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IN VITRO BIOLOGICAL CONTROL OF BRANCH CANKER (*MACROPHOMA THEIOCOLA*) DISEASE OF TEA

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A B S T R A C T

Antagonist microorganisms, such as *Trichoderma spp.* 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. An attempt was made to evaluate the in vitro biocontrol of branch canker (*Macrophoma theicola*) of tea plants by *Trichoderma spp*. Isolation of *Trichoderma spp.* and *M. theiocola* was done carefully. Pure culture of *Trichoderma spp.* and *M. theiocola* and their morphological characteristics were studied at different intervals. Five *M. theiocola* and five *Trichoderma* isolates were collected from mature tea plants and tea soils respectively of Bangladesh Tea Research Institute (BTRI) main farm area. The cultural morphology and antagonistic potentiality of *Trichoderma spp.* against branch canker pathogen (*M. theiocola*) were taken into consideration. *Trichoderma spp.* controls the growth of *M. theiocola* at different intervals. After 24 hour growth rate of *Trichoderma* was 9.3% and *M. theiocola* was 0.88%. The antagonistic potentialities of isolated *Trichoderma* against pathogens (*M. theiocola*) were observed at different intervals (24-120 hrs) and the percentage of inhibition was 82% which were observed after five days (120 hours) of inoculation. The *Trichoderma spp.* antagonizes the pathogens by several mechanisms such as antibiosis, competition, mycoparasitism or other form of direct exploitation. From this study it was revealed that, the *Trichoderma spp.* was highly effective to control the isolates of *M. theiocola* that is responsible for branch canker in tea cultivation.

Keywords: Biological agents, Branch canker, Inhibition activity, *Macrophoma theiocola*, *Trichoderma spp*.

INTRODUCTION

Tea being a perennial crop is prone to attack by many pests and diseases. The majority of the diseases in tea are of fungal origin. More than 400 pathogens cause various diseases in tea (Chen *et al.*, 1990) viz., foliage, stem and root. Branch canker is the most widely prevalent stem disease of tea and many other plants in all the tea growing areas of Bangladesh and North-east India. It is a wound parasite which gains its entrance into the frame of the bush through wounds, especially on the thicker branches caused by various agencies like pruning cuts, sun scorched lesions, damages with ragged surfaces made by carelessly chopping, sawing or wrenching off branches or by falling of shed tree wounds made by hail cattle etc. Chemical control measures have been considered as effective in controlling tea diseases so far (Premkumar and Baby, 2005). But, use of fungicides are not most desirable means of disease control as they are cost expensive, causes serious health hazard, environmental pollution and may induce pathogen resistance too (Conzalez and Collazo De Rivera, 1972; Ikediobi, 1985). Biocontrol is a potential, alternative, and eco-friendly way to control the disease which is one of the most interesting aspects of the science of the biological control is the study of the mechanisms employed by biocontrol agents to effect disease control (Howell, 2003). Several attempts were made to control various tea diseases by the application of biocontrol agents such as plants and animal extracts and several microbes (Ahmad *et al.,* 2013; Islam *et al.,* 2013; Hossain *et al.,* 2013; Ali *et al.,* 1993).

Trichoderma is the most important bio-control agent which has been used in different countries for several

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years (Amin *et al.,* 2010). *Trichoderma* species belong to small family of beneficial fungi that are commonly found in soils nearly all parts of the world. Pathogens that can be controlled by *Trichoderma* spp. include *Pythium, Phytophthora, Fusarium, Rhizoctonia, Sclerotia,* and *Pestalotia*. More than 100 different metabolites from *Trichoderma spp*., with known antimicrobial activities have been described so far, including antifungal cell wall degrading enzymes, peptaibols and broad-spectrum antibiotics such as gliotoxin (Howell *et al.,* 1993; Lorito *et al.,* 1996; Kim *et al.,* 2002; Wiest *et al.,* 2002; Pozo *et al.,* 2004). From the *in vitro* study on the biocontrol activity of *Trichoderma* against *Phomopsis theae* petch, infecting collar rot of tea, it was found that, *Trichoderma* was very effective against *P. theae* (Islam *et al.,* 2013).

The advantages of using *Trichoderma spp.* include: pathogens do not develop resistance against a bio-control agent, bio-control agents pose no health hazards, environmental hazards and leave no chemical residue on the produce. Several strains of the *Trichoderma spp*., are found to be effective biocontrol agents for the various plant pathogens (Amin *et al,* 2010) and they are characterized by rapid growth, abundant conidial formation and a high degree of ecological adaptability reported by Domsch *et al.* (1980), Papavizas (1985); Bissett (1991). *Trichoderma spp.* is capable to induce metabolic changes in plants that increase resistance to a wide range of plant-pathogenic microorganisms and viruses (Harman *et al.,* 2004). The mechanisms of mycoparasitism, antibiosis and competition afforded by *Trichoderma* sp., have been widely studied (Howell, 2003; Harman *et al.,* 2004). This study was focused on the need for screening the isolates of *Trichoderma* having broad spectrum of antagonistic against stem pathogen *M. theicola i*n order to bring efficient biocontrol of *Trichoderma* against branch canker (*M. theicola*) pathogen in tea.

MATERIALS AND METHODS

The experiment was conducted in the Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology and, Bangladesh Tea Research Institute (BTRI) Sylhet, Bangladesh, 2013.

Isolation of *Macrophoma theiocola*: *M. theiocola* attacked stems were collected from farm area of Bangladesh tea research institute (BTRI). The PDA media were inoculated with inocula of diseased stems in the petri plates. After that, petri plates were incubated for observing growth of *M. theiocola* and purified by repeated sub culturing and finally transferred to PDA slants. The cultures were sub cultured for getting pure form of the pathogen. The isolates were compared with type strain *M. theiocola* procured from MTCC.

Isolation of *Trichoderma spp.*: Soil samples were collected from 0-9 inches depth of different marks of tea areas under main farm of Bangladesh Tea Research Institute. All collected soil samples were mixed thoroughly to make a composite sample. 1 gram (dry weight basis) soil sample was taken from composite sample in test tube 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 plates. 20 ml of melted agar medium was poured in each plate and mixed with the suspension by giving a gentle whirling motion to the plate and allowed them to incubate in room temperature (Islam *et al.,* 2001). Sub culturing was performed and the culture of *Trichoderma* in pure form was maintained. Colony characterizations were done by observing the growth of the culture.

Interaction with Dual culture method: Potato dextrose agar (PDA) plates were inoculated with 5mm mycelial discs *M. theiocola* as well as the antagonist on diametrically, opposite points allowed them to incubate in room temperature for five days. Radial growth of the pathogen and antagonists were measured at 24hrs intervals and percentage inhibition was calculated using the following formula: $PI = \frac{(\mathrm{A}-\mathrm{B})}{\mathrm{A}} \times 100$

Where A is the colony diameter of the fungus in control plates (mm) and B is colony diameter of the fungus in dual cultured plates (mm), PI= Percent of Inhibition.

Statistical analysis: The statistical analysis was done by using MSTAT a computer package. Mean comparison among the different intervals was done by using DMRT.

RESULTS

The colony characteristics of four *Trichoderma* spp. isolates were observed different time intervals (24-120 hrs) (Table 01). Colony morphology of *Trichoderma* isolates were identically similar to each other. After 24 hrs of inoculation, all isolates were shown whitish mycelial growth and after 96 hrs they showed greenish white to dark greenish color (Table 01). Sporulation was started after 72 hrs of incubation at 28 ± 1^{0}C by all the isolates studied.

Table 1: Colony characters of five isolates of *Trichoderma spp*.

Isolates	24 hrs (1st day)	48 hrs (2nd day)	72 hrs (3rd day)	96 hrs (4th day)	120hrs (5thdays)
T1	Whitish mycelial growth	Thin whitish mycelial growth	Dark greenish color was found around the mycelial block	Old cultured dark greenish color and greenish white margin part	Dark green color
T2	A thin white mycelial growth	White mycelia growth was found	Light greenish colored was found the old part of the fungal culture	Greenish white mycellium	Greenish white colour
T3	White mycelia growth around the mycelial disc	Raised white mycelial growth	Greenish mycelial mat was formed	Dark greenish mycelial growth around the mycelial block	Dark green colour
T4	White mycelial growth	Same as 24 hrs but fluffy mycelial growth	Greenish mycelial growth was found and flattened mycelial growth around the mycelial block	Greenish white mycelium	Greenish white colour

The mycelial growths of *Trichoderma spp*. were increased with the increasing intervals of time (Table 02). The petri dishes were fully covered by the colony after 120 hrs of incubation of *Trichoderma spp.* on PDA media and average growth of *Trichoderma spp.* after five days incubation was 90.00mm (Table 02). Growth rate of mycelia of *Trichoderma spp.* in respect of different interval showed statistically significant (Table 02).

Table 2: Mycelial growth (mm) of *Tricoderma spp.* at different intervals.

Time after plate	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Average
	Average of three replication				
24 hrs	30	30	30	30	30e
48 hrs	48.33	50	50	50	49.58d
72 hrs	66.66	65	65	66.66	65.83c
96 hrs	81.66	80	81.66	81.66	81.25b
120 hrs	90	90	90	90	90a

CV= 1.10%, LSD= 1.072 ≈ 0.05

Values showing different letters are statistically different from each other

After inoculation on PDA media *Trichoderma spp.* grew gradually and covered the plates with white mycelia (Fig. 01).

Figure 1. Pure culture of *Trichoderma* spp.

The pathogen and antagonist grew until contacting each other and the growth of pathogen got decreased as soon as get contact with *Trichoderma spp.*

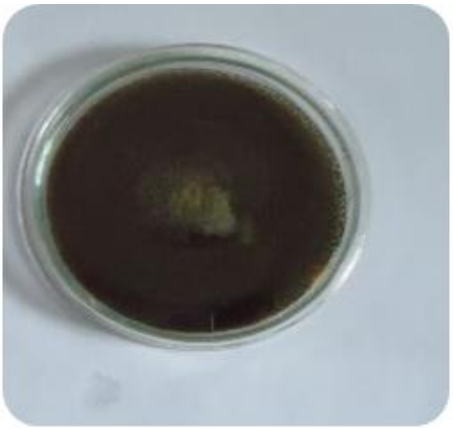

Figure 2. Pure culture of *Macrophoma Theiocola.*

In control plates the growth rate of *M. theiocola* increased gradually. After 24 hrs of inoculation the average growth was observed as 10.25 mm, after 48 hrs 34.99 mm, after 72 hrs 59.16 mm and after 96 hrs 73.91 mm and after 120 hrs 90 mm (Table 03). Growth rate of mycelia of *M. theiocola* in respect of different interval showed statistically significant (Table 03).

Table 04 reflects that, radial growth rates of *Trichoderma* isolates became slightly different at the time of contact with the pathogen. The average growth rates of *Trichoderma* were gradually increased and average growth rates of *M. theiocola* remained constant. After incubation of five days in PDA media the average growth of *trichoderma* spp. and *M. theoicola* were found73.8 mm and 16.2 mm respectively (Table 04).

Table 3. Mycelial growth (mm) *Mcrophoma theicola* at different intervals.

Time after plate	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Average
	Average of the three replication				
24 hrs	10.33	10	10.66	10	10.25[e]
48 hrs	33.33	35	36.66	35	34.99[d]
72 hrs	60	61.66	58.33	56.66	59.16[c]
96 hrs	73.66	74.33	74	73.66	73.91[b]
120 hrs	90	90	90	90	90[a]

CV= 2.17%, LSD= 1.794≈0.05

Values showing different letters are statistically different from each other.

Table 4. Mycelial growth (mm) of *Tricoderma spp.* and *Macrophoma theiocola* in dual culture at different intervals.

Dual culture plate	Radial growth (mm) of *Tricoderma spp.* and *Macrophoma theiocola* at different intervals									
	24 hrs DAI		48 hrs DAI		72 hrs DAI		96 hrs DAI		120 hrs DAI	
	T	M	T	M	T	M	T	M	T	M
Plate 1	8	0	18	6	33	10	55	13	73	17
Plate 2	9	2	18	6	35	9	56	13	74	16
Plate 3	8	1	19	6	34	9	56	14	74	16
Plate 4	8	0	17	5	34	10	55	14	74	16
Plate 5	9	1	18	6	35	10	55	13	74	16
Average	8.4	0.8	18	5.8	34.20	9.6	55.4	13.4	73.8	16.2

DAI=Days after incubation; (T= *Tricoderma spp.* and M=*Macrophoma theiocola*).

In dual culture plate the growth of pathogens were retarded due to the presence of *Tricoderma*. The percentage of mycellial growth of *Trichoderma spp.* and *M. theicola* in dual culture at different intervals were observed (Figure 3 and 04). After five days of incubation, only 18% growth of Macrophoma theicola and 82% of Tricoderma was occurred (Fig 05).

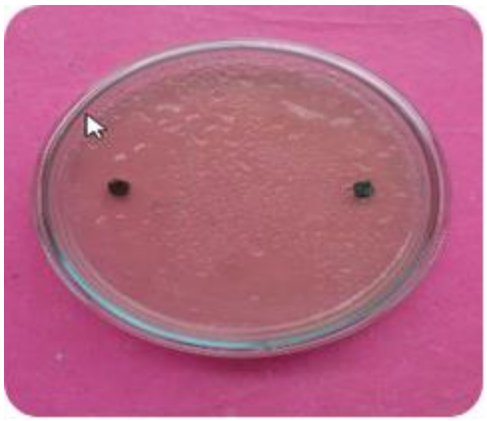

Figure 3. Dual culture

Figure 4. Dual culture of after 5 days.

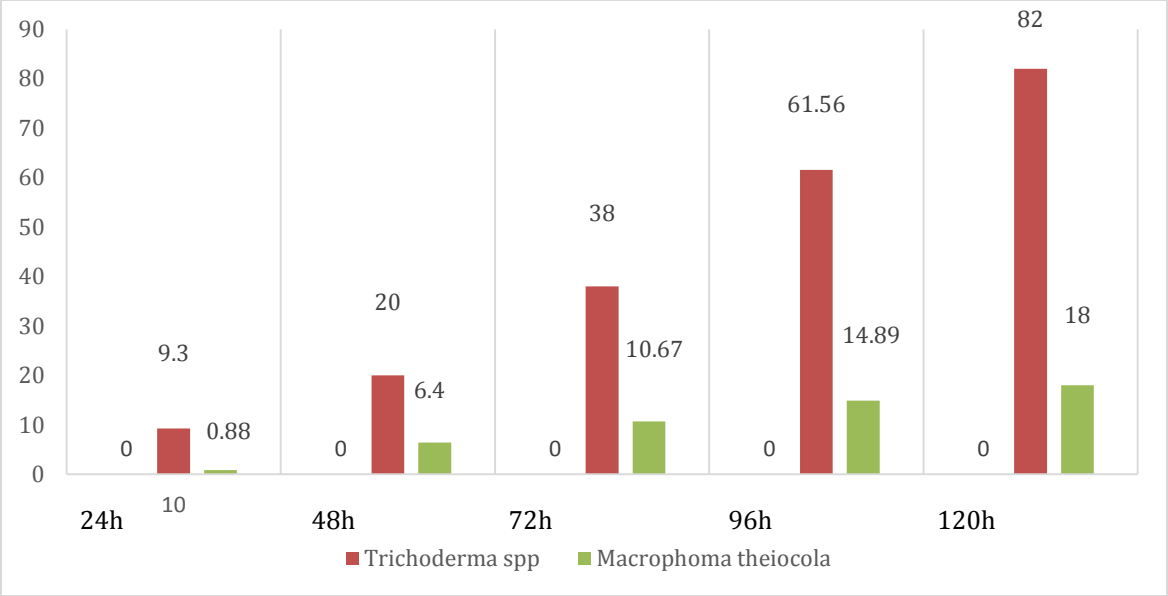

Figure 5. Comparative growth rate (%) of *Thrichoderma* & *Macrophoma* at different interval.

The graph showed different growth rate of *Trichoderma spp*. and *Macrophoma theiocola*. After 24 hours the growth rate of *Trichoderma* was 9.3% and *Macrophoma* was 0.88%. After 120 hrs the growth rate of *Trichoderma* was 82% and *Macrophoma* was 18%. It is clearly observed that *Trichoderma* growth is higher than *Macrophoma* (Figure 5).

DISCUSSION

The colony characterizations were done to isolate the *Trichoderma spp.* These same trends of results were observed in the *Trichoderma spp.* against the major fungal pathogen of Branch canker (Kuberan *et al.,* 2012). Such colony characteristics were clearly resembled to that *Trichoderma spp*. The mycoparasitism grew towards host, ran parallel and coiled around host hyphae by mycoparasitism producing the haustoria knob like structure with penetration peg, penetrated the pathogen hyphae and finally the cytoplasm of pathogens was lysed. Mycoparasitism includes both hyphal interaction and is the most vital mechanism of antagonism of fungal antagonist to give protection to the plants from the pathogen attack. Mycoparasitism as principle mechanism of biological control is favoured by many scientists (Elad *et al.,* 1983).

Table 02 showed the growth rate of the pure culture of *Trichoderma spp*. After 24 hour the rate is 30 mm and after 120 hour it is 90 mm. The CV value is 1.10% and LSD value is 1.072 ≈ 0.05. At the same time the growth rate of the pure culture of *Macrophoma theiocola* (Table 05) after 24 hours was 10.25 mm and after 120 hour it was 90 mm. It is clearly observed that *Tricoderma spp.* and *M. theiocola* covered the whole culture plate after 120 hours (5 days).

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 spp*., against stem pathogens of tea was studied. The efficacy of *Trichoderma* bioformulations in controlling some of the primary and secondary foliar and stem diseases has been reported (Papavizas *et al.,* 1985). 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 (Cook and Baker, 1983).

The radial growth rates of *Trichoderma* isolates were slightly different at the time of contact with the test pathogen (*Macrophoma theiocola*). The pathogen and antagonist grew until contacting them each other and the growth of pathogen got distributed as soon as get the contact with *Trichoderma*. The *Trichoderma* strains overgrew on the pathogen colony and complete invasion and sporulation occurred after four to five days.

Trihcoderma and *M. theiocola* showed an increasing and significant radial growth of mycelium at different

intervals (Table 04). In the dual culture experiment, the pathogen and antagonists grew until they came in contact with each other. Further growth of the pathogen was inhibited, while the antagonists continued their growth and completely covered the pathogen in about five days (Table 04). After 24 hrs of incubation the average growth of *M. theiocola* and *Trichoderma* was 0.8 mm and 8.4 mm respectively and after 48 hrs the average growth was 5.8 mm and 18 mm respectively and increasing in these trends. The average growth rate of *Tricoderma* is quite faster than *M. theiocola*. It indicates the inhibition on the growth of the pathogen was 82 % for *Trichoderma*. Inhibition of the growth of *M. theiocola* 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. (Tasiwall *et al.,*2009).

From the above discussion we can say that *Trichoderma spp.* control the growth *of M. theiocola*. So for the biocontrol of branch canker disease *Trichoderma spp*. was highly effective.

CONCLUSION

Trichoderma spp. plays an important role in controlling fungal plant pathogens, especially soil borne fungal pathogens. The use of *Trichoderma* based products is not only safe for the farmers and consumers but also good for the environment. In this study only morphologically *Trichoderma spp.* isolated based on the colony characterizations. It needs to identify the species clearly to produce formulations and market as a biocontrol product. From this study, we observe that inhibition on the growth of the pathogen was 82.00% for *Trichoderma.* It can be concluded that the *Trichoderma spp*., isolates reduced the growth of the isolates of *M. theiocola* significantly and therefore, can be incorporated into integrated disease management for controlling branch canker disease in tea. The degree of antagonism varied between and within species of *Trichoderma spp*., against the plant pathogens. *Trichoderma spp*. can be used for the biocontrol of branch canker disease in tea plant. However, much more work needs to be done to develop stable, cost effective, easy to produce and easy to apply formulations.

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