RESEARCH ARTICLE

Antagonistic effect of native *Trichoderma* isolates on economically important foliar pathogens of rubber

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ABSTRACT

Rubber foliar diseases play an important role in latex yield losses of rubber plantations in Sri Lanka. The frequent use of chemical fungicides to control causative pathogens leads to environmental pollution, become hazardous to human and may lead to the development of new chemical resist pathogenic strains. Trichoderma species are the most widely studied bio control agent (BCAs) against many economically important plant pathogens. Hence, an attempt was made to investigate the antagonistic effect of some native Trichoderma strains on the plant pathogenic fungi; Colletotrichum spp., Corynespora cassiicola, Phytophthora spp. and Drechslera heveae. Foliar pathogens were isolated and identified based on the symptoms, cultural and reproductive characteristics. Five Trichoderma strains isolated from different rubber growing soils in Sri Lanka were tested in vitro for their antagonistic effects against the four foliar pathogens. The results obtained from dual culture tests showed that all five Trichoderma strains were effective on the growth of four foliar pathogens. The test antagonists grew faster than the pathogen by limiting their growth. Trichoderma isolate A was the best antagonist against Drechslera heveae, Corynespora cassiicola and Colletotrichum spp. showing percentage of inhibition 75.6, 51.3 and 74.4%, respectively. Isolate B showed the best inhibition percentage (70.9%) against Phytophthora spp. All antagonists showed their lowest inhibition rates against Drechslera heveae. In conclusion, all the tested Trichoderma strains showed antagonistic effects against four foliar pathogens that were tested in this study.

Keywords: *Antagonists, Foliar Pathogens, Percentage of Inhibition, Trichoderma spp.*

INTRODUCTION

Hevea brasiliensis (Willd. A.L. Juss.) Muell.-Arg., commonly called Para rubber belongs to family Euphorbiaceae. Presently, the rubber extent in Sri Lanka is about 131,000 ha having 1.8% total contribution to the world market annually (Central Bank Report, 2013) while sustain the livelihood of over thousands of Sri Lankans by providing many ecological and socio-economic benefits to the country.

Rubber plant can easily get affected by a plethora of diseases caused by plant pathogens, which can lead to reduce the economic yield of the plant directly. Currently, more than sixty pathogens that are capable of attacking rubber

plantations in Sri Lanka have been identified (Jayasinghe and Fernando, 2009). Among all recorded foliar pathogens, the most infectious agent is fungi, which cause a significant latex yield loss (Brimer and Boland, 2003). The diseases of rubber can be categorised in to four major groups as leaf, stem, panel, and root depending on the plant parts affected. Conversely, among all the economically important rubber foliar diseases, the *Colletotrichum* leaf disease, the *Corynespora* leaf disease, the *Phytophthora* leaf fall disease and the birds eye spot leaf disease are the major leaf diseases, which can have a debilitating effect on yield.

The respective causative fungal pathogens for above foliar diseases should be effectively controlled for sustainability of the plantation without creating negative impacts towards both human and environment where the chemical control is being done since the beginning of the 19th century (Peiris, 1966). Application of biological control agents (BCAs) called "Antagonism" seems to be one of the promising disease control approaches currently used against many economically important plant pathogens (Cook, 1985). The term "Antagonism" refers to the action of any organism that suppresses the normal growth and activity of its effective plant pathogen over the time (Cook, 1983). Hence, an attempt was made to investigate the antagonistic effect of some native *Trichoderma* isolates on four strains of fungi; *Colletotrichum* spp., *Corynespora cassiicola, Phytophthora* spp. and *Drechslera heveae,* which are the causative agents of major economically important rubber foliar diseases in Sri Lanka.

The objectives of the study were therefore (i) to identify the antagonistic effect of some native *Tricoderma* isolates on four major foliar pathogens of rubber; *Colletotrichum* spp., *Corynespora cassiicola, Phytophthora* spp. and *Drechslera heveae* and (ii) to isolate and characterise foliar pathogens on their cultural and reproductive features

MATERIALS AND METHODS

Isolation of foliar pathogens

The causal pathogens of four foliar diseases were isolated from the leaves that showed symptoms diseases at Rubber Research Institute, Agalawatta. Infected areas (lesions) of freshly collected leaves were cut into sections having 1 cm^2 dimension. They were surface sterilised in a solution of 70% ethyl alcohol for 3 min and subsequently rinsed in sterile water. The cut sections were thereafter placed on sterilised Petri dishes having Potato Dextrose Agar (PDA) medium. All four cultures were incubated at room temperature under normal light and dark regimes. After two days, emerging mycelia were sub-cultured onto new PDA plates. The identity of each isolate was confirmed by microscopic examination for the shapes and sizes of conidia.

Cultural and reproductive characterisation

The pathogen cultures were identified based on their cultural and reproductive characteristics.

Colony morphology

Petri dishes with PDA were inoculated centrally with mycelial plugs of 5 mm in diameter taken from the edge of 5-d old cultures on PDA. These were sealed with para film and incubated at 25 °C with 12 h alternations of light and dark. Incubation was carried out until four pathogen colonies that reach the edge of Petri dishes. The colony characters; color, shape and the margin were recorded by using the laboratory hand lens.

Reproductive characterisation

Pathogen culture plates, which were incubated for ideal time periods (7-d old *Phytophthora*, 12-d old *Corynesphora cassiicola, Colletotrichum* spp. and *Drechslera heveae*) for the production of required amount of spore population were used to observe the characteristics (size, color, shape and branching pattern) of mycelia, conidia and conidiophores. Microscopic slides were prepared by pipetting 0.02 mL drop of the pathogen culture suspension on to clean glass slides and the slides were observed under the light microscope with 40X magnification. The size of conidia was measured using graticular micrometer and 10 repetitive measurements were taken to calculate the average size of conidium.

Growth rate

Mycelia plugs having exact 5.0 mm diameter were taken from the advancing margin of 7-d old cultures of all four tested foliar pathogens and placed at the center of petri plates containing 10 mL of PDA media. The plates were incubated at room temperature under the normal light and dark regimes. The growth was recorded daily by measuring colony diameter along two perpendicular lines. Four replicates were used in all experiments.

Calculation of spore production

Sterilised petri plates having 10 mL of PDA were inoculated in the center with mycelia plugs removed from actively growing pathogen cultures. Culture plates of *Corynespora cassiicola* (12-d old), *Drechsleraheveae* (12-d old), *Colletotrichum* spp. (10-d old) and *Phytophthora* spp. (7-d old) were flooded with 10 mL sterile distilled water and mechanically disturbed on the colony surface with a paint brush. The resulting suspensions were filtered through muslin cloth and 10 µL of the suspensions were pipetted out on to the surface of haemocytometer. Number of spores was counted under the light microscope with 40X magnification for *Phytophthora* spp. and *Colletotrichum* spp. and under 10X magnification for *C. cassiicola* and *D. heveae*. The concentration of spores in each suspension was calculated and adjusted to spores/mL. Four replicates were used in all experiments.

Screening of the antagonistic effect using dual culture test

Inhibition of the growth of foliar pathogen by the tested antagonists was carried on PDA using the direct opposition method recommended by Dennis and Webster (1971). Petri dishes with PDA medium were placed with 5 mm diameter disks of 5-d old *Trichoderma* mycelium and on the opposite side, 5 mm diameter disks of pathogen mycelium keeping 4 mm distance away from the antagonist. Ten day old *Colletotrichum* spp., *Corynespora cassiicola*, 16 d-old *Drechslera heveae* mycelia and 7-d old *Phytophthora* spp. mycelial plugs were used for the inoculation.

All four pathogens were dual cultured with five native *Trichoderma* strains obtained from Plant Pathology and Microbiology Department, Rubber Research Institute, Sri Lanka and each pathogen-antagonist culture was replicated for three times. Subsequently, four pathogens were individually cultured in PDA plates as control plates separately. The Petri dishes were incubated at 28±2 °C and the growth of the pathogen in both the test and control experiments were observed. The radius of both control pathogen culture and the pathogen in dual culture plate were measured after 6 d of inoculation. The inhibition of mycelial growth in percentage was calculated with the formula suggested by Fokkema (1978);

$$I = \frac{R1 - R2}{R1} \times 100\%$$

where,

I = Percentage of inhibition
R1 = Radius of the pathogen in control
R2 = Radius of the pathogen towards the antagonist

Statistical analysis

The five treatments for the present study were five *Trichoderma* spp. strains. They were distributed on a completely randomized design (CRD) with three replicates. The growth inhibition by antagonists was compared using mean separation using the Duncan Multiple Range Test (DMRT). Significance of the data was determined at $P = 0.05$.

RESULTS AND DISCUSSION

The cultural and reproductive characteristics were determined for the identification of each rubber foliar pathogen individually. The growth rate for each pathogen was as given in the table below (Table 1).

Table 1: Average growth rates of pathogens.

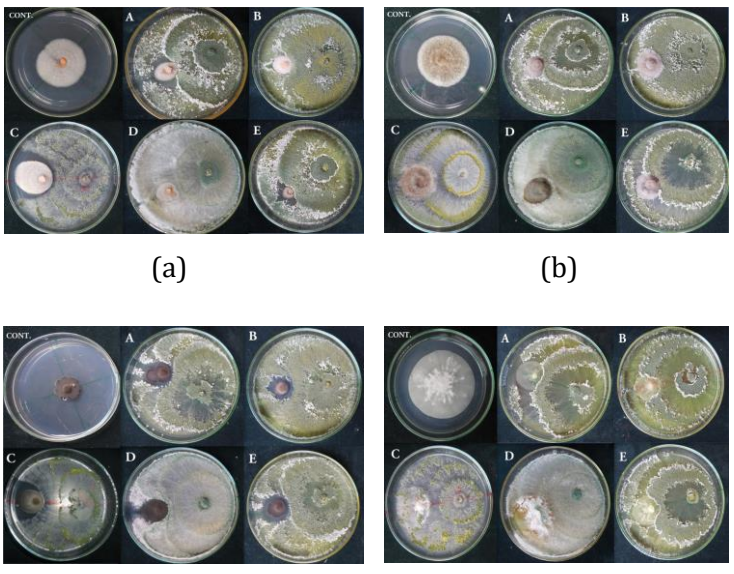
Pathogen	Mean growth rate (mm/d)
Colletotrichum spp.	3.90
Corynespora cassiicola	4.30
Drechslera heveae	2.43
Phytophthora spp.	5.32

The spore production ability of each foliar pathogen was determined by calculating the spore concentration as mentioned in the Table 2.

Table 2: Average spore concentrations of pathogens.

Pathogen	Average spore count (X)	Spore concentration (conidia/mL) (X x 10^4)
Colletotrichum spp.	108.0	108 x 10^4
Corynespora cassiicola	0.8	75 x 10^2
Drechslera heveae	3.8	38 x 10^3
Phytophthora spp.	12.0	12 x 10^4

The results obtained from the dual culture assay indicated that the colony diameters of all four foliar pathogens were significantly (P<0.05) affected by all five *Trichoderma* isolates (Plate 1).



(a) (b)

(c) (d)

Plate 1: Growth inhibitions of dual culture plates (a) *Colletotrichum* spp. (b) *Corynespora cassiicola* (c) *Drechslera hevea*, (d) *Phytophthora* spp.

The percentages of inhibition calculated for each *Trichoderma* isolate against foliar pathogens are as shown in the Table 3.

Table 3: Effect of *Trichoderma* isolates on the radial growth of foliar pathogens.

Pathogen	Antagonist	Inhibition (%)
Colletotrichum spp.	A	75.63
	B	67.51
	C	68.86
	D	62.09
	E	70.22
Corynespora cassiicola	A	74.47
	B	72.03
	C	64.74
	D	58.76
	E	48.93
Drechslera heveae	A	51.34
	B	45.93
	C	45.93
	D	49.99
	E	32.41
Phytophthora spp.	A	67.99
	B	70.99
	C	66.99
	D	61.99
	E	69.99

Radial growth of the pathogen was considerably hindered by all the test antagonists under the conditions of this study. A microorganism as a biological control agent may express different mechanisms against pathogens during their antagonistic activity; by weakening and destroying the pathogen by parasitism, by producing antimicrobial compounds, by competing for space and nutrients, by producing enzymes that attack the cell components of the pathogens. In this study, the antagonistic effect expressed by the *Trichoderma* spp. in dual culture method might be due to the one or combination effects of two or more above mechanisms. Further research has to be carried out to find the exact reason for the antagonism effect of *Tricoderma* spp. against the tested pathogens.

CONCLUSIONS

The pathogen cultures; *Corynesphora cassiicola*, *Colletotrichum* spp., *Phytophthora* spp. and *Drechslera heveae* were successfully identified using the macroscopic and microscopic features of cultural and reproductive characteristics. All the five *Trichoderma* isolates effectively checked against the growth of the four foliar

pathogens. *Trichoderma* isolate A was the best antagonist against *Drechslera heveae, Corynespora cassiicola* and *Colletotrichum* spp. showing percentage inhibition of 75.6, 51.3 and 74.5%, respectively. Isolate B showed the best inhibition rate (70.9%) against *Phytophthora* spp. All antagonists showed their lowest inhibition rates against *Drechslera heveae*. Therefore, the fungal strains can be used for further investigations under greenhouse and field conditions to confirm the feasibility of utilising biological control agent for the management of rubber foliar pathogens.

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