***In vitro* evaluation of antagonistic potential of native *Trichoderma spp*. against *Cochliobolusheterostrophus*causing maydis leaf blight of maize in Manipur**

ABSTRACT

The antagonistic potentialities of seven species of native *Trichoderma viz.,T. atroviride*, *T. koningiopsis*, *T.ovalisporum*, *Hypocrealixii*, *T. harzianum*, *T. asperellum* and *T. harzianum*were evaluated *in vitro* against *Cochliobolusheterostrophus*causing maydis leaf blight of maize. The current study of dual culture assay, revealed the percentages of mycelial growth inhibition of *C. heterostrophus*by *Hypocrealixii*, *T. harzianum*,*T. atroviridae*, *T. koningiopsis*, *T. ovalisporum*,*T. asperellu* and *T. harzianum*were 80.4%, 83.80%, 83.80%, 82.85%, 85.23%, 83.33% and 82.38% respectively. All the species considerably inhibited the growth of *Cochliobolusheterostrophus*pathogen. The outcomes direct that the extent of inhibition by all the seven species of *Trichoderma* provides use of excellent potential antagonists capable of reducing the growth of *C. heterostrophus*the casual pathogen ofmaydis leaf blight of maize.

Comment [R(1]: Under in vitro condition

Keywords: Maydis leaf blight,*Cochliobolusheterostrophus, Trichoderma spp*. dual culture, antagonism

INTRODUCTION

Maize or corn (*Zea mays L*.) is one of the most important cereal crops in the world and is grown in more than 150 countries. The major maize producing countries are USA, China, Brazil, Mexico, France and India. Maize is the third most important food grain in India next towheat and rice and it occupies an area of 9.26 million hectares having production of 2.4 milliontones with an average productivity of 2.57 t/ha. It belongs to family Poaceae (grass family)and tribe Maydaea. Maize is affected by as many as 112 diseases and 65 pathogens which include fungi, bacteria and viruses on a global basis. The diseases results not only in yield reduction but also deteriorate the value and quality of the grain. Among the wide disease spectrum the more serious diseases of maize are leaf blight, downy mildew, stalk rot and rust. Out of these, maydis leaf blight (MLB) also known as Southern corn leaf blight (SCLB) is considered as one of the most serious foliar disease caused by fungi *Cochliobolusheterostrophus* and has attained the status of the economically important disease (Malik *et al.,* 2017).

Comment [R(2]: References?? Needed

Synthetic chemicals used in management practise~~have~~ significant side effects, such as ~~pathogen resistance~~, residual toxicity, environmental pollution, high cost, and a higher carcinogenic risk than other pesticides, which could lead to avert biological effects on humans and animals, among other things (Brent and Hollomon, 1998; Schillberg*et.al.,* 2001). Organic farming and bio insecticides, are rising in popularity as a result of the inherent associated with chemical management. Biological control is the suppression of one organism's growth and infection/reproduction by another organism (Cook, 1993). Biological control is a pioneer in using alien or already-existing organisms in the eco system as natural enemies of pests and pathogens to limit their populations and negative consequences. In the absence of resistant/tolerant cultivars, utilising such sustainable approaches to

Comment [R(3]: Causes

Comment [R(4]: Development of resistance pathogens

Comment [R(5]: Effects

manage or prevent illness and disease-causing organisms results in an environmentally friendly environment.

Trichoderma spp., an anamorphic fungal genus, contains worldwide soil-inhabiting fungi that are a prominent component of the mycoflora in diverse ecosystems' soils (Harman *et al.,* 2004). *Trichoderma* contains a number of inhibitory mechanisms, including hyperparasitism, enzymes, competition, and induced systemic resistance (Lorito*et al.,* 1993). (De Mayer *et al.,* 1998). *Trichoderma spp.* is also known for producing a variety of volatile and non-volatile antibiotics that are antagonistic to other mycoflora. 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 *Cochliobolusheterostrophus,* which causes maize maydis leaf blight, were assessed.

Comment [N6]: Clearly state the problem statement.

MATERIALS AND METHODOLOGIES

Isolation of fungus

Comment [N7]: Mention the name of the pathogen

Plants with characteristic leaf blight symptoms were collected and examined under a microscope. The diseased samples were then lacerated into small pieces (between 0.5 and 1.0 cm) and washed twice under running tap water. Surface sterilisation was accomplished by dipping the pieces in 1% sodium hypochloride solution and then in three intervals washings was done. Blotting paper was used to dry the pieces. Finally, using sterile forceps, the fragments were aseptically put on potato dextrose agar (PDA) petridishes. The inoculated petridishes were then incubated for two days at 25±1° C in a BOD incubator for growth of the fungus. Using hyphal tip culture methods, (Kubick and Harman's key, 1998) purified fungus cultures were obtained.

Comment [N8]: Maize plants

Comment [N9]: Light microscope?

Comment [N10]: With what?

In vitro Evaluation of Antagonistic Potential of native *Trichoderma species* against growth*Cochliobolusheterostrophus*

Comment [N11]: Include "the growth of "

The antimicrobial potentials of seven native*Trichoderma spp.* against *Cochliobolusheterostrophus* were evaluated using the Bell*et al.,* (1982) dual culture technique. On PDA, the dual culture technique was performed by putting a 5mm diameter *C. heterostrophus* mycelial disc at one end of the petridish using a sterile cork borer and sterile needle, and a 5mm diameter *Trichoderma spp.*mycelial disc at the other end of the petridish at an angle of 180°.Petridish inoculated with the pathogen without any antagonist were used as control. The plates were then incubated at 25±1 °C in a BOD incubator. After an incubation time, the level of antagonistic activity by *Trichoderma spp.,* i.e., growth after contact with *C. hetrostrophus*, was determined by measuring fungal plant pathogen growth in a dual culture plate and a control plate. 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 formula(1927), the percent suppression of mycelial growth of the test fungus (*C. heterostrophus*) over control was computed.

Comment [N12]: Incubation time period is not mentioned

Comment [N13]: No. of replication & experimental design is not mentioned.

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

Where I = Percent inhibition,

C = linear growth of the fungus in control

T = linear growth of the fungus in treatment.

For antagonistic potential of bio control agents against *C. heterostrophus* dual culture technique is used given by Bell*et al.,* (1982), he gave different classes of scale for the growth of pathogen and antagonist as:-

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 seven native *Trichoderma spp.* which was studied by dual culture technique against *C. heterostrophus* causing maydis leaf blight of maize is tabulated and percent inhibition were tabulated and presented in Table 2, Plate 1, and Graph 1. Among seven*Trichoderma spp.* used *Trichodermaovalisporum* (KU904456) resulted in best mycelial growth inhibition by (85.23%) and the least per cent inhibition of 80.4% was shown by Hypocrealixii (KX0113223). However all the species showed a considerable mycelial growth inhibition i.e., *T.koningiopsis* (KU904460) by (82.85%), *T.harzianum* (KU904458) by (83.80%), *T.asperellum* (KU933476) by (83.33%) and *T. harzianum* (KU933471) by (82.38%), *T. atroviride* (KU933472) by (83.80%) respectively. Pyrones, Koninginins, Viridins, Nitrogen Heterocyclic Compounds, Azaphilones, Butenolides and Hydroxy-Lactones, Isocyano metabolites, Diketopiperazines, Peptaibols, and other secondary metabolites are produced by *Trichoderma spp* (Francesco Vinale et. al., 2014). Trichoderma produces heterogenic secondary metabolites, which cause myco parasitism, competition for nourishment (carbon, nitrogen, and also free space), and rapid colonisation. Trichoderma's unique characteristics enable it to act as a biocontrol agent against *C. heterostrophus.*

The Bell's scale classified the antagonism nature of *Trichoderma harzianum* (KU904458), *T. asperellum* (KU933476), *T.atroviride* (KU933472), *T. harzianum* (KU933471), *T.koningiopsis*

(KU904460), *T.ovalisporum* (KU904456) and*Hypocrealixii* (KX0113223)to class II where the antagonist over grew at least two thirds of the pathogen surface.

Table 1: List of bio control agents used

SI. No.	Isolate code	Bio control agent	Accession number
1.	NCIPMCAU-78	*T. harzianum*	KU904458
2.	NCIPMCAU-123	*T. asperellum*	KU933476
3.	NCIPMCAU-118	*T. atroviride*	KU933472
4.	NCIPMCAU-109	*T. harzianum*	KU933471
5.	NCIPMCAU-18	*T. koningiopsis*	KU904460
6.	NCIPMCAU-96	*T. ovalisporum*	KU904456
7.	NCIPMCAU-48	*Hypocrealixii*	KX0113223

Table 2*: In vitro* evaluation of bio control agents against growth of *C. heterostrophus*

SI. No.	Bio control agent	Bell's scale	Inhibition(%)*
1.	*T. harzianum* (KU904458)	Class II	83.80 (1.1) **
2.	*T. ovalisporum* (KU904456)	Class II	85.23 (1.0)
3.	*T. koningiopsis* (KU904460)	Class II	82.85 (1.2)
4.	*T. asperellum* (KU933476)	Class II	83.33 (1.2)
5.	*T. harzianum* (KU933471)	Class II	82.38 (1.3)
6.	*T. atroviride* (KU933472)	Class II	83.80 (1.1)
7.	*Hypocrealixii*(KX0113223)	Class II	80.4 (1.4)
	SE (d)		0.763
	CD $_{(P=0.05)}$		1.344

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

Comment [N14]: According to your study all the Trichoderma spp are in Class II. But It depends on the number of days of incubation. How do you clarify this?

Comment [N15]: Mention clearly when did you calculate inhibition %

Comment [N16]: ????????

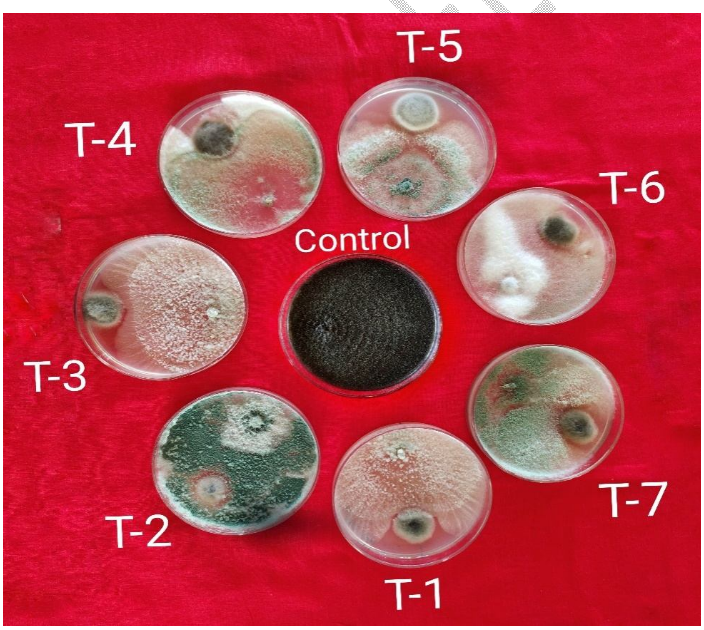


Plate 1: *In vitro* evaluation of bio control agents against growth of *C. heterostrophus*

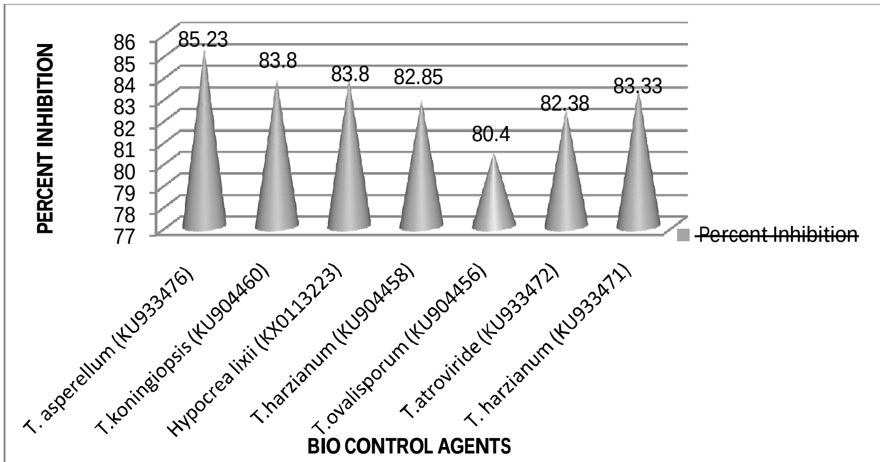
C. Control,

T- 1. *T. harzianum*(KU904458)   T- 2. *T. ovalisporum*(KU904456)

T- 3.*T. koningiopsis*(KU904460)   T- 4. *T. asperellum*(KU933476)

T- 5. *T. harzianum*(KU933471)   T-6. *T. atroviride*(KU933472)

T- 7. *Hypocrealixii* (KX0113223)



Graph 1: Per cent inhibition of mycelia growth of *C. heterostrophus* by *Trichoderma spp.*

CONCLUSION

Trichoderma spp. employed in this study had antagonistic effects on *C. heterostrophus* mycelial growth. *Trichoderma spp.* can be employed as a biocontrol agent for *C. heterostrophus*. As a result, with adequate field investigations, more research into these possible bioagents and their bioactive chemicals efficient against *C. heterostrophus*and can be used for future plant disease management to prevent maydis leaf blight of maize.

Comment [N17]: What is the type of antagonism you found according to your study

Comment [N18]: Conclusion should be based on your findings

References

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

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

3. Malik, V.K., GOGOI, R., Hooda, K.S. and Singh, M. Identification of multiple disease resistant maize accessions. *Indian Phytopathology.* 2017;70(1): 80-85.

4. Brent KJ, HollomonDW.Fungicide Resistance: the Assessment of Risk. FRAC, *Global Crop Protection Federation*, Brussels, 1998;48.

5. Cook RJ, 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual review of Phytopathology*. 31(1):53-80.

6. De Meyer G, Bigirimana J, Elad Y, Höfte M. Induced systemic resistance in *Trichoderma* harzianum T39 bio control of *Botrytis cinerea*. *European Journal of Plant Pathology*. 1998;104:279-286.

7. Francesco Vinale, KrishnapillaiSivasithamparam, Emilio L. Ghisalberti, Sheridan L Woo, Marco Nigro *et al*. *Trichoderma* Secondary Metabolites Active on Plants and Fungal Pathogens. *The Open Mycology Journal*. 2014;8(1, M5):127-139.

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

9. Kubicek CP, Harman GE.*Trichoderma* and Gliocladium. Taylor & Francis Ltd, 1 Gunpowder Square, London, EC4A 3DE 1998;3-30.

10. Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL *et al*. Chitinolytic enzymes produced by *Trichoderma harzianum*: antifungal activity of purified endochitinase and chitobiosidase. *Phytopathology.*1993;83:302-307.
11. Schillberg S, Zimmermann S, Zhang MY, Fischer R.*Antibody-Based Resistance to Plant Pathogens.* Trans. Res.2001;10:1-12.