Inhibitory potentialities of Bio-control agents on *Cochliobolus heterostrophus* inciting 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. Each species significantly slowed the *Cochliobolus heterostrophus* pathogen's expansion. The findings indicate that *C. heterostrophus*, a common pathogen associated with maize leaf blight, is capable of being inhibited to a significant degree by all seven species of *Trichoderma*, using them as effective potential antagonists.

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 top producers of maize are the United States, China, Brazil, Mexico, France, and India. With an area of 9.26 million hectares, maize is the third-most significant food grain in India after rice and wheat. It belongs to the Maydaea tribe and the family Poaceae (the grass family). Around the world, 65 pathogens, including fungus, bacteria, and viruses, harm maize and cause up to 112 illnesses. Diseases not only cause a decrease in output but also a decline in the grain's worth and quality.

The more significant diseases that affect maize are rust, downy mildew, stalk rot, and leaf blight. Out of these, *Cochliobolus heterostrophus*-caused maydis leaf blight (MLB), also known as Southern corn leaf blight (SCLB), is one of the most serious foliar diseases and has acquired the rank of an economically significant disease (Malik *et al*., 2017). Significant side effects from synthetic chemicals used in management practises include the emergence of resistant pathogens, lingering effects, environmental pollution, high cost, and a higher carcinogenic risk than other pesticides, which, among other things, could prevent biological effects on humans and animals (Brent and Hollomon, 1998; Schillberg *et.al.,* 2001). The inherent hazards of chemical management are driving an increase in interest in organic farming and bio pesticides. The prevention of one organism's growth, infection, and reproduction by another organism is known as biological control (Cook, 1993). The use of unfamiliar or already-present species in the eco system as natural enemies of pests and pathogens to reduce their numbers and harmful effects is known as biological control. Utilizing such sustainable methods to control or prevent illness and disease-causing organisms results in an environmentally friendly environment in the absence of resistant/tolerant cultivars.

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.

MATERIALS AND METHODOLOGIES

Isolation of *C. heterostrophus*

Maize plants with characteristic leaf blight symptoms were collected and examined under a light 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 with sterile water was done. Blotting paper was used to dry the pieces. Finally, using sterile forceps, the fragments were aseptically put on potato dextrose agar (PDA) Petri dishes. The inoculated Petri dishes 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 Efficacy of Antagonistic Potential of Bio-control agents against the growth of *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 Petri dish using a sterile cork borer and sterile needle, and a 5mm diameter *Trichoderma spp.* mycelial disc at the other end of the Petri dish at an angle of 180°. Petri dish 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 of 7 days, 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 with three replications using CRD. 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. *Trichoderma spp.* produces a variety of secondary metabolites, including Pyrones, Koninginins, Viridins, Nitrogen Heterocyclic Compounds, Azaphilones, Butenolides and Hydroxy-Lactones, Isocyano metabolites, Diketopiperazines, Peptaibols, and others (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

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	*Hypocrea lixii*	KX0113223

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

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

*Mean of three replications

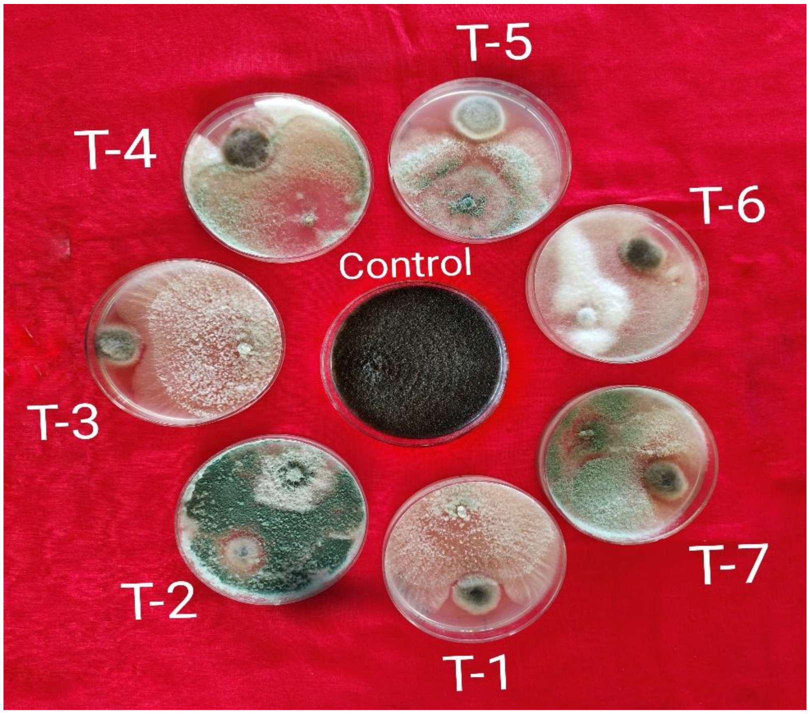


Plate 1: *In vitro* efficacy of bio control agents against growth of *C. heterostrophus*

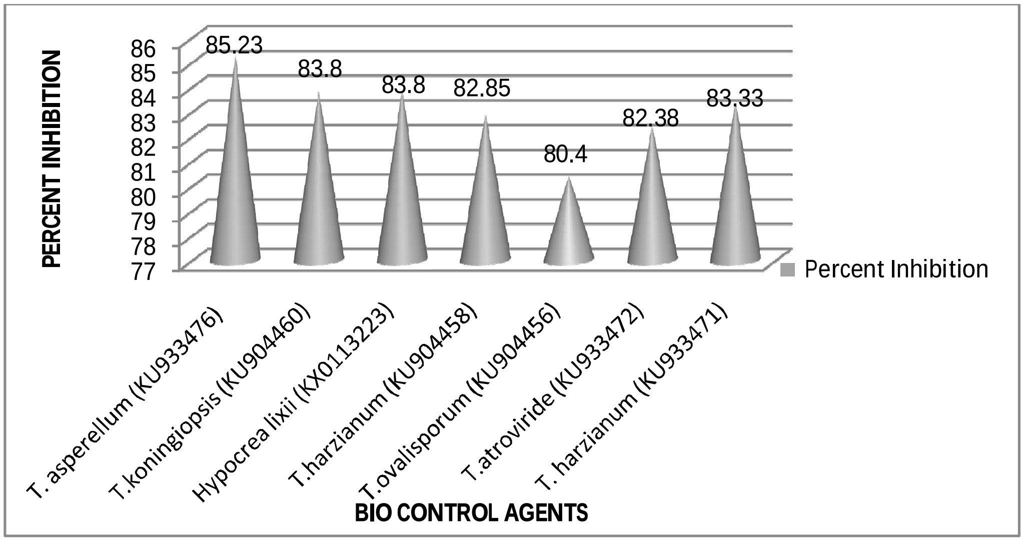
C. Control,

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 are effective on reducing mycelial growth of *C. heterostrophus*. *Trichoderma spp*. can be employed as a biocontrol agent for *C. heterostrophus.* As a result, among seven different *Trichoderma spp.* the highest per cent inhibition was shown by *T. asperellum* (85.23%). The least per cent inhibition was shown by *T. ovalisporum* (80.4%).

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