

Original Research Article

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Comparative Bioefficacy of Fungicides and *Trichoderma* spp. against *Pestalotiopsis theae*, Causing Grey Blight in Tea (*Camellia* sp.): An *In Vitro* Study

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Abstract

The grey blight, caused by *Pestalotiopsis theae* is a serious problem in almost all tea growing regions of India. A large number of tea cultivars were found to be susceptible to this disease. Evaluation of four selected fungicides (carbendazim 12% a.i + mancozeb 63% a.i., hexaconazole 5 EC, copper oxychloride 50 WP and copper hydroxide 77 WP) have been found effective in suppressing the pathogen to the considerable degree under *in vitro* conditions. Results indicated that, maximum control (70.8%) was achieved in the case of combi fungicidal formulation (carbendazim 12% a. i. + mancozeb 63% a. i.) followed by hexaconazole 5 EC and copper hydroxide 77 WP and copper oxychloride 50 WP, respectively. Effort has been made to evaluate the effectiveness of the indigenous *Trichoderma viride* (KBN-24) and *T. asperellum* (KBN-29) in inhibiting the mycelial growth of *P. theae* in dual culture method and the results indicated significant inhibition of the pathogen. Results on the compatibility study indicated that the tested fungicides adversely affected the growth of *T. viride* to varying degree. The maximum inhibition (79.0%) was noted in case of combi fungicide formulation followed by copper oxychloride (75.8%) and hexaconazole (70.4%). Thus the results of this current investigation highlighted the fact that the local *Trichoderma* isolates were found effective in controlling the targeted phytopathogen. However, use of such fungicides soon after the application of *Trichoderma* formulation, should be discouraged to have a better control of the disease.

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Introduction

The grey blight disease of tea (*Camellia* sp.) caused by *Pestalotiopsis theae* (Ascomycota, Amphispheariaceae), is one of the limiting factors which affect the quality and quantity of tea. It is capable of infecting several plants of economic importance including guava, mango etc. Its infection is manifested by the development various kinds of symptoms (Hopkins and McQuilken, 2000; Keith et al., 2006; Pirone, 1978; Kwee and Chong, 1990;

Xu et al., 1999; Tagne and Mathur, 2001; Sousa et al., 2004; Espinoza et al., 2008). In general, it infects the maintenance leaves of tea which ensures the nourishment to the young shoots and tender foliage that ultimately results in huge crop loss (Joshi et al. 2009, Horikawa 1986). Being a weak pathogen (Madar et al., 1991) it invades the tea leaves through stomata, lenticels, hydathodes and also through the mechanical wounds (Agrios, 2005). The genus *Pestalotiopsis* is anamorphic and does not possess sexual morphogenesis (Armstrong-

Cho and Banniza, 2006; Wulandari et al., 2009). Its sexual state or teleomorph have been identified as *Pestalospaeria* and *Neobroomella* (Barr, 1975; Kirk et al., 2008). The mode of infection has reported to be either by means of asexual conidia or fragmented spores (Espinoza et al., 2008). These conidia could survive during harsh weather conditions and may cause primary infection during favorable weather conditions. The plants subjected to abiotic stress are more susceptible to infection of this pathogen (Elliott et al., 2004; McQuilken and Hopkins, 2004; Keith et al., 2006). The source of the primary infection emanate from wild plantations (Keith et al., 2006), flowers (Pandey, 1990), crop debris, soil, contaminated nursery tools (McQuilken and Hopkins, 2004), splashed water droplets (Hopkins and McQuilken, 1997; Elliott et al., 2004) and also air borne spores (Xu et al., 1999).

For the successful control of this pathogen, different chemical fungicides have been used since last several years, but their frequent applications had invited numerous associated problems including the residues in made tea which is a burning issue. To minimize such problems, integrated disease management approach is most acceptable one, which is considered as the need of the hour that has gained wide popularity nationally as well as internationally, in different cropping systems. Biological control agents (BCAs) particularly

Trichoderma spp. could be an important component of such integration. This antagonistic genus has proven efficient in controlling a wide range of phytopathogens (Dominguesa et al., 2000) of different crops through different modes of action (Elad et al., 1982; Papavizas, 1985; Taylor, 1986; Ridout et al., 1988) in eco-friendly manner. Our present study has highlighted the disease incidence, pathogenicity of *Pestalotiopsis theae*. under lab as well as field conditions, bio-efficacy of different chemical fungicides and the indigenous isolates of *Trichoderma* spp. apart from the compatibility of fungicides with antagonist.

Materials and methods

Disease survey in tea plantation

To determine the frequency of grey blight disease occurrence, a total of ten tea gardens of Dooars region in West Bengal, India, were randomly selected and surveyed during active season (April to October, 2015). In each garden, from the selected fields, samples of maintenance foliage were randomly drawn and assessed for the presence of the disease. Similarly, at our experimental plot, sampling was done from thirty five different tea cultivars. The samples showing the presence of disease symptoms (Fig.1A and 1B) were categorized and considered as susceptible / disease prone cultivar in this survey.

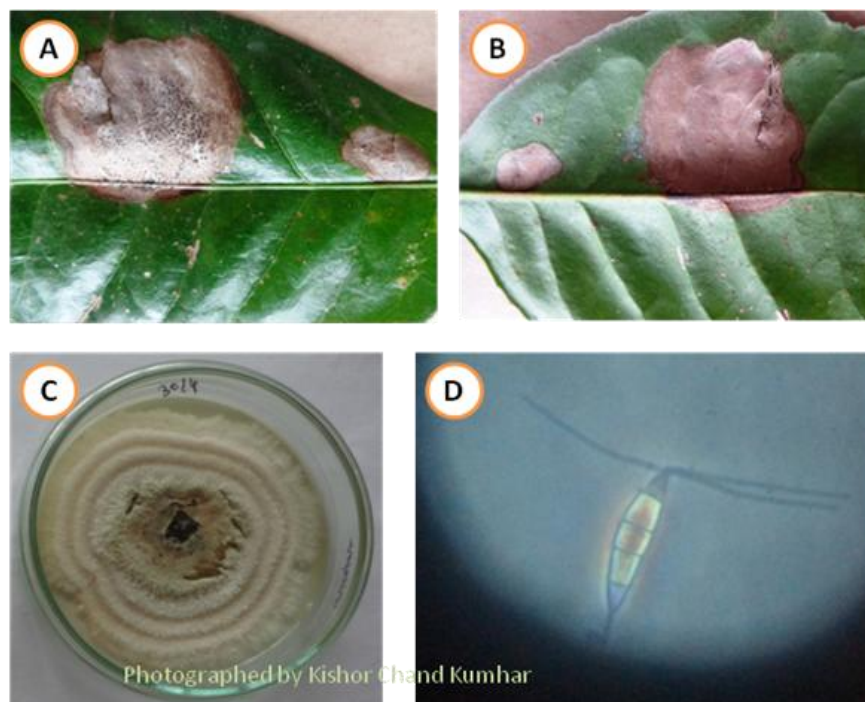


Fig. 1: Grey blight symptom on infected tea leaf (A: adaxial leaf surface; B: abaxial leaf surface) and isolated pathogen (C: mycelial colony; D: conidia of *P. theae*).

Isolation and determination of pathogenicity of pathogen

The isolation of the pathogen was done by adopting the technique of Vidhya Pallavi et al. (2012) with minor modifications. The *in vitro* and *in vivo* pathogenicity of pathogen was carried out by employing pin pricking method (Mondal et al., 2015) followed by inoculation. For this purpose, the conidial suspension was prepared from actively growing culture of *P. theae*. Twenty milliliter of distilled sterilized water was added to plate (90 mm diameter) and fungal biomass (conidia and mycelia) was harvested. This suspension was further diluted serially to get optimum conidial strength of 2×10^6 cfu/ml, and finally the leaves were inoculated with the fungus. Development of symptom was recorded up to one week for the confirmation of its virulence. The establishment of host – pathogen interaction was confirmed by visualizing the slide of infected leaf through compound microscope (Olympus – BX51) at 40X magnification.

In vitro bio-efficacy fungicides against pathogen

The bio-efficacy of a few fungicides against grey blight pathogen was carried out by poisoned food technique (Nene and Thapliyal, 1993) using potato dextrose agar medium (Hi-Media). Required quantities of fungicides were added in to medium and then it was poured in to plates (90 mm dia). After solidification, 5 mm diameter discs of pathogen's mycelia were inoculated in the centre of plates followed by incubation at $25 \pm 2^\circ\text{C}$ for two weeks. Each treatment was replicated five times. Growth inhibition was worked out by following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Colony diameter in control} - \text{Colony diameter in treatment}}{\text{Colony diameter in control}} \times 100$$

In vitro bio-efficacy of *Trichoderma* spp.

To assess the bio-efficacy of *Trichoderma* spp., dual culture technique (Stack et al., 1986) was employed. Five millimeter discs of both fungi (*Trichoderma* spp. and *P. theae*) were incubated at equidistance in the centre of the plates followed by incubation at $25 \pm 2^\circ\text{C}$ for one week. Each treatment was replicated five times. Pathogen's mycelial growth was measured after one week. Mycelial colony diameter was measured and growth inhibition was worked out using the above mentioned formula.

Fungicide compatibility with *Trichoderma* sp.

The recommended dose of each fungicide was evaluated by adopting poisoned food technique of Nene and Thapliyal (1993). Accordingly, required quantity of each fungicide was measured and admixed thoroughly in to hundred mL PDA medium before pouring it in to the plates (90 mm diameter). After solidification, 5 mm diameter mycelial discs of *T. viride* were inoculated in the centre of plates followed by incubation at $25 \pm 2^\circ\text{C}$. Each treatment was replicated five times. The colony diameter was measured followed by calculation of per cent growth inhibition using formula described earlier.

Statistical analysis

To compare critical difference, all experimental data were analyzed statistically through online package 'OPSTAT' of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India.

Results and discussion

Disease survey in tea plantation

Our study indicated that grey blight disease was ubiquitously prevalent in almost all surveyed tea garden of different locations. Similarly, at the experimental plot, sampling done from thirty five different tea cultivars (Table 1) showed the wide occurrence of grey blight pathogen irrespective of tea cultivar. Earlier researchers noted that certain tea cultivars such as Teen Ali-17/1/54 and TV-23 were highly susceptible, however cultivar CP-1 and TV-26 were less susceptible to grey blight pathogen (Chakraborty et al., 1995), which supported our findings. The disease susceptibility or tolerance of plant is governed by quantity of many anti-oxidative enzymes *i.e.* glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APO), peroxidase (POD) and polyphenol oxidase (PPO), which was found higher concentration in susceptible cultivars as reported by Palanisamy and Mandal (2014).

Isolation of pathogen and determination of its pathogenicity

The isolated pathogen, based cultural characteristics and conidial morphology, was identified as *P. theae*. The microscopic observations confirmed that its conidia were 5 celled. The conidial upper and lower cells were hyaline, however, rest median three cells were dark

colored. Its conidia possessed 2-3 setulae at the apex and one pedicel at the bottom as clear from Figs. 1C and 1D.

The ‘pin pricking method’ under lab conditions resulted in the successful establishment of the infection of this pathogen, which was manifested in the development of necrotized area in the form of ‘leaf spot’. The clear cut disease symptoms became evident only after 72 hrs of inoculation, which has undergone complete development within 7 days (Fig. 2A).

However, under field conditions, the leaf necrosis appeared after 72-96 hours around the inoculation site (Fig. 2B) and the prominent symptoms were visible only after 8-10 days of inoculation (Fig. 2C). Microscopic

visualization of adjoining infected area of leaves revealed that, there was positive host-pathogen interaction. Hence pathogen could successfully establish itself in to leaf tissues and efficiently produced conidia therein (Fig. 2 D). The re-isolation of pathogen from necrotized tissue justified the Koch’s postulates positive.

A few isolates of *Pestalotiopsis* spp. isolated from different tea growing areas of southern India which exhibited diversity in morphological characteristics including size and color of conidia as well as virulence have been reported (Joshi et al., 2009). Similarly a survey in mango orchards has been undertaken and isolation of this pathogen was carried out (Okigbo and Osuinde, 2003).

Table 1. Survey of grey blight disease of tea during 2015.

Selected tea garden	Total sample surveyed	Sample infected	Disease occurrence
Garden 1	5	5	+
Garden 2	5	5	+
Garden 3	5	5	+
Garden 4	5	5	+
Garden 5	5	5	+
Garden 6	5	5	+
Garden 7	5	5	+
Garden 8	5	5	+
Garden 9	5	5	+
Garden 10	5	5	+
TRA Experimental plot	35	35	+

+ indicated disease

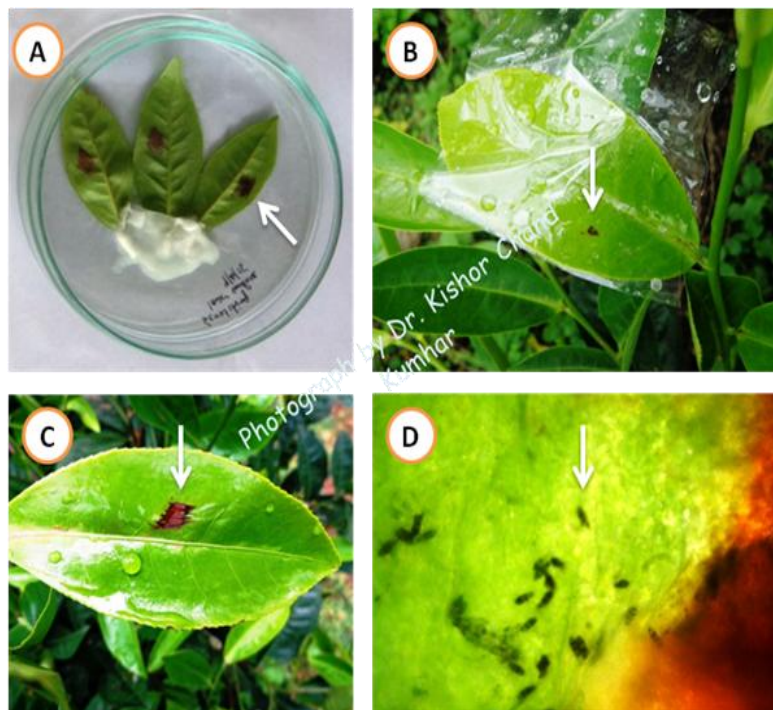


Fig. 2: Pathogenicity of *P. theae* (A: *In vitro*; B-D: *In vivo*).

In vitro bio-efficacy fungicides against pathogen

The results of the *in vitro* bio-efficacy of fungicides against pathogen indicated that all the four tested fungicides affected the growth of *P. theae* to a variable degree. The combi fungicidal formulation (carbendazim @ 12% a. i. plus mancozeb @ 63% a. i.) was found to be the most effective one in controlling the pathogen (76.4%) followed by hexaconazole copper hydroxide and copper oxychloride (Table 2), at their respective recommended doses. Ponmurugan et al. (2006) reported carbendazim as the most effective in controlling *P. theae* followed by dithane M-45. Several systemic as well as contact fungicides viz., tebuconazole, tridemorph, azoxystrobin, hexaconazole, triflumizole, bitertanol, copper oxychloride and copper hydroxide were tested against *P. theae* by Sarkar et al. (2009) and they found that bitertanol and hexaconazole were highly toxic to this pathogen when compared with copper fungicides.

In vitro bio-efficacy of *Trichoderma* spp.

The indigenous *Trichoderma* isolates inhibited the growth of *P. theae* to the tune of 53.8 to 62.5% after 7 days indicating the bio-efficacy of the tested isolates against the pathogen, *P. theae*. However, among the two isolates of *Trichoderma*, the maximum growth inhibition was noted in case of *T. asperellum* (KBN-29) followed by *T. viride* (KBN 24). However, both species were found statistically at par (Table 3). Vidhya Pallavi et al. (2010) noted that under lab conditions, *Trichoderma* spp. exhibited a very good antagonistic potentiality against the grey blight (*Pestalotiopsis* sp.) and wood rot (*Hypoxylon* sp.) pathogens of tea. Similarly, Naglot et al. (2015) tested several isolates of *Trichoderma* spp. and reported their antagonistic potency for the control of this pathogen and these findings are in agreement with our results.

Table 2: Growth inhibitory effect of fungicides on *P. theae*.

Fungicide	Fungicide conc. (ppm)	Fungicide quantity (g/mL) per 100 mL PDA	Per cent growth inhibition over control*
Copper oxychloride 50 WP	2500	0.25	69.4 (56.5 ± 1.3)
Carbendazim 12% a.i + mancozeb 63% a.i.	2500	0.25	76.4 (61.0 ± 1.1)
Hexaconazole 25 EC	1000	0.1	75.9 (60.6 ± 1.0)
Copper hydroxide 77 WP	2500	0.25	71.6 (57.8 ± 1.3)
C.D.			3.6
SE(m)			1.2
SE(d)			1.7
C.V.			4.5

*Values represent mean of 5 replications, figures in parenthesis are angular transformed values with ± standard error.

Table 3: Growth inhibitory effect of *Trichoderma* isolates on *P. theae*.

<i>Trichoderma</i> isolate	Growth inhibition (%) over control after one week*
<i>T. harzianum</i> (KBN-1/14)	57.0 (49.2 ± 0.9)
<i>T. harzianum</i> (KBN-2/14)	53.8 (47.2 ± 1.0)
<i>T. viride</i> (KBN-24)	61.5 (51.5 ± 0.7)
<i>T. asperellum</i> (KBN-29)	62.5 (50.8 ± 1.5)
C.D.	3.2
SE(m)	1.0
SE(d)	1.5
C.V.	4.6

*Values represent mean of 5 replications, figures in parenthesis are angular transformed values with ± standard error

Fungicide compatibility with *Trichoderma* sp.

Result of present investigation on the susceptibility showed that, the recommended concentrations of all tested fungicides inhibited the growth of *T. viride* (Table 4). However, the maximum inhibition (79.0%)

was noted in case of combi fungicide formulation followed by copper oxychloride (75.8%) and hexaconazole (70.4%). Earlier reports also indicated that various triazole fungicides like hexaconazole, propiconazole and penconazole had high inhibitory influence against *T. harzianum* at varying

concentrations (Narayana and Srivastava, 2003). Similarly toxic effect of hexaconazole on *T. viride* and *T. harzianum* was also observed by Johnson (2001). Sarkar et al. (2010) found that systemic fungicides, hexaconazole was the most toxic for *T. harzianum*,

however contact fungicides viz., copper oxychloride and copper hydroxide were lesser toxic to this antagonistic fungus. Bagwan (2010) noted that, thiram, copper oxychloride and mancozeb were less toxic against *T. harzianum* and *T. viride*.

Table 4. Compatibility of fungicides with *T. viride*.

Fungicide	Fungicide concentration (ppm)	Per cent inhibition of antagonist's growth after 96 hrs*
Carbendazim 12% plus mancozeb 63%	2500	79.0 (62.8 ± 1.2)
Copper hydroxide 77 WP	2500	61.6 (51.7 ± 1.5)
Copper oxychloride 50 WP	2500	75.8 (60.5 ± 0.5)
Hexaconazole 25 EC	1000	70.4 (57.1 ± 1.3)
C.D.	-	3.6
SE(m)	-	1.2
SE(d)	-	1.7
C.V.	-	4.6

*Values represent mean of 5 replications, figures in parenthesis are angular transformed values with ± standard error

Conclusion

The findings of the present study showed that, the grey blight disease has a wide occurrence in tea gardens of Dooars region. This pathogen is capable of infecting a large number of tea cultivars with varying degree of infection. Amongst the different fungicides tested, combination of carbendazim plus mancozeb proved to be better for the control of *P. theae*. The indigenous *Trichoderma* isolates could also effectively control this pathogen. Our study also suggested that, this disease could be efficiently managed by adopting integrated management approach, however taking in to account of the susceptibility of the *Trichoderma* spp. to the fungicides, care should be taken to ensure sufficient time gap between the applications of fungicide and *Trichoderma* formulation for achieving better control of the disease.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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