

## ***In vitro* evaluation of antagonistic potential of native *Trichoderma* spp. against *Cochliobolus heterostrophus* 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*, *Hypocrea lixii*, *T. harzianum*, *T. asperellum* and *T. harzianum* were evaluated *in vitro* against *Cochliobolus heterostrophus* causing maydis leaf blight of maize. The current study of dual culture assay, revealed the percentages of mycelial growth inhibition of *C. heterostrophus* by *Hypocrea lixii*, *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 *Cochliobolus heterostrophus* 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 of maydis leaf blight of maize.

**Keywords:** Maydis leaf blight, *Cochliobolus heterostrophus*, *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 to wheat and rice and it occupies an area of 9.26 million hectares having production of 2.4 million tones 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 *Cochliobolus heterostrophus* and has attained the status of the economically important disease (Malik *et al.*, 2017).

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 dangers 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

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 *Cochliobolus heterostrophus*, which causes maize maydis leaf blight, were assessed.

## **MATERIALS AND METHODOLOGIES**

### **Isolation of fungus**

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.

### ***In vitro* Evaluation of Antagonistic Potential of native *Trichoderma species* against growth *Cochliobolus heterostrophus***

The antimicrobial potentials of seven native *Trichoderma spp.* against *Cochliobolus heterostrophus* 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. heterostrophus*, 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 per cent suppression of mycelial growth of the test fungus (*C. heterostrophus*) 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.

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 per cent inhibition were tabulated and presented in Table 2, Plate 1, and Graph 1. Among seven *Trichoderma spp.* used *Trichoderma ovalisporum* (KU904456) resulted in best mycelial growth inhibition by (85.23%) and the least per cent inhibition of 80.4% was shown by *Hypocrea lixii* (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, Isocyno 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 *Hypocrea lixii* (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**

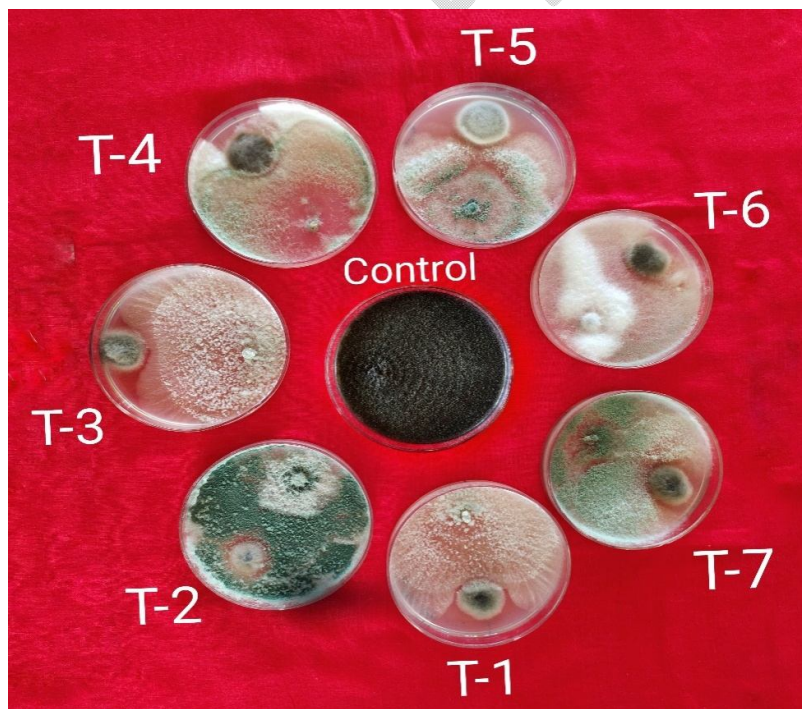
Sl. No.	Isolate code	Bio control agent	Accession number
1.	NCIPMCAU-78	<i>T. harzianum</i>	KU904458
2.	NCIPMCAU-123	<i>T. asperellum</i>	KU933476
3.	NCIPMCAU-118	<i>T. atroviride</i>	KU933472
4.	NCIPMCAU-109	<i>T. harzianum</i>	KU933471
5.	NCIPMCAU-18	<i>T. koningiopsis</i>	KU904460
6.	NCIPMCAU-96	<i>T.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

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

Sl. No.	Bio control agent	Bell's scale	Inhibition (%) *
1.	<i>T. harzianum</i> (KU904458)	Class II	83.80 (1.1) **
2.	<i>T. ovalisporum</i> (KU904456)	Class II	85.23 (1.0)
3.	<i>T. koningiopsis</i> (KU904460)	Class II	82.85 (1.2)
4.	<i>T. asperellum</i> (KU933476)	Class II	83.33 (1.2)
5.	<i>T. harzianum</i> (KU933471)	Class II	82.38 (1.3)
6.	<i>T. atroviride</i> (KU933472)	Class II	83.80 (1.1)
7.	<i>Hypocrea lixii</i> (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



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

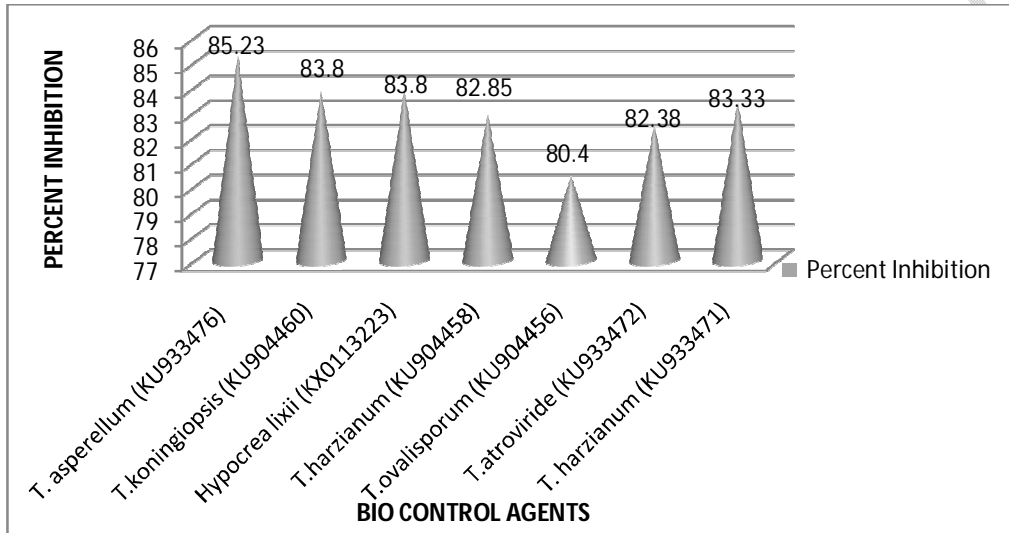
C. Control,

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. *Hypocrea lixii* (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.

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