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Research Article

*IN VITRO E*VALUATION OF FOUR NATIVE *TRICHODERMA* SPP ISOLATES AGAINST TEA PATHOGENS

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ABSTRACT
Four native *Trichoderma spp* (three *T. viride* and one *T.harzianum)* isolated from tea ecosystem of North Bengal were evaluated against two fungal pathogens (*Corticium invisum, (DTRDC isolate)* and *Pestalotiopsis theae (NBU isolate)* of tea, *Camellia sinensis* (L).O.Kuntze *in vitro.* All the *Trichoderma* species exerted inhibitory influence on the radial growth of *C.invisum* and *P.theae* depending on isolate of *Trichoderma*. *T.viride isolate 1, 2* and *3* caused inhibition of radial growth of *P.theae* in the range of 55-63 and *C.invisum* in the range of 55-66 percent. Maximum inhibition of radial growth of *C.invisum* (77%) and *P.theae (75%)* was caused by *T.harzianum.*Hyphae of all *Trichoderma species* grew over the colony of *P.theae* and *C.invisum* to a greater or lesser extent depending on the species.

Keywords: *Tea, Trichoderma harzianum, Corticium invisum, Pestalotiopsis theae,* Biocontrol, *Camellia sinensis*

INTRODUCTION
The development of fungi for biological control of plant diseases has attracted significant amount of interest in the recent years and subsequent research led to the discovery of many potential fungal biocontrol agents some of which have reached the stage of commercialized (Butt *et al.,* 2001; Kabalak *et al.*, 2010). The ability of *Trichoderma spp* to parasitize and kill destructive plant pathogens have attracted attention of agricultural scientists, farmers, policy makers worldwide and large body of information on biological control of plant pathogens by *Trichoderma* have accumulated in the recent past (Weindling, 1932; Bliss, 1951; Rifai, 1969; Samuel, 1996; Mukherjee *et al.,* 2013; Jaklitsch, 2014; Bissett *et al.,* 2015). The genus *Trichoderma* (Teleomorph: *Hypocrea*) was established by Persoon (1794) in Germany and out of four isolates one was *T.viride* . In India there are about 32 commercial companies producing over 500 formulation of biopesticides (NAAS -2013). *Trichoderma* has been used in India against 87 different agricultural crops including 70 soil borne and 18 foliar pathogens (Sharma *et al* 2014).
Some of the products based on *Trichoderma* used in India are, Biozim, Phalada 105, Sunagroderma Honitor, Trichogard, Niprot, Bioderma, Biovidi, Eswin Tricho, Biohit, Tricontrol, Ecoderm, Phalada 106 TV, Sunagroderma, Defense SF (Rabindra and Grzywacz, 2010). Recent reports on *Trichoderma* or endophytic *Trichoderma* from different parts of the world have demonstrated the role of *Trichoderma* as plant growth promotion and induction of defense response on host plants in addition to antagonistic action (Chang *et al.,* 1986; Harman *et al.,* 2004; Vinale *et al.,* 2009). Considering the multifarious benefits of *Trichoderma* Harman (2011) termed *Trichoderma* as "multifunctional fungal plant symbiont".

Review of Work Done on Trichoderma in Tea
The first experimental use of *Trichoderma* in tea, *C.sinensis*(L). O.Kuntze was initiated at Tocklai during 1983 with a formulation (Bintab-T) obtained from UK. Pellets of Bintab-T were drilled inside infected tea branch and treated plants showed improvement over untreated control (Anon, 1983-84). Native *Trichoderma* isolate no. 1 applied on pruning cuts provided effective control of branch canker (Anon, 1986-87). Topical application of *T.viride* reduced *Poria* branch canker in tea under field conditions (Anon, 1987-88). Mass production process of *Trichoderma* was standardized at Tocklai (1988-89). A spray of 10% suspension of *Trichoderma* provided effective control of Poria and caused 25% reduction of black rot disease under field condition (Anon, 1995-96). Application of *Trichoderma* on planting pits

during transplanting reduced mortality due to root rots (Anon, 1996-97). *Trichoderma* application in tea cuttings in nursery enhances callus development and promotes root growth (Anon, 2010-11).

Prunning is an essential agronomic operation carried out in tea plantation which exposes the cut surfaces of tea stem vulnerable for infection. Copper fungicide is applied to cut surfaces as protective measure against entry of Poria and other fungi. Tea industry in India of late uses *Trichoderma* extensively for protection of pruning cuts, *Fusarium* die back, root diseases, as planting pit mixture and enriching compost (PPC, 2014; Borthakur and Dutta, 2005). Native *Trichoderma* spp occur in tea soils (Radhakrishnan *et al.,* 2006), pruning litter of tea (Debnath, 2015) and also in air over tea plantation in North East India (Debnath, 2016).

It is believed that introduction of *Trichoderma* in Tea Industry in NE India has caused significant reduction of copper fungicides used for protection of pruning cuts. Black rot is a primary leaf disease of tea in North East India while grey blight is considered a secondary disease of maintenance foliage (Sarmah, 1960). It was observed that application of *Trichoderma spp* against black rot disease caused by *Corticium invisum* provides 25 percent reduction of disease in plains of NE India under field condition (ASR –TRA, 1995-96). Of late there has been report of damaging impact of grey blight disease on tea in South India. Joshi *et al., (*2009) working on occurrence of *Pestalotiopsis* on tea plants in South India showed that *P. longisetae* has been found to be the dominant species causing crop loss as high as 17 %. Prem Kumar (2011) studied disease of Tea in South India due to grey blight infestation and suggested chemical and biological control measures. Information on species diversity of local *Trichoderma* isolates and evaluation against tea pathogens occurring in high elevation tea areas of NE India (Darjeeling) appeared to be lacking.

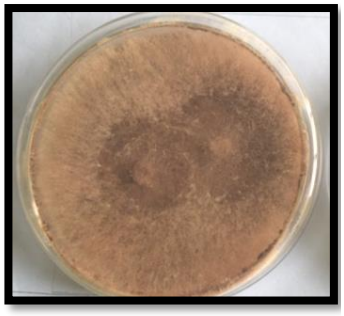
In the present study four local isolates of *Trichoderma spp* were evaluated i*n vitro* against grey blight and black rot pathogen of tea, *Camellia sinensis*(L). O.Kuntze.

MATERIALS AND METHODS

Source of Test Fungus

The grey blight fungus, *Pestalotiopsis theae* was isolated from diseased tea leaf collected from Experimental plots of North Bengal University, Siliguri .

***C. invisum* (DTRDC Isolate) 2 Days Old**



***C. invisum* (DTRDC Isolate) 15 Days Old**

***P. theae* (NBU Isolate)**

The black rot fungus, *Corticium Invisum* Petch was collected from Darjeeling Tea Research and Development Centre (DTRDC), Kurseong. Both the fungi were maintained on potato dextrose agar. *C. invisum* and *P. theae* varied in their growth rate and attained a colony diameter of 45 and 40 mm in four days in PDA respectively.

Sources of *Trichoderma Viride Isolates*

1. Isolate no 1. Collected from Poria infected tea stem, NBU, Siliguri
2. Isolate no 2. Isolated from Old blister blight affected area of DTRDC Kurseong, Darjeeling
3. Isolate no 3. Isolated from air during air sampling at North Bengal University
4. Isolate no 4. Plating of old tea leaf collected from DTDRDC, Kurseong, Darjeeling

Plate 1: Cultural Characteristics of Four Isolates of *T. viride*

Strian: 1 **Strain: 2** **Strain: 3** **Strain: 4**

Tentative identification of *Trichoderma* isolates was made by comparing the microscopic observation with the relevant identification sources (Samuel *et al.*, 2012) and also comments from Dr P. N. Chowdhry ex Senior Mycologist IARI New Delhi-12.

Growth Characteristics of Four isolates of *Trichoderma spp* at 25-28^{0}C, and 8 hours light incubation under normal conditions.

Isolate No 1: *T.viride*, Colony fast growing, flat, spreading (12mm/day) white initially later turn light green due to sporulation. Spreading aerial mycelium covering the plate. Reverse light coloured. Conidiophore irregularly branched, phialides sigmoid (8-14x2.4-3µ) Conidia globose to obvoid, rough wall, green (4-4.8x 3.5-4µ).

Isolate No 2: *T.viride*, Colony on PDA fast growing, flat, spreading (13mm/day) white initially later turn light green due to sporulation. Reverse light coloured. Conidiophore irregularly branched, phialides sigmoid (8-14x2.4-3µ) Conidia globose to obvoid, rough wall, green (4-4.8x 3.5-4µ).

Isolate No 3: *T.viride*, Colony fast growing, flat, spreading (13mm/day) white initially later turn light green due to sporulation. Reverse light coloured. Conidiophore irregularly branched, phialides sigmoid (8-14x2.4-3µ) Conidia globose to obvoid, rough wall, green (4-4.8x 3.5-4µ).

Isolate No 4: *T.harzianum*, Colony fast growing, flat, spreading (15mm/day) white initially later turn deep green due to sporulation. Spore production early and profuse. Conidiophore regularly branched, phialides ampuliform (3.5-7.5x2.5-3.8µ) Conidia globose to obvoid, rough wall, green (1.7-3.2.8x1.3-2.5µ).

Evaluation of Antagonistic Activity

Antagonistic activity was determined by dual culture methods as per Rama *et al.*, (2000). *Trichoderma spp* and test fungus were raised on Potato Dextrose agar separately. A mycelial disc (8 mm) obtained from the margin of 3-day-old actively growing colony of test fungus (*C.invisum* and *P.theae)* was placed on a fresh PDA plate 2 cm from the center of petriplate and antagonist (*Trichoderma spp)* were placed opposite to test fungus at 4 cm apart in the petridish. The radial growth of the pathogens in dual culture and control plates was measured after 4 days of incubation at 28± ^{0}C. However, the observation continued upto 8 days of growth to observe the colony interaction. The per cent inhibition of pathogen was calculated as described by Vincent and Budge, (1990). The growth inhibition was calculated by using the formula:

Percent Inhibition: 100 x (r1 –r2)/r1.

Where, r1 = diameter of fungal colony in control; r2 = diameter of fungal colony in dual inoculation

Reaction Types in Dual Culture

The degree of antagonisms or reaction types between each *Trichoderma* and test pathogen in dual culture was scored on scale of 1-5 as proposed by Bell *et al.*, (1982), which is, R1= complete overgrowth; R2 = 75 % overgrowth; R3 = 50% overgrowth; R4 = growth inhibition at line of contact; R5 = pathogen

overgrowing antagonist. The observation was recoded and shown in table 1 for *P. theae* and *C.invisum* in table 1.

RESULTS AND DISCUSSION

Table 1: Inhibition of Growth of *P.theae* and *C.invisum* by Four Isolate of *T.viride* (After 4 Days of Incubation)

***Trichoderma Viride* Isolate**	***Pestalotiopsis Theae (NBU Isolate)***		***Corticium Invisum (DTRDC Isolate)***		
	Percent Inhibition Over Untreated Control	**Colony Interaction (Reaction Types**	**Percent Inhibition Over Untreated Control**	**Colony Interaction (Reaction Types)**
1	55(18)	R2	55(20)	R2
2	55(18)	R2	60(18)	R2
3	63(15)	R3	66(15)	R1
4	75(10)	R1	77(10)	R1

(* Figure in the brackets denotes radial growth (mm) in treated plates)

It was evident from table 1 and plate 1 that four isolates of *Trichoderma* varied in their ability to inhibit grey blight pathogen *P. theae.* Profuse sporulation was produced by strain no 4. The maximum inhibition of *P. theae* (75%) was conferred by *T. harzianam* isolate no 4 followed by isolate no 3. Microscopic observation revealed parasitization of hyphae of *P. theae.* The isolate no 1 and 2 offered 55 percent inhibition of vegetative growth after 4 days of incubation in dual culture.
Reaction types also varied depending on the *Trichoderma* strain. *T. harzianum* isolate no 4 covered entire surface of vegetative growth within 7 days of incubation showing reaction type R1. Strain no 1 and 2 showed reaction type R2 indicating 50 percent overgrowth of *Trichoderma* mycelium over host. There were however no zone of inhibition.

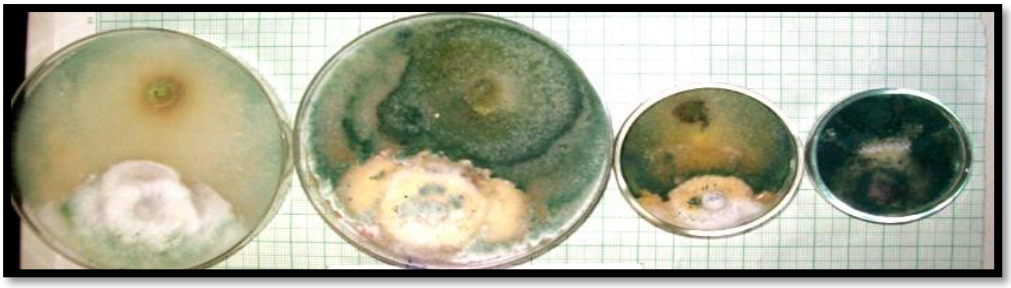


Plate 1. A: Inhibition of *P. theae* by Four Isolate of *Trichoderma*

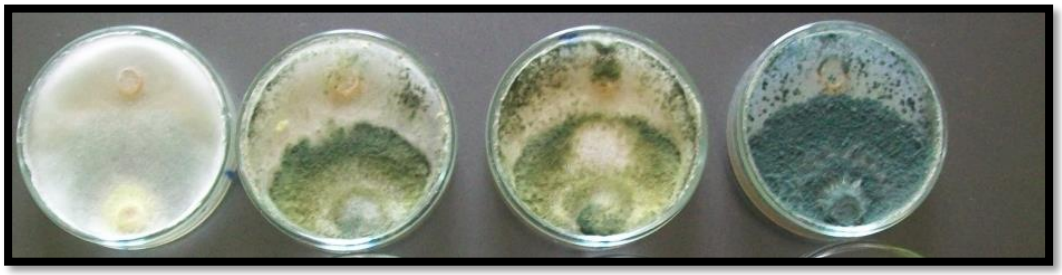


Plate 1. B: Inhibition of *C. invisum* by Four Isolate of *Trichoderma*

It was evident from table 1, that four isolates of *T.viride* vary in their ability to inhibit *C.invisum.* Maximum inhibition of vegetative hyphae (77%) was caused by strain no 4 followed by strain no 3, 2 and 1. Microscopic observation revealed parasitization of hyphae of *C.invisum.* Profuse sporulation was

produced by strain no 4. Reaction types also varied depending on the source of strain. *Trichoderma* isolate no 4 and 3 covered entire surface of vegetative growth within 7 days of incubation showing reaction type R1. Strain no 1 and 2 showed reaction type R2 indicating 50 percent overgrowth of *Trichoderma* mycelium over host.

Microbial pest control agents (MCPA) have been developed for over 100 years but very few microbes have been put to use for commercial purposes. Report of poor performances under field conditions is attributed largely due to lack of selection of appropriate bioagents. Considering the species diversity of fungal biocontrol agents and its developmental cost it is highly imperative to select appropriate and effective biocontrol *Trichoderma* for control of target pathogen (Ravensberg, 2002).

Conclusion

Trichoderma viride isolates varied in their ability to inhibit tea pathogen. The present study also emphasizes the importance of selection of effective and competent *Trichoderma spp* with antagonistic and growth promoting ability before their use in the commercial tea plantation. Development of appropriate *Trichoderma* strain for field application requires a series of steps on which success of field application largely depends (Ravensberg, 2011). Those steps are, discovery and identity of effective strain, selection *in vitro* and *in vivo*, including disease control, rhizosphere competent strain and growth promotion, temperature and pH tolerance, strain specificity, amenability to mass culture, formulation, impact on natural enemies, long term survival in agroecosystem and compatibility with agrochemicals, Shelf life and cfu content. Isolate no 4. *T. harzianum* is found to be novel and promising and as such bears high prospect to undertake further trials for development as biocontrol agents to be included in IPM programme for management of tea diseases in NE India.

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