Acta Phytopathologica et Entomologica Hungarica 50 (2), pp. 179–186 (2015)
DOI: 10.1556/038.50.2015.2.3

Biocontrol Potentiality of Isolated *Trichoderma* spp. against *Pestalozzia theae* Saw. in Tea

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(Received: 5 January 2015; accepted: 7 March 2015)

Isolates of *Pestalozzia theae* Saw. and *Trichoderma* spp. were collected from Bangladesh Tea Research Institute (BTRI) farm area, Bangladesh. The cultural morphology and antagonistic potentiality of *Trichoderma* spp. against grey blight pathogen *Pestalozzia theae* was studied for tea cultivation. The antagonistic potentiality of *Trichoderma* spp., against *Pestalozzia theae* showed maximum (inhibition 84.45 ± 0.77%) after 72 hrs of inoculation under *in vitro* condition followed by 76.02 ± 3.50% after 24 hrs of inoculation. This study revealed that *Trichoderma* strain was highly effective to control *Pestalozzia theae*, the causal agent of grey blight disease of tea.

Keywords: *Trichoderma* spp., *Pestalozzia theae*, biocontrol, tea.

Diseases are one of the most common barriers for the production of tea. More than 400 pathogens cause various diseases in tea (Thoudam and Dutta, 2012) viz., foliage, stem and root infections. Different pathogens like bacteria, algae, viruses and fungi can cause heavy losses. The majority of the diseases in tea are of fungal origin. The species in the genus *Pestalotiopsis* have received considerable attention in recent years, not only because of their role as plant pathogens but also as commonly isolated endophytes which have been shown to produce a wide range of chemically novel diverse metabolites (Maharachchikumbura et al., 2011). Species of *Pestalotiopsis* genus are not highly host-specific and taxa may have the ability to infect a wide range of hosts (Keith et al., 2006). Species of *Pestalotiopsis* cause a variety of diseases in plants, including canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and leaf spots (Pirone, 1978; Tagne and Mathur, 2001; Sousa et al., 2004; Espinoza et al., 2008; Deng et al., 2013). Pathogenic species of *Pestalotiopsis* initially make contact with the host where the infection occurs, probably by means of the conidia or fragmented spores (Espinoza et al., 2008). These inocula may survive during harsh weather conditions and may cause primary infections. Secondary inoculum produced on diseased tissue

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may cause secondary infections and increase the severity of the disease. *Pestalotiopsis* spp. are also considered to be weak pathogens (Madar et al., 1991), which penetrate the host through natural openings such as stoma, lenticels and hydathodes (Agrios, 2005).

Among the tea diseases, grey blight is a foliar disease caused by *Pestalozzia theae* Saw. (Premkumar and Muraleedharan, 2009). The two main reasons responsible for invasion of grey blight pathogen through leaf damage are scorch and hail. Severe infections cause defoliation, resulting in considerable damage (Rangaswami and Mahadevan, 1998). This pathogen can be controlled by several methods such as cultural practices, chemical control, genetical modification of tea plant species for creating tolerant varieties and biological control.

Although chemical control measures and inventing tolerant varieties are effective in controlling tea diseases, they are, however, expensive and cause serious environmental pollution as well as may induce pathogen resistance after a certain period (Gonzalez and Collazo de Rivera, 1972). In this context, biocontrol is a potential alternative way to control the diseases. Nowadays biological control has been considered as one of the most interesting aspects of the science to find out the mechanisms employed by biocontrol agents for effective disease control (Howell, 2003). The mode of action of biocontrol agents (beneficial fungi, bacteria) can be competition for nutrients, root exudates, space or infection sites on the roots (wounds), stimulation of root growths, induction of resistance in plants, mycoparasitism. Generally, bio-control products work as preventatives rather than knockdowns of diseases such as fungicides. Biological control is being investigated in several countries and various organisms are studied, such as some species of *Trichoderma* to control various diseases, some non-pathogenic strains of *Fusarium* species to suppress *Fusarium* wilt, *Bacillus subtilis* against a number of root diseases, etc.

Presently, *Trichoderma* spp. are among the most important biocontrol agents which are commonly found in soils worldwide. 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. Among the biocontrol agents the filamentous fungal genus *Trichoderma* is of great economic importance as sources of enzymes and antibiotics. Antagonistic microorganisms, such as *Trichoderma* species reduce growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion (Cook and Baker, 1983). Pathogens that can be controlled by *Trichoderma* spp. include *Pythium, Phytophthora, Fusarium, Rhizoctonia, Sclerotinia* and *Pestalotia.* 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 several researchers (Domsch et al., 1980; Papavizas, 1985; Bissett, 1991). *Trichoderma* spp. are capable of inducing metabolic changes in plants that increases resistance to a wide range of plant pathogenic microorganisms (Harman et al., 2004). The mechanisms of mycoparasitism, antibiosis and competition afforded by *Trichoderma* spp., have been widely studied (Howell, 2003; Harman et al., 2004). *Trichoderma* spp. can directly impact other fungi: after sensing a suitable fungal host, they respond with the production of antibiotic compounds, formation of specialized structures and degradation of the host cell wall, followed by the assimilation of its cellular content, a

process known as mycoparasitism (Chet and Chernin, 2002; Limón and Codón, 2004). In fact, more than 100 different metabolites from *Trichoderma* spp. with known antimicrobial activities have been described, 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). Our emphasis in this present study is to find out the antagonistic potentiality of *Trichoderma* spp., as a biocontrol agent against the foliar pathogen *Pestalozzia theae*, the causal agent of grey blight disease in tea.

Materials and Methods

Infected leaves were collected from BTRI farm area, Srimongal, Bangladesh and washed gently with distilled water and then dried by placing them between folds of filter papers. The isolation of the respective pathogen was carried out *in vitro* using water agar medium followed by potato dextrose agar (PDA) medium. The colony of the sporulating state was purified by single spore isolation and those of non-sporulating isolates by hyphal tip method (Goh, 1999). The pathogen was confirmed as *Pestalozzia theae* by observing its colony characteristics. The fungal antagonists (*Trichoderma* spp.) were isolated from tea phylloplane (CV. BT7) collected from same area by leaf washing technique (Balasuriya and Kalaichelvan, 2000; Kuberan et al., 2012) and purified by single spore method in PDA medium. The cultures of both *P. theae* and *Trichoderma* spp. were maintained on PDA slants at 4 °C. The isolate of *Trichoderma* was inoculated against *P. theae* (dual culture) under *in vitro* conditions in PDA plates considering diametrically opposite points and were incubated at 25 ± 2 °C for five days. At the same time, the *Trichoderma* strain and *P. theae* were incubated individually (monoculture) to compare the results with those of dual culture. Every treatment had four replications.

Hyphal interaction between the antagonist and the test pathogen in the dual culture was observed. Mycelial mats were lifted gently from the zone of interaction in dual culture plates with the help of a needle and placed in a drop of lactophenol cotton blue on a microscopic slide and observed under microscope (Elad et al., 1983). Radical and mycelial growth of the pathogen and the antagonistic fungus were measured at 24 hrs intervals both from individual and dual cultures and percent inhibition was calculated. *P. theae* grown individually was considered as control (Dennis and Webster, 1971). Percent growth inhibition was calculated by using following formula (Dubey et al., 2007; Kuberan et al., 2012):

$$I = C - \frac{T}{C} \times 100$$

where I = percent growth inhibition, C = colony diameter/radial growth of *P. theae* in control (monoculture) and T = the colony diameter/radial growth of *P. theae* in dual culture with *Trichoderma* spp.

Prior to commencing statistical analyses, all data were checked for normalcy, and equal variance was measured using the Levene's test. All data were presented as mean ± SD (n = 4). The effects of *Trichoderma* spp. treatment on *P. theae* were analyzed

by one-way ANOVA followed by Tukey's test ($\alpha = 0.05$). All statistical analyses were performed using SPSS for Windows (Version 13.0, SPSS, Inc., Chicago, IL, USA).

Results and Discussion

The morphological characteristics of *Trichoderma* spp. isolate colonies (Fig. 1a) were similar to that of *Trichoderma harzianium*, which is commonly used against the major fungal pathogen of brown blight (Kuberan et al., 2012) and can effectively man-

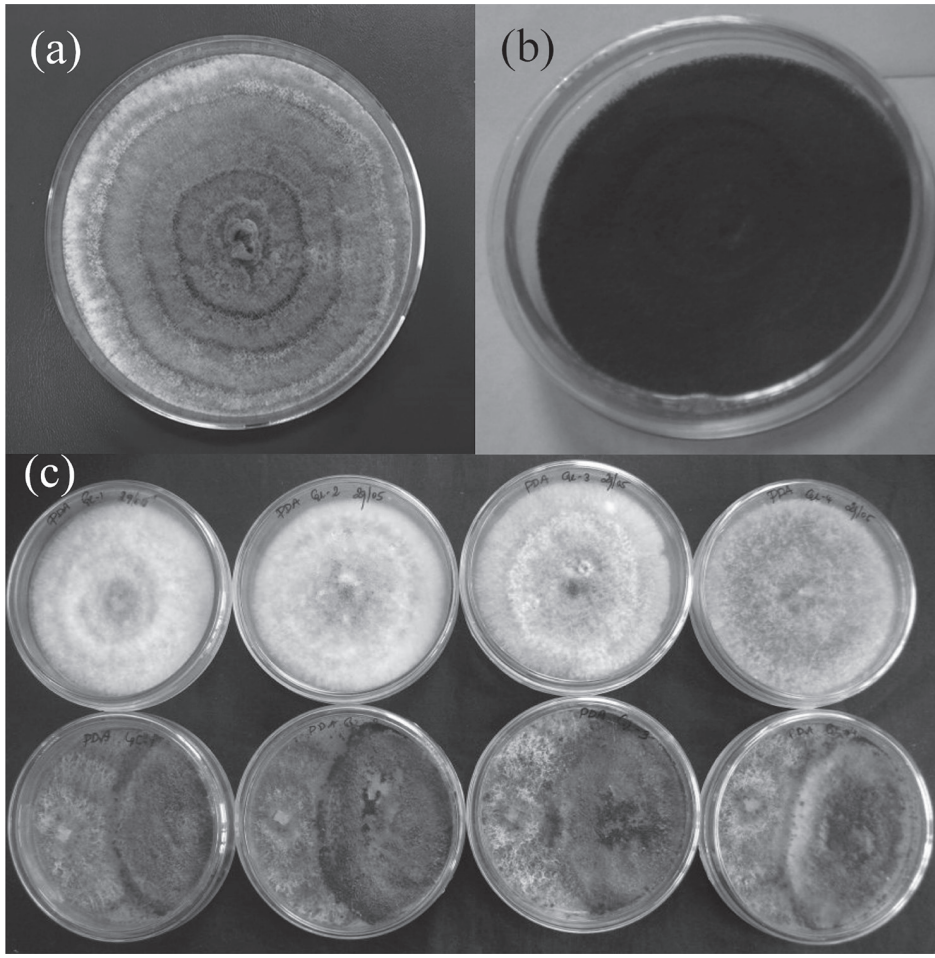

Fig. 1. (a) Monoculture of *Trichoderma* spp. (b) Monoculture of *Pestalozzia theae*. (c) Growth inhibition of *P. theae* by *Trichoderma* spp. Plates of first row having *P. theae* free from *Trichoderma* and plates of second row having *Trichoderma* as antagonistic to *P. theae* where the isolate of *Trichoderma* was inoculated against *P. theae* (dual culture) under *in vitro* conditions in PDA plates considering diametrically opposite points and allowed them to incubate at 25 ± 2 °C for five days

Table 1

Mycelial growth (mm) of *Pestalozzia theae* and *Trichoderma* spp. in monoculture and dual culture, and percent inhibition of *P. theae* by *Trichoderma*. The cultures of both *P. theae* and *Trichoderma* spp. were maintained on PDA slants at 4 °C

	24 hr		48 hr		72 hr		96 hr		120 hr	
	P. theae	*Trichoderma*	*P. theae*	*Trichoderma*	*P. theae*	*Trichoderma*	*P. theae*	*Trichoderma*	*P. theae*	*Trichoderma*
Monoculture	22.6 ± 0.55c	18.4 ± 0.55c	48.2 ± 0.45b	41.8 ± 0.45b	69.8 ± 0.45b	65.6 ± 0.55b	82.4 ± 0.55a	78.2 ± 0.45ab	90 ± 0.00a	89.8 ± 0.45a
Dual culture	4.4 ± 0.55b	11.2 ± 0.45c	7.4 ± 0.55ab	20.6 ± 0.55c	10.2 ± 0.45a	35.2 ± 0.45bc	13.2 ± 0.45a	56.8 ± 0.45b	14.2 ± 0.45a	75.8 ± 0.45a
% Inhibition	76.02 ± 3.50b		82.30 ± 1.24ab		84.45 ± 0.77a		83.12 ± 0.60a		84.19 ± 0.48a	

Different letters indicate significant differences at P = 0.05 according to Tukey's post-hoc test

age *Meloidogyne incognita* (Pandey et al., 2011). In monocultures of *Trichoderma* and *P. theae*, the radial growth of the colonies increased gradually and after a five-day incubation the petri dishes were fully covered (Fig. 1b). The growth of mycelia of *P. theae* and *Trichoderma* in mono and dual cultures, respectively, increased gradually with time and reached their maximum at 120 hrs (until the end of the observation) (Table 1). In dual culture, the radial growth of *P. theae* stopped when came in contact with *Trichoderma* spp. (Fig. 1c). However, *Trichoderma* spp. continued its radial growth after contact with *P. theae*, though at a slower rate that of its monoculture. The *Trichoderma* strains overgrew on the pathogen colony and complete invasion and sporulation occurred after four to five days. The antagonistic potentiality of *Trichoderma* spp. against *P. theae* showed maximum (inhibition $84.45 \pm 0.77\%$) after 72 hrs of inoculation under *in vitro* condition followed by $76.02 \pm 3.50\%$ after 24 hrs of inoculation (Table 1). Inhibition of the growth of *P. theae* might be due to the diffusible metabolites secreted by the antagonists (*Trichoderma* spp.). The mycoparasite grew towards host, ran parallel and coiled around host hyphae producing the haustoria, a knob-like structure with penetration peg. Then it penetrated the pathogen hyphae and finally the cytoplasm of the pathogen 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.

The antagonists completely inhibited the mycelia growth of pathogens by inducing swelling and plasmolysis of the cells (Tasiwal et al., 2010). Grey blight disease caused by the fungus *Pestalozzia theae* is the most common foliar disease in mature tea. This disease causes a serious problem in all tea growing areas of the world. Antagonist microorganisms, such as *Trichoderma* spp. reduce growth as well as survival or infections caused by pathogens with different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion (Cook and Baker, 1983).

It can be concluded that the *Trichoderma* spp. effectively reduced the growth of *Pestalozzia theae* and therefore, it is possible to consider to incorporate these fungi into the integrated disease management for controlling grey blight disease in tea in the future. So, much more work is needed for isolating *Trichoderma* spp. and develop stable, cost effective biocontrol method for tea diseases control before field application.

Acknowledgement

Authors are thankful to Dr. Mainuddin Ahmed, Director, Bangladesh Tea Research Institute, Srimangal, Moulvibazar for encouragement and providing lab facility for conducting this experiment.

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