

***In-vitro* Sensitivity Test of native *Trichoderma* spp. against growth of *Rhizoctonia solani* f.sp. *sasakii* causing Banded Leaf and Sheath Blight of Maize in Manipur**

ABSTRACT

The antagonistic potential of seven species of native *Trichoderma* against *R. solani* were evaluated in dual culture method which includes *T. asperellum*, *T. koningiopsis*, *Hypocrea lixii*, *T. harzianum* (*mention accession number*), *T. ovalisporum*, *T. harzianum* (*mention accession number*), *T. atroviridae*. Among them *T. asperellum* showed highest inhibition percentage (78.09%), followed by *Hypocrea lixii* by (70.95%), *T. ovalisporum* by (68.76%), *T. atroviridae* by (54.22%), *T. harzianum* by (64.76%), *T. koningiopsis* by (64.76%) and *T. harzianum* by (62.38%) respectively. However, all the species considerably inhibited the growth of *R. solani*.

Key words: Banded Leaf and Sheath Blight, *R. solani*, *Trichoderma*, Dual culture

INTRODUCTION

Maize (*Zea mays* L.; 2n=20) is a Mexican and Central American crop belongs to family Poaceae is major staple food crop grown worldwide. It was introduced to India in the beginning of 17th century. Globally, it is known as "Queen of Cereals". It is now one of the important crops in India occupying fifth place in area and third place in production. Worldwide production of maize in 2020-2021 is about 1,125 million metric tons (Statista, 2021). The maize crop is attacked by a variety of fungal, bacterial, and viral diseases, with banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* f.sp. *sasakii* (*Thanatephorus cucumeris*) being one of the most serious and leading causes of low yield. *R. solani* has a unique combination of competitive saprophytic abilities and high pathogenic potential, making it a tenacious and deadly plant pathogen. As a result, effective control techniques for banded leaf and sheath blight are critical to minimising crop destruction and avoiding economically significant crop losses (Singh and Shahi, 2012).

Various fungicides are advised for the treatment of this disease. However, employing these expensive chemicals to control disease is not a good idea. Despite their high cost, many chemicals have negative consequences for our ecosystem. As a result, cost-effective and environmentally acceptable solutions for controlling plant diseases are required. Although developing resistant varieties is ideal, there is a scarcity of sources of resistance to this *virusefungus* (Sharma *et al.*, 2002). Biological control is one such effective technique that has gained a lot of attention, however biocontrol agents alone are not adequate to achieve satisfactory results against this infection. *Trichoderma* spp. is an anamorphic fungal genus that encompasses cosmopolitan soil-inhabiting fungi that constitute a prominent component of the mycoflora in soils across the globe. (Harman *et al.*, 2004). Most *Trichoderma* species produce volatile and non-

volatile metabolic chemicals such as tricholin, massoilactone, heptelidic acid, gliovirin, 6-penthy-pyrone, harzianic acid, glisoprenins, peptaibols, alamethicins, and others that are toxic to the target pathogen (Qualhato *et al.*, 2013). Antibiotics and hydrolytic enzymes work together to create maximum antagonism, rather than acting independently (Monte, 2001). Different species of *Trichoderma* produce compounds at distinct sizes and have different mechanisms of action against infections. As a result, the antagonistic potentialities of native *Trichoderma* species against *Rhizoctonia solani*, which causes banded leaf and sheath blight in maize, were assessed.

MATERIALS AND METHODOLOGIES

Isolation of fungus

Maize plants with banded leaf and sheath blight symptoms were harvested and examined under a microscope. The diseased samples were then lacerated into small pieces (about 0.5 to 1.0 cm) and washed twice under running tap water. Surface sterilisation was accomplished by dipping the pieces in a 1 % sodium hypochlorite solution and then rinsing them with sterile distilled water three times in one minute intervals. Using sterilized blotting paper, the pieces were dried. Finally, sterile forceps were used to arrange the fragments aseptically on to sterilised potato dextrose agar (PDA) petri dishes. The inoculated petri dishes were ~~then~~ incubated for two days in a BOD incubator at 25±1° C to check for fungal growth.

In vitro Evaluation of Antagonistic effect of *Trichoderma* spp. against growth of *Rhizoctonia solani* f.sp. *sasakii*

The anti~~baeteria~~fungal potentials of *Trichoderma* spp. against *Rhizoctonia solani* were examined by Bell *et al.*, 1982. The dual culture technique was performed on PDA using a sterile cork borer and sterile needle, with a 5mm diameter *R. solani* mycelial disc at one end of the petri plate and a 5mm diameter *Trichoderma* spp. mycelial disc at the other end of the same petri plate. A petri plate with no antagonist was utilised as a control. In a BOD incubator, the plates were then incubated at 25±1° C. The level of antagonistic action of *Trichoderma* spp., i.e., growth after contact with *R. solani* f.sp. *sasakii*, was determined by measuring fungal plant pathogen growth in a dual culture plate and a control plate after an incubation period. Each treatment was tested three times. The biocontrol agents that were utilised are listed in (Table.1). The biocontrol agents (various native *Trichoderma* spp.) employed in this investigation were obtained from the Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal. Using Vincent's (1927) formula, the percent suppression of mycelial growth of the test fungus (*R. solani*) over control was computed.

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

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 pathogen (100% overgrowth)
Class II : The antagonist overgrew at least 2/3rd of the pathogen surface (75% over growth)
Class III : The antagonist colonized on half of the growth of the pathogen surface (50% over growth)
Class IV : The pathogen and the antagonist locked at the point of contact.
Class V : The pathogen overgrew the mycoparasite.
Class VI : The pathogen and antagonist form inhibition zone

RESULTS AND DISCUSSIONS

The study tabulated the differential capacity of seven native *Trichoderma* spp. against *R.solani*, which causes banded leaf and sheath blight in maize, using a dual culture approach, and percent inhibition was ~~tabulated and documented~~ mentioned in Table.2, Plate.1, and Graph.1. Among tested *Trichoderma* spp. Highest mycelial growth inhibition was observed with *T. asperellum* (KU933476) by (78.09%). However all the species showed a considerable mycelial growth inhibition i.e., *Hypocrea lixii* (KX0113223) by (70.95%), *T. ovalisporum* (KU904456) by (68.76%), *T. atroviridae* (KU933472) by (54.22%), *T. harzianum* (KU904458) by (64.76%), *T. koningiopsis* (KU904460) by (64.76%) and *T. harzianum* (KU933471) by (62.38%) respectively. The highest percent of inhibition 78.09% was shown by *T. asperellum* (KU933476) and the least percent inhibition of 62.38% was shown by *T. harzianum* (KU933471). Similar findings were recorded by (Chakrapani *et al.*, 2020). *Trichoderma* fungi are forced to compete for nutrients and space with many other organisms as a result of their colonisation of various settings. *Trichoderma* spp. have well-developed and diversified processes that aid in the colonisation of several ecological niches. Hyperparasitism occurs when an antagonist comes into direct touch with a pathogen and includes steps such as pathogen recognition, assault, progressive pathogen cell penetration, and death (Vinale *et al.*, 2008).

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Table 1: List of bio control agents used

Sl. No.	Isolate code	Bio control agent	Accession number
1	CAUNCIPM-18	<i>T. koningiopsis</i>	KU904460
2	CAUNCIPM-48	<i>Hypocrea lixii</i>	KX0113223
3	CAUNCIPM-78	<i>T. harzianum</i>	KU904458
4	CAUNCIPM-96	<i>T. ovalisporum</i>	KU904456
5	CAUNCIPM-109	<i>T. harzianum</i>	KU933471
6	CAUNCIPM-118	<i>T. atroviradae</i>	KU933472
7	CAUNCIPM-123	<i>T. asperellum</i>	KU933476

Table 2: *In vitro* evaluation of *Trichoderma* spp. against *R. solani*

Sl. No.	Bio control agent	Bell's scale	Inhibition (%) [*]
1	<i>T. asperellum</i> (KU933476)	Class II	78.09 (1.53)**
2	<i>T. koningiopsis</i> (KU904460)	Class III	64.76 (2.46)
3	<i>Hypocrea lixii</i> (KX0113223)	Class III	70.95(2.03)
4	<i>T. harzianum</i> (KU904458)	Class III	64.76 (2.43)
5	<i>T. ovalisporum</i> (KU904456)	Class III	68.76 (2.0)
6	<i>T. harzianum</i> (KU933471)	Class III	62.38 (2.57)
7	<i>T. atroviradae</i> (KU933472)	Class III	66.19 (2.60)
SE (d)			0.7636
CD _(P=0.05)			1.3447

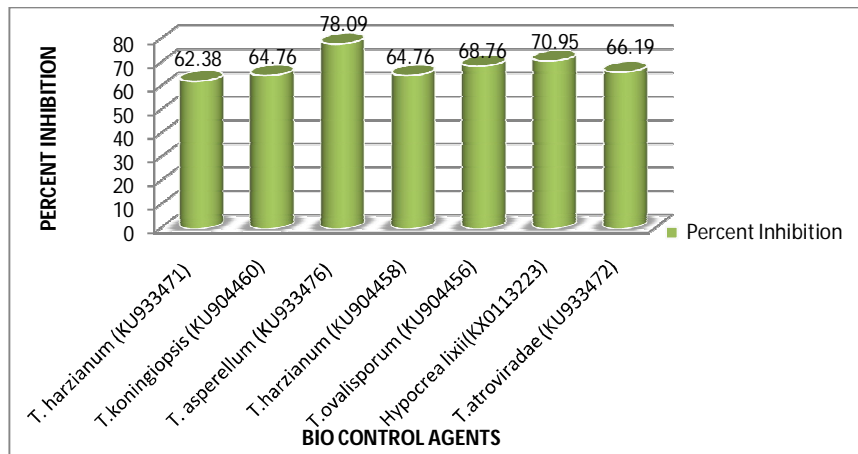
*Mean of three replications

**figures in parent thesis in cm



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

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|---------------------------------------|---------------------------------------|
| C. Control, | 1. <i>T. asperellum</i> (KU933476), |
| 2. <i>T. harzianum</i> (KU933471), | 3. <i>T. atroviridae</i> (KU933472) |
| 4. <i>T. ovalisporum</i> (KU904456), | 5. <i>T. harzianum</i> (KU904458), |
| 6. <i>Hypocrea lixii</i> (KX0113223), | 7. <i>T. koningiopsis</i> (KU904460). |



-Graph 1: Percent inhibition of mycelial growth of *R.solani* by *Trichoderma* spp. graph must be deleted as same data mentioned in the table.

CONCLUSION

All of the *Trichoderma* spp. employed in this study showed antagonism when it came to inhibiting *R. solani*'s mycelial growth. *Trichoderma* spp. can be employed as a biocontrol agent for *R. solani*, according to these findings. As a result, with adequate field investigations, further investigation using these prospective bioagents and their bioactive components effective against *R. solani* can be explored for future plant disease management to prevent banded leaf and sheath blight of maize.

References

Bell DK, Wells HD, Markham CR. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathology*. 1982;72(4):379-382.

Chakrapani, K., Sinha, B., Chanu, W.T., Chakma, T. and Siram, T. Assessing *in vitro* antifungal activity of plant extracts against *Rhizoctonia solani* causing sheath blight of rice (*Oryza sativa*. L). *Journal of Pharmacognosy and Phytochemistry*. 2020;9(1):1497-1501.

Das, B.C., Bora, L.C., Phookan, A.K and Bhagabati. Antagonistic effects of *Aspergillus terreus*, *Trichoderma viride* on sheath blight of rice. *Oryza*. 1996;33:62-65.

Harman GE, Howell CR, Viterbo A, Chet I and M. Lorito. *Trichoderma* species opportunistic, avirulent plant symbionts. *Nature Review of Microbiology*. 2004;2:43–56.

<https://www.statista.com/statistics/1156213/global-corn-production/> 08/07/21.

Monte, E. Understanding *Trichoderma*: between biotechnology and microbial ecology. *International Microbiology*. 2001;4:1-4.

Morton, D.J and Stroube, W.H. Antagonistic and stimulating effects of soil microorganisms upon *Sclerotium*. *Phytopathology*. 1955;45:415-420.

Qualhato, T.F., Lopes, F.A.C., Steindorff, A.S., Brandão, R.S., Jesuino, R.S.A., Ulhoa, C.J. Mycoparasitism studies of *Trichoderma* species against three phytopathogenic fungi: evaluation of antagonism and hydrolytic enzyme production. *Biotechnology Letters*. 2013;35:1461-1468.

Sharma, R.C., Vasal, S.K., Gonzalez, F., Batsa, B.K. and Singh, N.N. Redressal of banded leaf and sheath blight of maize through breeding, chemical and biocontrol agents. In Proceed of the 8th Asian regional maize workshop: new technologies for the New Millennium, Bangkok, 2002; pp. 391-397.

Singh, A. and Shahi, J.P. Banded leaf and sheath blight: an emerging disease of maize (*Zea mays* L). *Maydica*., 2012;57(3):215-219.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L., Lorito, M. *Trichoderma*–plant–pathogen interactions. *Soil Biology and Biochemistry*. 2008;40:1-10.

Vincent, J. M. Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, 1927;159:850.

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