) [17] and recently in Australia (Lanoiselet *et al*. 2001) [12]. In the USA, the disease was reported in California (Gunnell and Webster, 1984) [7]. The teleomorph of *R. oryzae-sativae* was described as *Ceratobasidium oryzae-sativae* (Gunnell and Webster, 1987) [6]. Aggregate sheath spot lesions first appear on the lower leaf sheaths at the water line during the tillering stage. Lesions are circular to elliptical with grey-green to straw-coloured centers surrounded by distinct brown margins. Frequently, additional margins from around the initial lesion, producing a series of concentric bands. A strip of light-coloured necrotic cells runs down the lesion centre. Later in the season, secondary infections frequently occur well above the water line.

Trichoderma is a fungal genus that is found in the soil. 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. 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) [15] antibiosis, and competition for nutrients with the pathogen (Harman and Kubicek, 1998) [9]. The present research work carried out to understand the effect of *Trichoderma* spp. On the growth of *Rhizoctonia oryzae-sativae* in *in-vitro*.

Materials and Methodologies

Isolation of fungus

The infected rice plant showing typical symptoms of aggregate sheath spot were collected and were 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 oryzae-sativae* (MH255604 & MH255605). All plates were kept at 25 ± 2°C for 3-4 days and from these plates pure cultures of *R. oryzae-sativae* 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 oryzae-sativae*

Dual culture method: *In vitro* antagonistic activity of *Trichoderma* spp. against *Rhizoctonia oryzae-sativae* was studied in dual culture technique by following the method by Kucuk and Kivanc (2003) [11]. Petridishes containing sterile PDA were inoculated with a 5mm diameter plug of 4- day-old pure culture of antagonistic fungi and pathogens. One mycelial disc of each fungus was placed on opposite poles of PDA plates using sterile cork borer and sterile needle and incubated at 25°C in incubator and radial growth of pathogen was measured at 24hr intervals. A petridish without antagonist served as control. Each treatment was replicated thrice. The bio control agents 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 over control was calculated by using the formula suggested by Dennis and Webster (1971) [2].

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

Where,

I = Per cent 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.

The list of Bio-control agents used is listed with Isolate code and 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. atroviridae)* - KU933472

Results and Discussions

Differential biocontrol ability among the antagonists was noticed against *Rhizoctonia oryzae-sativae* (Fig.1) and percent inhibition also calculated (Fig.2). Results showed that among the six potential antagonists, *T. atroviride* and *T. harzianum* proved to be the most potent bioagents against *R. oryzae-sativae.* 6 days of incubation showed different degrees of mycelial growth inhibition of *R. oryzae-sativae* (Fig.3). *T. harzianum* was most potent in reducing growth of the pathogen than *T. atroviride* (Saikia *et al*., 1995) [18]. However all the species showed a considerable mycelial growth inhibition i.e., *T. harzianum* by (75.68%), *T. atroviradae* by *(*70.19%), *T. koningiopsis* by *(*68.23%), *T. ovalisporum* by (64.31%), *Hypocrea lixii by (60.0%), T. asperellum* by *(*58.43%) and respectively. The highest percent of inhibition 75.68% was shown by *T. harzianum* and the least percent inhibition of 58.43% was shown by *T. asperellum*. According to Papavizas and Lumsden (1982) [14]; (Devaki *et al*. 1992) [4], the mechanisms involved in the control of pathogens by *Trichoderma* spp. are probably due to antibiosis, lysis, competition and mycoparasitism. The mode of mycoparasitism was observed to be entirely different between *T. viride* and *R. solani* (Pandey *et al*., 2005) [10]. *T. harzianum* hyphae coiled around the pathogen. Later on pegging started and knob like haustoria formed inside the hyphae of *R. solani*. The cytoplasmic contents may be taken through the haustoria and only cell wall was clearly visible without cytoplasm. Baker and Cook (1979) [1] have reported that the enzymes may be produced that digest the mycelial walls and septal walls or antibiotics may be formed that inhibit growth or cause endolysis. Dennis and Webster (1971) [3] 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 β-glucans, thereby destroying cell wall integrity (Elad, 2000; Devaki *et al*., 1992) [4, 5]. These may also play a major role in mycoparasitism because of changes in cell wall integrity.

The Bell's scale classified the antagonism nature of *T. harzianum* to class II where the antagonist over grew at least two thirds of the pathogen surface. And the rest other antagonists *T. atroviridae, T. koningiopsis, T. ovalisporum, Hypocrea lixii and T. asperellum* are classified into Class III because the antagonist colonized on half of the growth of the pathogen.

Conclusion

The present investigation showed that tested *Trichoderma* isolates reduced the growth of *R. oryzae-sativae* pathogen significantly. However, this study was conducted in laboratory conditions and it will be effective for future plant disease management to control aggregate sheath spot of rice with proper field studies. The degree of antagonism varied between and within species of *Trichoderma* against the pathogen. Biocontrol agents can be incorporated for integrated disease management of many soil borne plant pathogens for sustainable crop production.

Table 1: Evaluation of *Trichoderma* isolates against *R. oryzae-sativae* using dual culture

Sl. No.	Bio control agent	Bell's scale	Inhibition (%)*
1.	*T. harzianum* (KU904458)	Class II	75.68
2.	*T. atroviridae* (KU933472)	Class III	70.19
3.	*T. koningiopsis* (KU904460)	Class III	68.23
4.	*T. ovalisporum* (KU904456)	Class III	64.31
5.	*Hypocrea lixii* (KX0113223)	Class III	60.0
6.	*T. asperellum* (KU933475)	Class III	58.43
SE (d)			1.32
$CD_{(P=0.05)}$			2.97

*Mean of three replications

Fig 1: Dual culture technique of bio control agents against the growth of *R. oryzae-sativae*

1. *Hypocrea lixii* 2. *T. asperellum* 3. *T.koningiopsis*
4. *T.ovalisporum* 5. *T.harzianum* 6. *T.atroviridae*

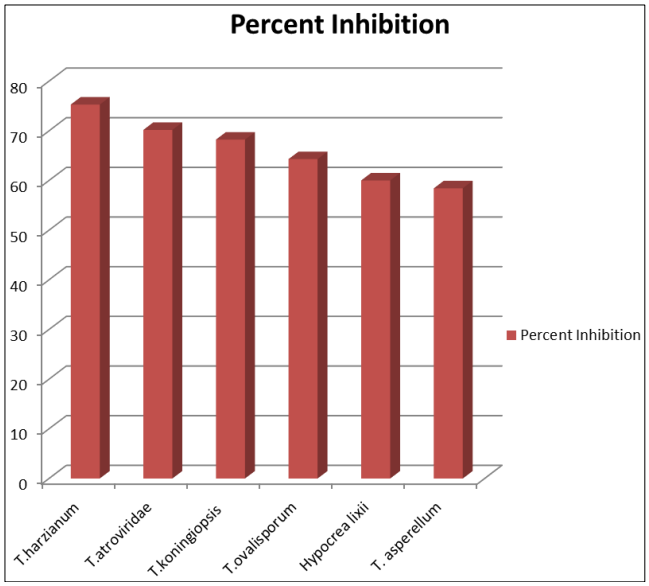

Fig 2: Percent inhibition of mycelial growth in culture plate

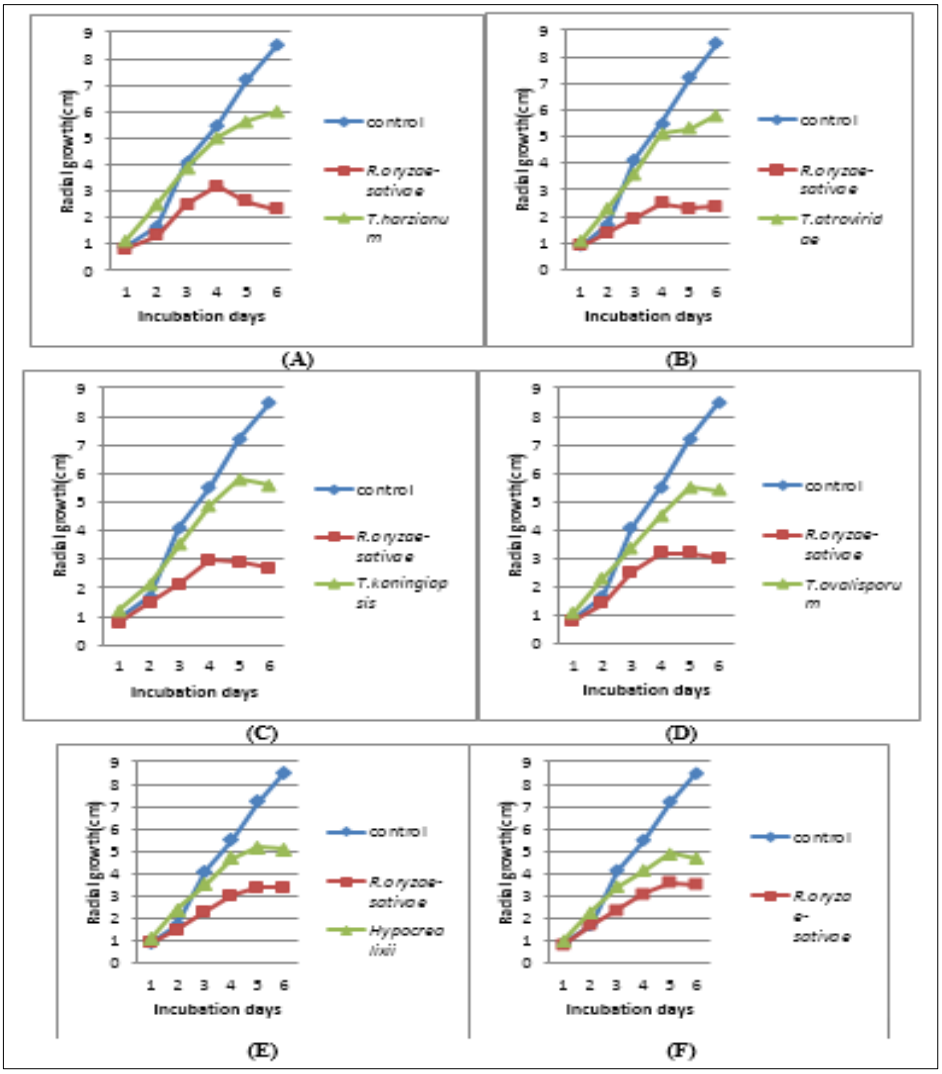

Fig 3: Growth pattern of *R. oryzae-sativae* and the biological control agents in terms of radius of the colony

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