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Antagonistic Potential of Some Isolated Soil Fungi Against Brown Root Rot Disease of Tea in Barak Valley of Assam

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As tea is a long duration perennial crop it is very much prone to attack by several pathogens. Brown root rot disease (*Fomeslamoensis* (Murr.) Sacc. and Trott) is the one of the important primary root disease of teawhich, inhibits plant growth, causes yellowing and wilting of leaves, defoliation, branch dieback, and affect plant death.An antagonist is broadly defined as an opponent or adversary. In biological control of plant pathogen, antagonists are biological control agents (BCA) with the potential to interfere in the life process of plant pathogens. In the present work more than 20 fungal isolates were tried and out of which four isolate have exhibited antagonistic activity against the pathogen *F. lamoensis*.These four selectedisolates were found to be fast growing and on coming close to the pathogen then into it, eventually overgrew, except *Penicillium* sp. No clear inhibition zone was noticed in between isolates and *F. lamoensis*. The genus *Trichoderma* showed 'F' type of colony interaction. Among of these *Trichodermaviride* and *T. citrinoviride* showed highest antagonism followed by *Aspergillusniger* and *Penicillium spp*.

Key words: Antagonism, Biological control agent, Brown root rot, Colony interaction, Tea.

Tea is one of the most common and cheapest beverages in the world. Tea manufactured from the crop shoots comprising two to three leaves and a bud of a perennial shrub, which belongs to the genus Camellia of the family theaceae. It is commercially grown in more than 30 countries, predominantly grown in Asia followed by Africa and to a very small extent in Europe, South America and Australia .India has more than 4,40,000ha under tea cultivation and is the largest producer and consumer of black tea in the world. As it is a long duration plantation crop, it become largely prone to attack by several pathogens (Saha et al.2005) and it has become a happy hunting ground for the disease causing organisms since the beginning of tea plantations. Disease problems were found to be an integral part of the tea plant, which is under monoculture for over 150 years in North Eastern (N.E) region of India including Barak Valley of Assam (Dutta and Borthakur 1991) The annual loss of production have been estimated around 10-15% in Southern Indian tea plantation (Ponmurugan and Baby, 2008). Petch describes Brown root rot disease (*Fomes lamoensis* (Murr.) Sacc.andTrott.) as the earliest known root disease of tea. Symptoms of

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brown root rot disease are: slow plant growth, yellowing and wilting of leaves, defoliation, branch dieback, and plant death. The disease is more common in sandy soil, sand and stone particles remain encrusted in the root system (Banerjee 1993). The fungus causing brown root disease of tea was first described in Singapore by Corner in 1932 as *Fomes noxius* and reclassified by Cunningham in 1965 as *Phellinus noxius*. Corner speculated that the fungus was the cause of brown root rot of rubber trees and tea bushes. Symptoms of brown root rot disease are similar to those caused by other root rot pathogens, slow plant growth, yellowing and wilting of leaves, defoliation, branch dieback, and plant death. These aboveground symptoms are caused by a root and but rot hinders uptake and transport of water and nutrients from the soil. Biological control is the reduction of inoculums density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonist (Baker and Cook,1974).

MATERIALS AND METHODS

Isolation of Brown root rot (*Fomes lamaoensis*) causing organisms

Diseased plant material were collected from Rose Kandy Tea Estate, Cachar. Small pieces were made from the infectedportion of the root. They were sterilized with mercuric chloride(0.2%)(saturated solution in ethyl alcohol) or sodium hypochloride (1%) and placed in the sterile petriplates containing PDA medium. Growth of the fungus was observed and the same was sub cultured in the sterile PDA slants. Antagonism study for the biological control of the test pathogen was done *in vitro* using some isolated fungi from tea soil.

Collection of soil samples

Soil samples were collected multi location sites from the Rosekandy Tea Estate situated in Cachar District of Assam. Samples were collected in plastic bags and bought to the laboratory without sealing, and then these samples were air dried, homogenized and sieved (200 mesh) to get uniform samples.

Isolation of soil fungi using Dilution plate method

10gm of the soil was taken in a 250ml conical flask containing 100ml sterile water. This stock solution was thoroughly hand shaken for about 10-15minute.The dilution in the flask was treated as 1:100. Subsequently, dilution i,e 1:10000, are prepared for the isolation of fungi. 1ml of the prepared solution as described above were inoculated in the respective petridishes. The respective media put in the petridishes (10ml each approx) and shaken gently for few seconds. While pouring the media in petridishes, care was taken to keep the cover of the petridishes close to prevent contamination. The plates for fungus were incubated for 5-7days at 25±2°c. After the incubation period the growth of fungi was observed and the colony was calculated as follows:

Total population=Total no. of colonies× inoculum×dilution factor/Dry wt. of soil(gram)

Different species of fungi were identified with the help of the literature available (Gilman1956;Barnett and Hunter 1972). The culture media used during the experimental period are as follows:

Rose Bengal Agar media(Tsao,1964)

Agar-17.0gm, Dextrose-10.0gm, Yeast extract-0.50gm, KH_2PO_4-0.50gm, $MgSO_4,7H_2O$-0.50gm, Peptone-0.50gm, Rose Bengal-0.05gm, Distilled Water-1000ml, Streptomycin-0.03gm.

Potato Dextrose Agar Media

Peeled Potato-200gm, Dextrose-20gm, Agar-20gm, $MgSO_4,7H_2O$-0.20gm, $CaCO_3$-0.20gm Distilled water 1000ml.

In vitro antagonism study

To ascertain whether antagonism existed between the test fungi and the pathogens, a 4mm disc of the antagonistic fungi was placed in the petridishes containing sterile potato dextrose agar medium at 2cm apart from the pathogen. Three plates were prepared for each fungus. Respective controls were also made without the test fungi. All the plates were separately incubated at 25±1^{0}C for 6 days and measured the growth of the pathogen against the tested fungi 2 days intervals and at the same time antagonistic colony interaction were examined thereafter. The kind and degree of antagonism was determined according to the classification of Skidmore and Dickinson (1976).Once the organisms grow towards each

other, one of the following reactions may be observed:

A – Mutual intermingling of growth, B – The antagonist obliterated the test fungi, C – Prominent inhibition zone but the test organism formed clear colony, D – Very prominent inhibition zone but the test organism formed clear colony, E – The antagonist inhibited the growth of the test pathogen at a particular point, F – Overgrowing by the antagonist, G – Intermingling growth in which the test organism ceased to grow/ overgrown, H – Very insignificant inhibition and I – No inhibition.

RESULTS AND DISCUSSION

Total Population (colony) of fungi

It was observed that site 4 showed highest number of fungal colonies whereas least one was found in site 7. The total fungal population from the all sites counted as 17.47×10^4 indicates good population of fungi in tea rhizosphere soil, habitat. Among the different tea plantation soil sampling sites of the Rosekandy Tea Estate from where the isolation and identification of the fungal species was done, almost in all sites *Trichoderma sp* were abundant, whereas the genus *Aspergillus* and *Penicillium* were found to be dominant.

Antagonism Study

The interaction between the colony of pathogen *Fomes lamoensis* and soil fungi mainly *Penicillium* sp *Aspergillus*, *Trichoderma viride, Trichoderma citrinoviride* was observed. All the isolates were fast growing and on come close to the pathogen and eventually overgrew them, except *Penicillium* sp. No clear inhibition zone wasobserved in between the isolates and *F. lamoensis*. The genus *Trichoderma* showed 'F' type of colony interaction observed (Table 3).

From the result, it was also observed that the radial growth of *F. lamoensis* inhibit by *T. viride* followed by *T. citrinoviride, Aspergillus niger* respectively (Fig 1). All the isolates tested significantly at Pd"0.05 level as compared to control. Isolates *Penicillium* sp have found no significant effect on radial growth of *F. lamoensis*.

An antagonist is broadly defined as an opponent or adversary. In biological control of

Table 1.

Site	No. of colonies	Mean of colonies	Total population
1	10.33±0.88		
2	19.33 ±0.66		
3	23.33 ± 1.20	19.66	19.66×10^4
4	28.66 ± 0.88		
5	16.66 ± 1.45		
LSD at 5% level	0.638		

Values are average from three different sets
Values are Mean ± SE

Table 2.

Site	Fungal species
Young plantation	*Curvularia geniculate, Mucor sp., Penicillium, Aspergillusniger, Trichodermacitrinoviride, Aspergillusclavatus, Fusariumoxysporum*
Old plantation	*Verticilliumsp.,Aspergillusflavus, Mucor*sp, *Aspergillusniger, penicilliumsp, Aspergillusclavatus*
Cold slope	*Aspergillusniger, Trichodermasp, Curvularia geniculate, Mucorflavus, Penicilliumrubrum, Zygorhynchus sp., Verticillium sp.*
Hot slope	*Trichodermaviride, Aspergillusfumigatus, Nigrospora sp., Rhizophus sp., Penicilliumgranulatum, penicilliumrubrum, Fusarium sp.*
Nursery	*Verticillium sp. Aspergillusniger, Mucorsp.,Tricodermaharzanium*

Table 3.

Name of the fungi	Colony interaction	Radial growth of the *F. lamoensis*(cm) against the fungal isolates 2nd Day	4th Day	6th Day
Penicillium sp.	A	5.63±0.14	5.73±0.14	5.56±0.23
Aspergillusniger	E	3.30±0.20	3.90±0.12	4.06±0.03
Trichodermaviride	F	1.10±0.11	1.26±0.08	0.93±0.08
Trichodermacitrinoviride	F	1.46±0.13	1.60±0.11	1.66±0.08
Control	I	5.36±0.43	6.86±0.18	8.660.16
LSD at 5% level	0.568			

plant pathogen using by fungi, antagonists are biological agents with the potential to interfere in the life process of plant pathogens. Antagonist may be applied to soil to a) Destroy pathogen inoculums, b) Prevent recolonization of treated soil by a pathogen or c) Protect germinating seeds and roots from infection. Most of the soil fungi isolated from the Tea agroecosystem are known to servive saprophytically in nature. Release of inhibitory substances/metabolites produced by *Trichoderma viride* into the host organism is known to result in direct inhibition of growth of pathogen by disintegrating the hyphal wall resulting the penetration, absorption and lysis of the mycelium. Due to several adverse effect of chemical control of pest and diseases the attention of biological control is now increased by using some beneficial microorganism. This may be alteration of chemical control at large. Several soil fungi such as *Aspergillus flavus*, *Alternaria alternaria*, *Penicillium aurantiogriseum*, *Coniothyriumminitans*, *Gliocadium* sp and *Tricoderma* sp (Royse and Ries, 1978; Sinaga 1986; Adebanjo and Bankole, 2004; Rabeendran et al., 2006).Members of *Trchoderma* sp known to be active hypoparasites of several soil fungi and hence it is use as a biocontrol agent (Ekefan et al. 2009). Control of plant diseases by the used of antagonistic microorganisms can be affective means (Cook, 1993). An extensive study has been done on the Interaction of biocontrol agent and plant pathogen on commercially important crops is promising (Vesseur et al., 1990). Various plant diseases have been successfully controlled through bacterial and fungal antagonists (Cook and Baker, 1983: Campbell, 1989). The *in vitro*

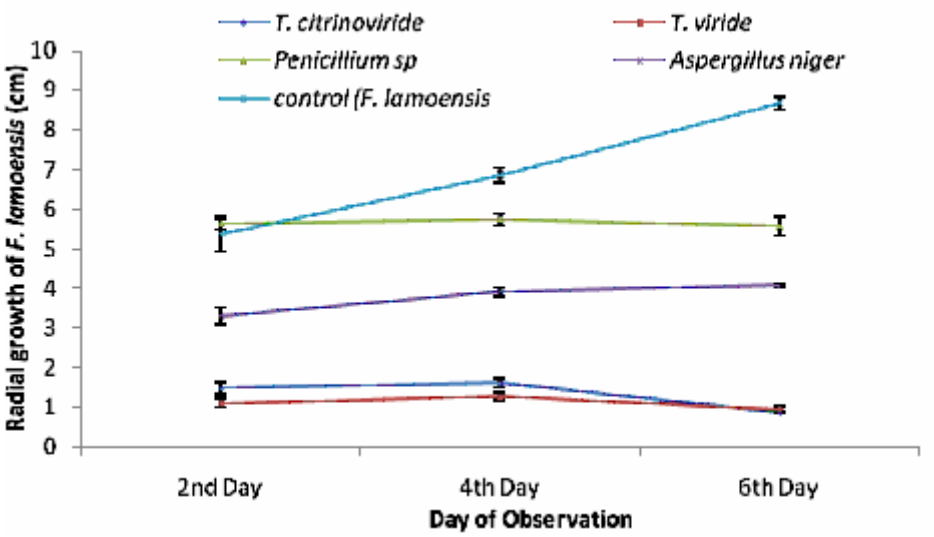

Fig. 1. Effect of fungi isolate on the radial growth of *F. lamoensis*

antagonism of *Trichoderma* sp. against certain primary root pathogens of tea (Baby and Chandramouli, 1996) as well as efficacy of *Thrichorderma* bioformulations in controlling some of the primary and secondary root diseases (Borthakur and Dutta, 1992), and *Phomopsis* canker (Ponmurugan and Baby, 2007) diseases have been reported.

In this study in the*in vitro* investigation of selective fungal isolates *T. viride* and *T. citrinoviride* have been found potential antagonistic effect against the tea root pathogen *F. lamoensis*. They have produced excellent result *in vitro* to reduce the radial growth of the pathogen eventually overgrowing it. It is also reported that *T. harzianium* suppress the growth of *Pythium aphanidermatum* and *P. myriotylum* killing the mycelium within three days of inoculation as the test organisms were not recover in the area grown over by the antagonists (Devaki et al. (1992).

Tea leaves, besides providing the healthiest drink, are now-a-days being exploited for other innovative aspects like extraction of active principles that give protection from cancer and other diseases. The anti-oxidant properties of tea have been largely attributed to the high amount of phenolics in tea leaves. Therefore, metabolism of the phenolics under naturally occurring biotic stress in tea plants is very important. Therefore, further work should be taken up to explore the possibility of the use of the antagonists from soil for the biological control of the tea root pathogen at large.

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