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In vitro study on the biocontrol activity of *Trichoderma* against *Phomopsis theae* petch, infecting collar rot of tea in Bangladesh

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ABSTRACT: An attempt was made to study the biocontrol activity of *Trichoderma* against *Phomopsis theae* Petch, a causing agent of Collar Rot of tea plant during 2006–2010 under *in vitro* condition. The pathogen was isolated from collar rot infected tea plants collected from main farm and its experimental farm of Bangladesh Tea Research Institute (BTRI), Srimangal, Bangladesh. In order to isolate *Trichoderma*, soil samples were collected from 15 different marks of tea plantation of BTRI at a depth of 0–9 inches. *Trichoderma* was isolated by serial dilution technique. The pathogen, *P. theae* was isolated from collar canker affected tea plants by tissue plating technique. Interaction of the antagonists with the pathogen was studied by dual culture method. *Phomopsis theae* and *Trichoderma* showed separately an increasing and significant radial growth of mycelium at different intervals. The pathogen and antagonist grew until they came in contact with each other in dual culture. Growth of the pathogen was inhibited, while the antagonist continued their growth and completely covered the pathogen. After 5 days of plating, only 12.67% growth of *P. theae* and 87.33% of *Trichoderma* were observed. It indicates that the percent inhibition on the growth of the pathogen was 85.92%

KEYWORDS: *Pathogen; Collar rot;* Trichoderma; *Bangladesh Tea Research Institute; Dual Culture Method*

Introduction

Trichoderma spp. are free-living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environments. They produce or release a variety of compounds that induce localised or systemic resistance responses in plants. *Trichoderma* strains have long been recognized as biological agents, for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients. Antagonist microorganisms, such as *Trichoderma*, reduce growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion. According to Daami-Remadi *et al.*,[1] soil-borne plant pathogens such as *Rhizoctonia solani*, *Sclerotium sclerotium*, *Phytium* spp., *Stereum purpureum*, *Botrytis cinerea*, *Phomopsis viticola* and *Fusarium* spp. are being successfully controlled by *Trichoderma* species.

Trichoderma species belongs to a small family of beneficial fungi that are commonly found in soils in nearly all parts of the world. Basically, *Trichoderma* species live on plant debris and organic matter in soil, while some strains are parasitic on other fungi. The quest for biological control of plant pests and pathogens continues to instigate research and development in numerous fields. This is specially the case in plantation crops like tea (*Camellia sinensis* (L.) O. Kuntze). This foliage crop tends to endure severe stress both physically and physiologically owing to the frequent harvest of shoot.[2] Among the various diseases that affect tea plants, Collar rot disease caused by the fungus *Phomopsis theae* Petch is the most common stem disease both in young and mature tea in Bangladesh. This disease is a serious problem in all tea-growing areas of the world leading to replanting debacle.[3-5] The yield has been estimated as 10–15% in Southern Indian tea plantation.[6] Despite its economic impact, effective preventive measures are available, other than pruning to healthy wood and application of copper fungicides on prune cuts.[7] Islam *et al.*[8] reported that Carbendazim and Calixin were highly effective in completely inhibiting the growth of *Phomopsis theae* at the lowest concentration of 50 and 100 ppm, respectively. Application of fungicides is mostly toxic and pollutes the atmosphere by spreading out in the air and accumulating in the soil. Random and extreme use of chemical fungicides for seed and soil management has led to increase of pathogen resistance.[9] Using biocontrol agents could be an alternative to chemicals in the management of fungal diseases. Several commercial biocontrol agents both

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Table 1: Mycelial Growth (mm) of *Phomopsis theae* at Different Intervals

Culture plate of *P. theae*	Radial growth (mm) of *Phomopsis theae* at different intervals				
	24 hrs DAP	48 hrs DAP	72 hrs DAP	96 hrs DAP	120 hrs DAP
Isolate 1	16	35	58	75	90
Isolate 2	15	35	58	75	89
Isolate 3	15	36	58	76	89
Isolate 4	15	36	57	76	90
Isolate 5	16	36	58	76	90
Average	15.4 e	35.6 d	57.8 c	75.6 b	89.6 a
LSD ≈ 0.05			0.367		

bacteria and fungi have been registered and are available as commercial products for controlling of various diseases.[10] The disease is of great importance as the area under replanting and new clearing is increasing in recent years. Collar canker disease caused by the fungus *Phomopsis theae* Petch is the most common stem disease in young tea. The disease has great economic importance of the area under replanting, and new clearings with clonal tea are increasing in recent years. The disease is prevalent in young tea plantations planted with clonal tea and to a certain extent also in mature tea fields. There are two ways by which bio control agents can suppress the plant pathogen: (i) Production of antibiotics or (ii) Production of hydrolytic enzymes. In the present study, attempts were made to study the biocontrol activity of *Trichoderma* against *Phomopsis theae* under *in vitro* conditions.

Materials and Methods

Sample Collection

Collar rot infected tea plants were collected from main farm and its experimental farm of Bangladesh Tea Research Institute, Srimangal.

Preparation of Culture Media

Potato Dextrose Agar (PDA) media was prepared in the laboratory of Plant Pathology. Media and necessary glassware were sterilized in autoclave.

Isolation of *Trichoderma*

According to Islam *et al.*,[11] soils were collected from 15 different marks of tea areas under main farm of Bangladesh Tea Research Institute at 0–10 inches. All collected soil samples were mixed thoroughly to make a composite sample. As working samples, 1 gm (dry weight basis) soil was taken from composite sample and mixed into 9 ml of sterile distilled water. Then, 1 ml of suspension was taken into another tube containing 9 ml of sterile distilled water. This serial dilution technique was continued up to 1: 10,000. From the final dilution (1: 10,000), 1 ml suspension was transferred to each of the five petri dishes. The melted agar medium (20 ml) was poured in each plate and mixed with the suspension by giving a gentle whirling motion to the plate and allowed them to incubate at room temperature.[12] Sub-culturing was performed, and the culture of *Trichoderma* in pure form was maintained.

Isolation of *Phomopsis theae*

The pathogen, *P. theae* was isolated from collar canker affected tea plants collected from main farm and its experimental farm of Bangladesh Tea Research Institute, Srimangal. Diseased specimens were kept in moist chamber to develop fruiting bodies. Actively growing mycelial tips of the fungus were transferred to PDA medium and purified by repeated sub-culturing and finally transferred to PDA slants. The cultures were sub-cultured for every 3 months.

Interaction with Dual Culture Method

Potato dextrose agar (PDA) plates are inoculated with 5-mm mycelial discs of *P. theae* as well as the antagonist on diametrically, opposite points. Since the pathogen under study is slow growing, antagonists are inoculated only after the pathogen colony grew to 20–30 mm in diameter. Radial growth of the pathogen and antagonists are measured at 24 hr intervals and per cent inhibition calculated using the following formula:

$$PI = \frac{(A - B)}{A}\ 100$$

Where A is colony diameter of the fungus in control plates (mm) and B is colony diameter of the fungus in dual cultured plates (mm).

Results and Discussion

Phomopsis theae and *Trichoderma* showed an increasing and significant radial growth of mycelium at different intervals (Tables 1 and 2). In the dual culture experiment, the pathogen and antagonist grew until they came in con-

Table 2: Mycelial Growth (mm) of *Trichoderma* at Different Intervals

Culture plate of *Trichoderma*	Radial growth (mm) of *Trichoderma* at different intervals				
	24 hrs DAP	48 hrs DAP	72 hrs DAP	96 hrs DAP	120 hrs DAP
Isolate 1	25	47	69	79	90
Isolate 2	26	48	70	80	90
Isolate 3	26	48	70	80	90
Isolate 4	26	47	69	80	90
Isolate 5	25	48	69	79	90
Average	25.6 e	47.6 d	69.4 c	79.6 b	90 a
LSD ≈ 0.05			0.409		

Table 3: Mycelial Growth (mm) of *Trichoderma* and *Phomopsis theae* in Dual Culture at Different Intervals

Dual culture plate	Radial growth (mm) of *Trichoderma* and *Phomopsis theae* at different intervals									
	24 hrs DAP		48 hrs DAP		72 hrs DAP		96 hrs DAP		120 hrs DAP	
	T	*P*	*T*	*P*	*T*	*P*	*T*	*P*	*T*	*P*
Plate 1	12	3	25	7	45	9	59	11	79	11
Plate 2	13	3	25	8	45	10	59	12	79	11
Plate 3	13	4	26	7	45	9	58	11	78	12
Plate 4	13	4	26	7	45	10	58	12	78	12
Plate 5	13	4	26	7	46	9	58	11	79	11
Average	12.8	3.6	25.6	7.2	45.2	9.4	58.4	11.4	78.6	11.4

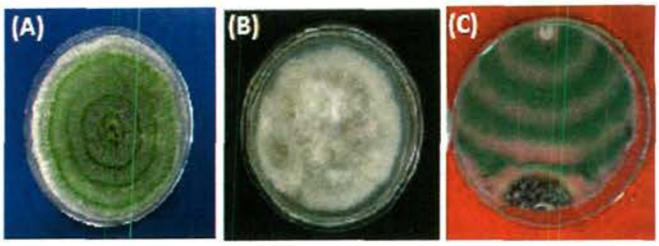

Figure 2. (**A**) Pure culture of *Trichoderma*, (**B**) Pure culture of *Phomopsis theae* and (**C**) Growth inhibition of *Phomopsis theae* by *Trichoderma*.

tact with each other. Further growth of the pathogen was inhibited, while the antagonists continued their growth and completely covered the pathogen in about 5 days (Table 3). After 5 days of plating, only 12.67% growth of *P. theae* and 87.33% of *Trichoderma* was observed (Fig. 1). It indicates that the inhibition on the growth of the pathogen was 85.92% for *Trichoderma*. Inhibition of the growth of *P. theae* might be due to the diffusible metabolites secreted by the antagonists. The antagonists completely inhibited the mycelia growth of antibiotics which induced swelling and plasmolysis of the cells.

Antibiosis and parasitism play an important role in biocontrol of plant diseases. A large number of plant diseases are successfully controlled through bacterial and fungal antagonism. The *in vitro* antagonism of *Trichoderma* sp. against root pathogens of tea was studied. The efficacy of *Trichoderma* bioformulations in controlling some of the primary and secondary root diseases has been reported. Collar canker disease caused by the fungus *Phomopsis theae* Petch is the most common stem disease in young tea. This disease is a serious problem in all tea-growing areas of the world. There are two ways by which biocontrol agents can suppress the plant pathogen: (i) Production of antibiotics or (ii) Production of hydrolytic enzymes. A large number of plant diseases have been successfully controlled through bacterial and fungal antagonists. It has been reported that the interaction between *P. theae* and fungal antagonists such as *Trichoderma* was studied *in vitro* through dual culture and antibiosis techniques (Fig. 2), which revealed that pathogen growth was suppressed significantly. The biocontrol agents from plant protection species is the filamentous fungal genus *Trichoderma* which is of great economic importance as sources of enzymes and antibiotics. Antagonist microorganisms, such as *Trichoderma* reduce growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion.

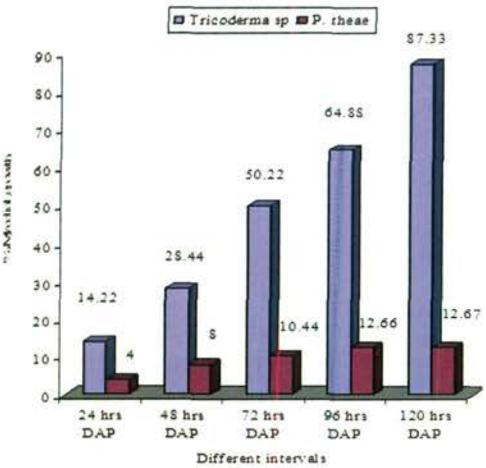

Figure 1. Percent mycelial growth of *Trichoderma* sp. and *Phomopsis theae* in dual culture at different intervals.

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