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Linda Lalduhzuali
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Bireswar Sinha
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Sobita Devi
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

V Bhuvaneswari
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Tokmem Siram
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

K Dinesh
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Correspondence
Linda Lalduhzuali
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

In vitro evaluation of antagonistic potential of native *Trichoderma* spp. Against *Lasiodiplodia theobromae* causing Tree bean decline in Manipur

Linda Lalduhzuali, Bireswar Sinha, Sobita Devi, V Bhuvaneswari, Tokmem Siram and K Dinesh

Abstract
The antagonistic potentialities of nine species of native Trichoderma viz., *T. asperellum* (KU933475), *T. koningiopsis* (KU904460), *Hypocrea lixii* (KX0113223), *T. harzianum* (KU904458), *T. ovalisporum* (KU904456), *T. atroviradae* (KU933472), were evaluated *in vitro* against *Lasiodiplodia theobromae* causing tree bean decline in Manipur. The current study of dual culture assay, revealed the percentages of mycelial growth inhibition of *L. theobromae* by *T. asperellum* (KU933475), *T. koningiopsis* (KU904460), *Hypocrea lixii* (KX0113223), *T. harzianum* (KU904458), *T. ovalisporum* (KU904456), *T. atroviradae (*KU933472*)*,T69 *T. harzianum* (KU933468), *T. asperellum* (KU933476), *T. harzianum* (KU933471) were 55.29%, 61.57%, 56.86%, 72.94%, 62.75%, 60%, 57.25%, 51.76% and 61.18% respectively. All the species considerably inhibited the growth of *Lasiodiplodia theobromae* pathogen. The outcomes direct that the extent of inhibition by all the nine species of *Trichoderma* provides use of excellent potential antagonists capable of reducing the growth of *Lasiodiplodia theobromae* on tree bean decline.

Keywords: Treebean decline, *Parkia roxburghii*, *Lasiodiplodia theobromae*, *Trichoderma spp*. dual culture, antagonism

Introduction
Tree bean (*Parkia roxburghii* G. Don Syn. *P. timoriana* (DC.) Merr.) of leguminosae family (Subfamily momosoideae) is an important tree vegetable of South East Asia, especially North Eastern India. It is distributed in India, Bangladesh, Myanmar, Java, Thailand, Egypt and the Malaysian region. In North East India, tree bean is found in Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura. It is also known as Supota, Kharial (Hindi); Manipur-urohi, Khorial (Assamese); Manipuri seem (Bengali); Zawngtah (Mizo); Yongchak (Manipuri);Aoelgap (Garo); Bire-phang (Kachari); Themuk-arang (Mikir); Unkamn-pinching (Naga); Shivalingadamara (Kannada) and Unkampinching (Marathi). There are no known varieties or cultivar of tree bean available in India. The trees are usually grown under semi-wild condition or in homestead backyard gardens. The forest area provides a number of tree bean plants whose pod make an important contribution to the diet of local people. Due to lack of organized plantation, there is a demand-supply imbalance of tree been in North Eastern States, especially in Manipur and hence, a considerable volume of tree been is imported from Myanmar.

Tree bean is a multipurpose tree species having a variety of uses, *viz.*, antibacterial (Zuhud *et al.* 2001) [13], alleopathy (Magani *et al*. 2009) [8], human food (Longvah and Deosthale 1998) [7], tanning, face wash and shampoo, firewood and paper pulp (RFRI 2015). The bark is reported to be suitable for tanning; it is used for dyeing nets in Philippines. The timber is also used as firewood. The tree bean being a fast growing leguminous species is suitable for reclamation of abandoned jhum land. Being a legume it also helps to enrich the soil through nitrogen fixation. Despite a variety of uses, unfortunately the tree bean plants are under threat and there is vast decline in their population in some parts of NE India, especially in Manipur.

Trichoderma, a filamentous soil borne saprophytic fungus is known to be one of the best candidate of biological agents for the management of soil borne plant pathogens. Mode of action of this fungus include mycoparasitism, antibiosis, competition for nutrient and space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients and induced resistance. The antagonistic action of

Trichoderma species against phytopathogenic fungi might be due to either by secretion of extra cellular hydrolytic enzyme (Di Pietro *et al.* 1993; Schirmbock *et al.* 1994) [5, 10] or by the production of antibiotics (Dennis and Webster, 1971a, b; Claydon *et al.* 1987) [3-4, 2]. The growing interest in nonchemical methods of pest and disease management are solely due to environmental and health hazards. The use of *Trichoderma* based products is not only safe for the farmers and consumers but it is also good for the environment.

In Manipur, tree bean decline is common but so far not much previous research have been done in relation to pathogen regarding the tree bean decline pathogen and its proper management in this region. Therefore native Trichoderma species were evaluated for their antagonistic potentialities against *Lasiodiplodia theobromae* causing tree been decline in Manipur.

Materials and Methodologies

Isolation of fungus

Samples collected from symptomatic plants were washed under tap water, dried and briefly flamed. Small pieces of wood were taken from the interface between healthy and diseased plant tissue, submerged in 1 % sodium hypochlorite for 2 min, and washed twice in sterile distilled water. Wood pieces were plated on potato dextrose agar medium and incubated at 28±2ºC for two days and observed for growth of the fungus.

In vitro Evaluation of Antagonistic Potential of *Trichoderma* spp agents against growth of *Lasiodiplodia theobromae*

Antagonistic potentials of *Trichoderma* spp. against *Lasiodiplodia theobromae* 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 *Lasiodiplodia theobromae* 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 *Lasiodiplodia theobromae* 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 (*Lasiodiplodia theobromae*) over control was calculated by using the formula suggested by Vincent (1927) [12]

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

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.

Results and Discussions

The study demonstrated the differential ability of nine native *Trichoderma* spp. which was studied by dual culture technique against *Lasiodiplodia theobromae* causing tree bean decline is tabulated and percent inhibition were tabulated and recorded in Table.2 and Plate.1. Among nine *Trichoderma* spp. used *T. harzianum* (KU904458) resulted in best mycelial growth inhibition by (72.94%). However all the species showed a considerable mycelial growth inhibition i.e. *T. ovalisporum* (KU904456) by (62.75%), *T. koningiopsis* (KU904460) by (61.57%), *T. harzianum* (KU933471) by (61.10%), *T. atroviradae* (KU933472) by *(*60%), *T. harzianum* (KU933468) by *(*57.25%), *Hypocrea lixii* (KX0113223) by (56.86), *T. asperellum* (KU933475) by (55.29%), and *T. asperellum* (KU933476) by (51.76%) respectively. The highest percent of inhibition 72.94% was shown by *T. harzianum* (KU904458) and the least percent inhibition of 51.76% was shown b*y T. asperellum* (KU933476). 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). 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 *Lasiodiplodia theobromae*.

The Bell's scale classified the antagonism nature of *Hypocrea lixii* (KX0113223*), T. koningiopsis* (KU904460*), T. harzianum (*KU904458), *T. ovalisporum* (KU904456), *T. asperellum* (KU933475), *T. atroviradae* (KU933472), *T. harzianum* (KU933471), *T. harzianum* (KU933468) and *T. asperellum* (KU933476) to Class III where the antagonist colonized on half of the growth of the pathogen.

Table 1: List of *Trichoderma* spp. evaluated against *L. theobromae*

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
7	CAUNCIPM-69	*T. harzianum*	KU933468
8	CAUNCIPM-109	*T. harzianum*	KU933471
9	CAUNCIPM-123	*T. asperellum*	KU933476

Table 2: *In vitro* evaluation of *Trichoderma* spp. against growth of *L. theobromae*

Sl. No.	Bio control agent	Bell's scale	Inhibition (%)*
1	*Hypocrea lixii* (KX0113223)	Class III	56.86
2	*T. harzianum* (KU933468)	Class III	57.25
3	*T. asperellum* (KU933475)	Class III	55.29
4	*T. koningiopsis* (KU904460)	Class III	61.57
5	*T. harzianum* (KU933471)	Class III	61.18
6	*T. ovalisporum* (KU904456)	Class III	62.75
7	*T. atroviradae* (KU933472)	Class III	60
8	*T. harzianum* (KU904458)	Class III	72.94
9	*T. asperellum* (KU933476)	Class III	51.76
SE (d)			3.24
$CD_{(P=0.05)}$			6.81

*Mean of three replications

C. Control, 1. *Hypocrea lixii* (KX0113223)), 2. *T. harzianum* (KU933468) 3. *T. asperellum* (KU933475), 4. *T. koningiopsis* (KU904460) 5. *T. harzianum* (KU933471) 6. *T. ovalisporum* (KU904456)) 7. *T. atroviradae* (KU933472) 8. *T. harzianum* (KU904458) 9. *T. asperellum* (KU933476)

Plate 1: *In vitro* evaluation of bio control agents against growth of *L. theobromae*

Conclusion

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

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