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Biocontrol potential of endophytic fungi in tea (*Camellia sinensis* (L.) Kuntze) leaves against selected fungal phytopathogens

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ABSTRACT

Aims: Endophytic fungi are a diverse group of microorganisms that stay asymptomatically in the healthy tissues of the host. Many fungal endophytes are associated with the tea plant (*Camellia sinensis*) and the pathogens of the tea plant have the potential to grow as endophytes or act as latent pathogens during the initial growth of the plant. The present study aimed at screening tea endophytic fungi with the potential for control of a few critical phytopathogens, *Fusarium* sp., *Lasiodiplodia theobromae*, *Pestalotiopsis* sp. and *Sclerotinia sclerotiorum* while evaluating the efficiency of growth inhibition of these phytopathogens by the endophytic fungal isolates using *in vitro* assays.
Methodology and results: Five endophytic fungal strains; *Colletotrichum gloeosporioides*, *C. siamense*, *Daldinia eschscholtzii*, *Pseudopestalotiopsis chinensis* and *Phyllosticta capitalensis* isolated from leaves of *C. sinensis* in Sri Lanka were evaluated for growth inhibition against plant pathogens; *Fusarium* sp., *L. theobromae*, *Pestalotiopsis* sp. and *S. sclerotiorum* using the dual culture assay and volatile compound-mediated inhibition assay. All the fungal endophytes used in this study exhibited antifungal activity against *Fusarium* sp., *Pestalotiopsis* sp. and *S. sclerotiorum* in the dual culture assay on PDA. *Daldinia eschscholtzii* (67.30%) and *C. gloeosporioides* (61.54%) showed strong antagonistic activity against *S. sclerotiorum*, while *P. capitalensis* (42.30%) demonstrated moderate activity. All the endophytic fungal strains showed moderate antifungal activities against *Fusarium* sp. The percentage growth inhibitions of *Pestalotiopsis* sp. by all the endophytic isolates tested were below 25.00%. In the volatile compound-mediated inhibition assay, none of the endophytic isolates showed visible inhibition against the phytopathogens used.
Conclusion, significance and impact of study: In this study, the fungal endophytes which showed potential antagonistic activity against the tested phytopathogens can be used to develop commercialized products of fungal biocontrol agents (BCAs) for controlling plant fungal diseases.

Keywords: Antagonistic fungi, biocontrol agents, dual cultures, endophytes, fungal pathogens

INTRODUCTION

Tea (*Camellia sinensis* (L.) Kuntze) is a widely cultivated important cash crop throughout the tropical and subtropical regions, including Asia and Africa. It is the most popular beverage in the world and is reported to have a wide range of beneficial physiological and medicinal properties (Namita *et al*., 2012; Win *et al*., 2021).

Endophytic fungi are a diverse group of microorganisms that reside asymptomatically in the healthy tissues of the host. They are well known for their bioactive secondary metabolites that may support the host in defense and survival against pathogenic and insect attacks, stress tolerance and disease resistance (Strobel and Daisy, 2003; Rodriguez *et al*., 2009; Rabha *et al*., 2014; Win *et al*., 2021). Many fungal endophytes are associated with the tea plant and the pathogens of the tea plant have the potential to grow as endophytes or act as latent pathogens during the initial growth of the plant (Fang *et al*., 2013; Xie *et al*., 2020; Thambugala *et al*., 2021). Only when the environmental conditions are favorable for these endophytes or due to a secondary infection or when there are mechanical damages to the plant, these endophytes enter their pathogenic mode and cause diseases (Fisher and Petrini, 1992; Slippers and Wingfield, 2007). Fungal endophytes associated with *C. sinensis* show a higher biocontrol activity against a wide range of pathogenic fungi and bacteria (Zhu *et al*., 2009; Su *et al*., 2010; Rabha *et al*., 2014; Kehelpannala *et al*., 2018). Some tea endophytic fungi have shown potential plant growth-promoting ability as well (Nath *et al*., 2015).

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Endophytic fungus, *C. gloeosporioides* isolated from tea leaves was evaluated for growth inhibition against tea pathogens *Pestalotiopsis theae* and *C. camelliae* by Rabha *et al*. (2014). In that study, *C*. *gloeosporioides* isolate showed strong and moderate antagonistic activity against *P*. *theae* and *C*. *camelliae* respectively. Zhu *et al*. (2014) evaluated the biocontrol potential of two endophytic fungal strains, *Pseudocercospora kaki* and *Penicillium sclerotiorum*, isolated from tea leaves in China, against the rice blast pathogen *Magnaporthe grisea*. The results showed that the endophytic fungal culture broth and its ethyl acetate extract of the dual culture have a stronger inhibition activity against *M*. *grisea* than the monocultures of *P*. *kaki* and *P*. *sclerotiorum*.

Biological control of plant diseases using fungal biocontrol agents (BCAs) has developed considerably in recent years and the applications of BCAs have also been increased for the improvement of agro-industrial processes (Thambugala *et al*., 2020). The present study aims to screen tea endophytic fungi with the potential for control of a few important phytopathogens, *Fusarium* sp., *L. theobromae*, *Pestalotiopsis* sp. and *S. sclerotiorum* while evaluating the efficiency of growth inhibition of these phytopathogens by the endophytic fungal isolates using *in vitro* assays.

MATERIALS AND METHODS

Isolates

Four phytopathogenic fungal isolates, *Fusarium* sp., *L. theobromae*, *Pestalotiopsis* sp. and *S. sclerotiorum* and five tea endophytic fungal isolates *C. gloeosporioides* (UKBC 048), *C. siamense* (UKBC 030), *D. eschscholtzii* (UKBC 036), *P. chinensis* (UKBC 035) and *P. capitalensis* (UKBC 043) were obtained from the Culture Collection of the University of Kelaniya (UKBC), Sri Lanka.

In vitro antifungal activity of the endophytic fungi against selected fungal phytopathogens

Dual culture assay

Antifungal activity of each endophytic isolate against *Fusarium* sp., *L. theobromae*, *Pestalotiopsis* sp. and *S. sclerotiorum* was tested in dual cultures on PDA (Potato Dextrose Agar). Mycelial plugs (5 mm) of the pathogen and the endophytic fungus were inoculated on opposite sides of PDA plates (9 cm in diameter) at approximately one centimeter from the edge and incubated at room temperature (25 ± 2 ºC) for seven days. Plates inoculated only with the pathogens served as controls. Triplicate media plates will be maintained for each treatment. An endophytic fungus capable of producing an inhibition zone against each inoculant of the pathogen in the dual cultures was considered an antagonistic fungus and was selected for further experimentation. The inhibition percentage (I%) was calculated using the formula proposed by Imtiaj and Lee (2008).

$I\% = r_1 - r_2/r_1 \times 100$

r_1 = The radial growth of the pathogen on the control plate; r_2 = The radial growth of the pathogen on the dual culture plate, where both the test endophyte and the pathogen were inoculated.

Volatile compound-mediated inhibition assay

The methods described by Dennis and Webster (1971) and Li *et al*. (2018) were employed with slight modifications to determine how volatile compounds (VCs) of tea endophytes affect the growth of selected phytopathogens.

The 5 mm diameter mycelial plug of 7 days-old culture obtained from the margin of each endophytic fungal isolate was centrally placed on the PDA plates and incubated at 25 ± 1 ºC for 48 h. In control plates, a 5 mm diameter of sterile PDA plug was placed in the dish as done above. At the end of the incubation period, the top of each plate was replaced with the bottom of the PDA plate inoculated centrally with a 5 mm diameter mycelial plug of the pathogen isolates and held together with adhesive tape. Three replicates were set for each treatment. Radial growths of the pathogens were recorded each day and percent inhibitions of average mycelial growth in relation to the growth of their controls were calculated using the above-mentioned formula.

RESULTS

The antagonism of *C. gloeosporioides* (UKBC 048), *C*. *siamense* (UKBC 030), *D. eschscholtzii* (UKBC 036), *P. chinensis* (UKBC 035) and *P. capitalensis* (UKBC 043) against pathogens, *Fusarium* sp., *L. theobromae*, *Pestalotiopsis* sp. and *S. sclerotiorum* was investigated in dual culture tests. All the fungal endophytes used in this study exhibited antifungal activity against *Fusarium* sp., *Pestalotiopsis* sp. and *S. sclerotiorum* to some extent, with the formation of inhibition zones ranging from 4 to 18 mm in width between the antagonistic fungi and the pathogen in the dual cultures on PDA (Table 1 and Figure 1 and 2). No antifungal activity or a clear inhibition zone was shown against *Pestalotiopsis* sp. and *L. theobromae* (data not shown). In addition, no significant growth inhibition was recorded for all phytopathogenic isolates in the volatile compound-mediated inhibition assay (data not shown). The percentage growth inhibition of *Fusarium* sp. was significantly increased (50.00% or more) when all endophytic isolates and *Fusarium* sp. (pathogen) are cultured in dual culture plates. However, significant growth inhibition of *S*. *sclerotiorum* was recorded only for *C*. *gloeosporioides* and *D*. *eschscholtzii* (Table 1). The highest rate of growth inhibition against *Fusarium* sp. and *S. sclerotiorum* were recorded in *C*. *siamense* (56.73%) and *D*. *eschscholtzii* (67.30%) dual culture plates, respectively. The percentage growth inhibition of *Pestalotiopsis* sp. against all the endophytic isolates was below 25.00 % (data not shown).

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Table 1: Percentage growth inhibition (%) of phytopathogens in the dual culture with fungal endophytes on PDA at 7 days.

Endophytic fungi	Growth inhibition (%) of phytopathogens	
	Fusarium sp.	*S. sclerotiorum*
Colletotrichum gloeosporioides	50.96	61.54
Colletotrichum siamense	56.73	36.36
Daldinia eschscholtzii	50.00	67.30
Pseudopestalotiopsis chinensis	53.85	6.00
Phyllosticta capitalensis	50.00	42.30

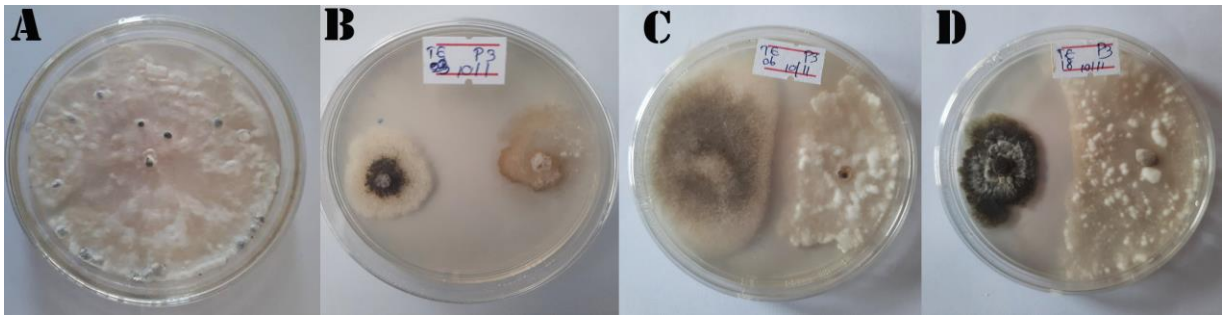

Figure 1: Mycoparasitism of the endophytic fungi used in this study against *S*. *sclerotiorum* at seven days. A. Control. B. *Colletotrichum gloeosporioides*. C. *C*. *siamense*. D. *Phyllosticta capitalensis*.

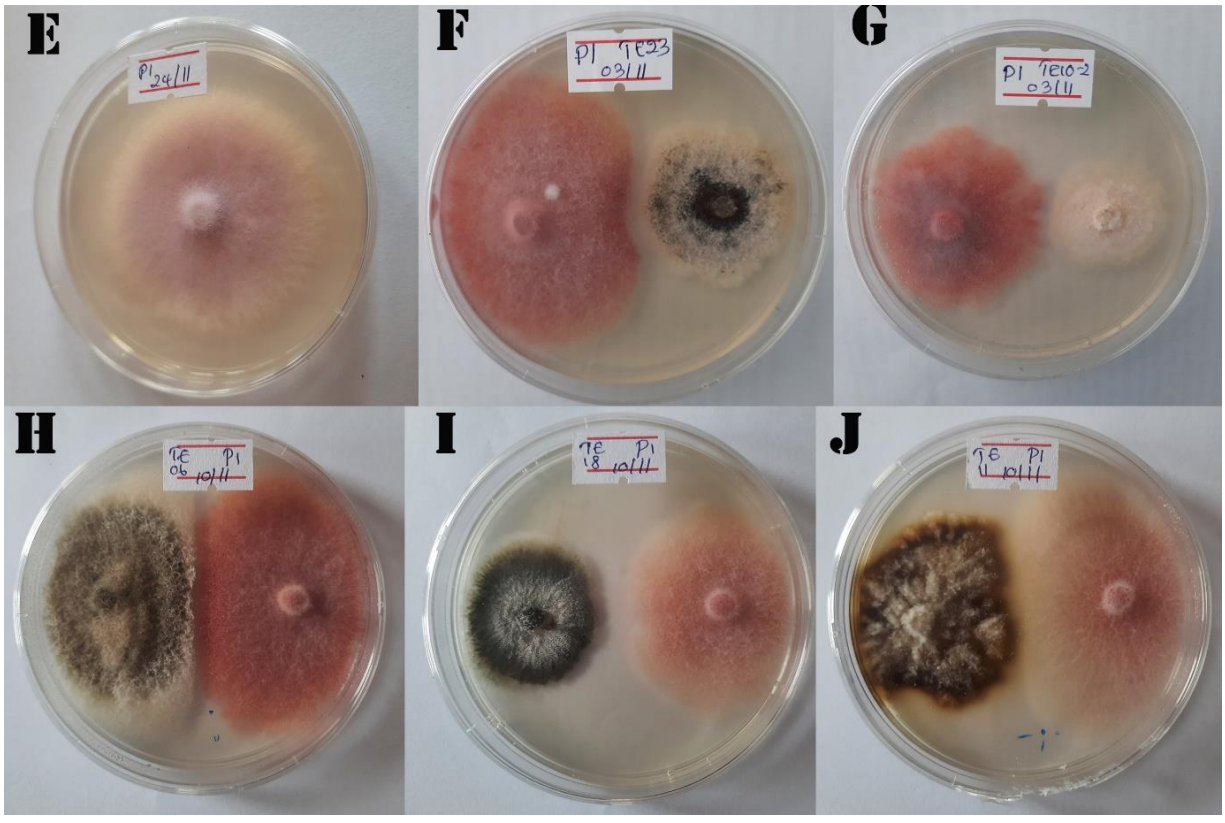

Figure 2: Mycoparasitism of the endophytic fungi used in this study against *Fusarium* sp. at seven days. E. Control. F. *Colletotrichum gloeosporioides*. G. *Pseudopestalotiopsis chinensis*. H. *C*. *siamense*. I. *Phyllosticta capitalensis*. J. *Daldinia eschscholtzii*.

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DISCUSSION

This study identified several tea endophytic fungal isolates obtained from our previous study (Thambugala *et al*., 2021) as potential candidates for controlling *Fusarium* sp. and *S. sclerotiorum*. Fifty percent or more percentage growth inhibition of *Fusarium* sp. was recorded in dual culture plates of all endophytic isolates. However, significant growth inhibition of *S. sclerotiorum* was recorded only for *C. gloeosporioides* and *D. eschscholtzii* (Table 1).

Sclerotinia sclerotiorum is a cosmopolitan, necrotrophic and soil-borne plant pathogen, causing diseases in a wide range of vegetable, ornamental and field crops. This species causes damping-off on seedlings and root and collar-rot in plants and remains dormant forming sclerotia for years in the soil or in host plant debris (El-Gali, 2018; Mahalingam *et al*., 2018). *Fusarium* is a genus of filamentous fungi that contains many important plant pathogens causing blights, cankers, rots and wilts, while almost all *Fusarium* species synthesize mycotoxins (Ma *et al*., 2013; Perincherry *et al*., 2019; Jayawardena *et al*., 2021; Crous *et al*. 2022). However, *Fusarium* is identified as a common and most abundant plant pathogenic genus, to which frequently BCAs are applied for disease control (Thambugala *et al*., 2020). *Sclerotinia* and *Fusarium* species may be controlled through biological agents and many types of research have been carried out to identify effective biocontrol agents for these pathogens (Larkin and Fravel, 1998; Schisler *et al*., 2002; Fernando *et al*., 2007, Kamal *et al*., 2016).

Only a limited number of research have been carried out to evaluate the bio-control potential of tea endophytes in the world. Therefore, this is the first comprehensive study of identifying the biocontrol capabilities of tea endophytes. Recent studies have shown the potential applications of *C. gloeosporioides*, *D. eschscholtzii* and *P. capitalensis* (isolated from different hosts other than *Camellia sinensis*) as biocontrol agents against plant fungal diseases (Sreeja *et al*., 2016; Tran *et al*., 2019; Thambugala *et al*., 2020). For instance, Sreeja *et al*. (2016) carried out an *in vitro* evaluation using fungal endophytes isolated from black pepper against *Phytophthora capsici* which causes Phytophthora foot rot of black pepper and they observed 72% mycelial growth inhibition in a dual culture plate assay. However, the applications of *C. siamense* and *P. chinensis* as bio-control agents are rarely known. In the present study, *C. siamense* and *P. chinensis* exhibit moderate antifungal activity against *Fusarium* sp. The findings from this study open and facilitate several possibilities for future research in the field of biocontrol and biotechnology. Furthermore, fungal extracts and secondary metabolites produced by these fungal species will also play a significant role in future bio-control methods of plant pathogens.

CONCLUSION

Daldinia eschscholtzii and *C. gloeosporioides* showed strong antagonistic activity against *S. sclerotiorum*, while *P. capitalensis* demonstrated moderate activity. All the endophytic fungal strains used in this study showed moderate antifungal activities against *Fusarium* sp. The percentage growth inhibitions of *Pestalotiopsis* sp. by all the endophytic isolates tested were low. The fungal endophytes that showed potential antagonistic activity against the tested phytopathogens in this study can be used to develop commercialized products of fungal biocontrol agents (BCAs) for controlling plant fungal diseases.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare relevant to this article's content.

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