International Journal of Agriculture, Environment and Bioresearch

Vol. 4, No. 01; 2019

ISSN: 2456-8643

ANTAGONISTIC EFFECT OF SOME NATIVE TRICHODERMA ISOLATES ON ECONOMICALLY IMPORTANT FUNGAL PATHOGENS OF TEA (Camellia Sinensis (L.) O. Kuntze) IN SRI LANKA

PDPMD Silva and G. Chandrasena

[1]Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University of Sri Lanka, Badulla, 90 000, Sri Lanka

ABSTRACT

Tea plants are subjected to serious fungal diseases which cause significant yield losses. The frequent use of chemical fungicides to control causative pathogens may lead negative impacts to both human and environment. Trichoderma species are noted for their bio control capabilities against many economically important plant pathogens. Hence, an attempt was made to investigate the antagonistic effect of three (03) Trichoderma strains (Trichoderma asperellum, Trichoderma viride and Trichoderma harzianum) on six (06) fungal pathogens (Colletotrichum camelliae, Pestalotia theae, Calonectria theae, Rhizoctonia solani, Phomopsis theae, Macrophoma theicola) of Tea. Isolated pathogens of Tea and tested antagonists were identified at their species level based on their phenotypic traits such as colony morphology, growth rate and vegetative and reproductive structures in In-vitro conditions and the pathogenicity of each isolates were confirmed using pathogenicity test at In-vivo conditions. Antagonistic effect of tested Trichoderma strains against isolated pathogens were calculated in terms of Percentage of Inhibition resulted from the Dual Culture Test. The percentage of inhibition of Trichoderma asperellum, Trichoderma viride and Trichoderma harzianum against all six pathogens were calculated in-between 63.41% to 79.76%, 75.75% to 88.88%, and 73.11% to 82.51% and each were significant at p values 0.05 as 0.0000, 0.018 and 0.001 respectively. In conclusion, all three antagonists showed more than 50% antagonism over the six pathogens concerned as they grew in faster rate than the pathogens and the Trichoderma viride was identified as the best antagonist against all tested pathogens of Tea.

Keywords: Antagonistic Effect, Trichoderma spp., Tea, Fungal Pathogens, Dual Culture Test

1. INTRODUCTION

Tea (*Camellia sinensis*) is the most popular and natural beverage produced from young leaves of the commercially cultivated tea plant. The majority of the recorded leaf diseases of tea and all the economically important ones are caused by fungi (Handbook on Tea, 2008). Crop losses due to these diseases are substantial as they lead to loss the yield and income. The use of some systemic fungicides, besides being expensive and creating risks to the environment, is not totally effective and may lead to the appearance of new, resistant strains of pathogens. It is therefore necessary to develop alternative ways of disease control. One such alternative is biological control, in which microorganisms are selected for their ability to antagonize pathogens.

Vol. 4, No. 01; 2019

ISSN: 2456-8643

Trichoderma species are ubiquitous soil-borne Ascomycetes noted for their bio control capabilities against many economically important plant pathogens (Kumara *et al*., 2017). Antagonist microorganisms, such as *Trichoderma* can reduce growth, survival or infections caused by pathogens by disturbing different mechanisms of them like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion. And also they have ability to increase root growth and development, crop productivity, resistance to abiotic stresses, uptake and use of nutrients and to make more favorable micro climate to crop (Kumara *et al*., 2017). Therefore, the present experiment will undertake to evaluate the efficacy of inhibition of mycelial growth of *Colletotrichum camelliae, Pestalotia theae, Calonectria theae, Rhizoctonia solani, Phomopsis theae,* and *Macrophoma theicola* by some native *Trichoderma* isolates (*Trichoderma asperellum, Trichoderma viride* and *Trichoderma harzianum*) thereby to use them in future as biological control agents for combatting Brown blight, Grey blight, Black blight, Collar and Branch Canker, Stem and Branch Canker diseases respectively in Tea fields as an economically feasible and environmentally friendly disease management methodology.

Objective

The main objective of the study was to identify the antagonistic effect of some native *Tricoderma* isolates on economically important fungal pathogens of Tea.

Thus, the specific objectives were to isolate and identified some native *Tricoderma* isolates and fungal pathogens of Tea based on their cultural and reproductive features using in vitro techniques and to prove the pathogenicity of each pathogen before testing them for Antagonism.

2.MATERIALS AND METHODS

Collection of Test Pathogens and Antagonists

The test pathogens, *Colletotrichum camelliae, Pestalotia theae, Calonectria theae, Rhizoctonia solani, Phomopsis theae, Macrophoma theicola* were collected from the diseases tea leaves based on the disease symptoms of Brown blight, Grey blight, Black blight, Collar and Branch Canker, Stem and Branch Canker diseases accordingly. Tested antagonists (*Trichoderma asperellum, Trichoderma viride* and *Trichoderma harzianum*) were collected from the soil samples of respective tea lands around Passara region, Sri Lanka.

Isolation and Identification of Test Pathogens and Antagonists

The causal pathogens of six (06) Tea diseases were isolated from the leaves that showed the symptoms of diseases at the Microbiology Laboratory, Uva Wellassa University of Sri Lanka. Infected areas of freshly collected leaves were cut into sections having 1 cm^2 dimension. They were surface sterilized in a solution of 70% Ethyl alcohol for 3 minutes and subsequently rinsed in sterilized water. The cut sections were thereafter placed on sterilized Petri dishes having 25 ml of Potato Dextrose Agar (PDA) medium. All six pathogens were isolates on three replicates and cultures were incubated at room temperature under normal light and dark regimes. After two days, emerging mycelia were sub-cultured on to new PDA plates for purification. The identity of each isolate was confirmed by observing Macroscopic and Microscopic features of Mycelia and Conidia of each pathogen with reference to the pure culture of each organism obtained from Tea Research Institute, Sri Lanka. The pathogenicity of the tested organisms were also confirmed by using Koch's Postulates on Healthy Tea plants in the plant house at Uva Wellassa University of Sri Lanka while maintaining the environment condition similarly to the selected tea fields.

Vol. 4, No. 01; 2019

ISSN: 2456-8643

The collected soil samples were used to isolate the antagonists using the mono-spore cultures technique following the serial dilutions and PDA culture medium. Further, *Trichoderma* strains were identified comparing the macro and micro colony morphology of pure cultures obtained from the Horticultural Research and Development Institute, Gannoruwa, Sri Lanka.

Screening of the Antagonistic Effect using Dual Culture Test

The antagonistic activity of *Trichoderma* isolates against test organisms were tested by using Dual Culture Method described by Cherif and Benhamou, (1990). Petri dishes with 25 ml of PDA medium were placed with 5 mm diameter disks of 5 days old pure *Trichoderma* mycelium and on the opposite side, 5 mm diameter disks of pathogen mycelium keeping 4 mm distance away from the antagonist. Approximately, 10 days old *Colletotrichum camelliae, Pestalotia theae, Calonectria theae,* and *7* days old *Rhizoctonia solani, Phomopsis theae, Macrophoma theicola* were used for the inoculation. All six pathogens were dual cultured with three native *Trichoderma* strains and each pathogen-antagonist culture was replicated for three times. Subsequently, six pathogens were individually cultured in PDA plates as control plates. The petri dishes were incubated at 28 ± 2 ℃ and the growth of the pathogen in both the test and control experiments were measured 5, 7 and 9 days from the inoculation.

The effect of *Trichoderma* strains on fungal pathogens of Tea was determined by the percentage of mycelia growth inhibition in centimeter calculated with the follow formula;

$$\text{Inhibition (\%)} = [(D1-D2) / D1] \times 100$$

Where;

D1 = radius growth of the phyto-pathogen in the absence of antagonist

D2 = radius growth of the phyto-pathogen in the presence of antagonist

The days of contact between fungal pathogen and the antagonist and the antagonistic ability of each *Trichoderma* isolates were also measured according to the methodology proposed by Bell *et al*. (1982).

Statistical Analysis

The test was conducted in completely randomized design (CRD) with three replicates. The growth inhibition by antagonists were compared using mean separation using the Turkey Multiple Range Test using Minitab 17. Significance of the data was determined at $p = 0.05$.

3.RESULTS

Individual colony morphology for each tested antagonists and fungal pathogens of Tea are shown in the Fig 1 and 2 respectively.

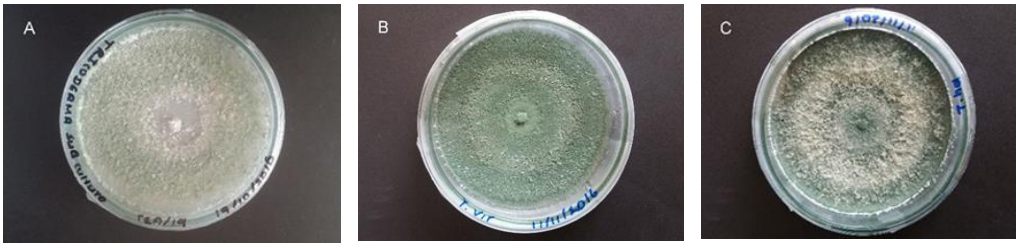

Figure 1: Colony morphology of *Trichoderma* strains used in the study
(A: *Trichoderma asperellum,* B: *Trichoderma viride,* C: *Trichoderma harzianum)*

Vol. 4, No. 01; 2019

ISSN: 2456-8643

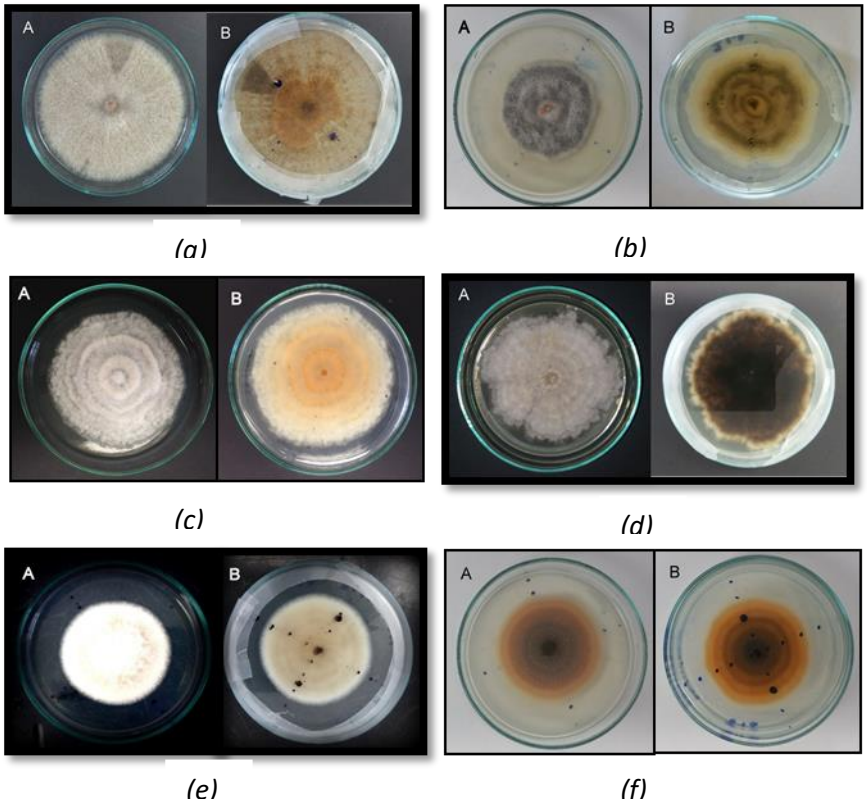

Figure 2: Colony morphology of fungal pathogens used in the study (A: Upper surface B: Lower surface *(a) Colletotrichum camelliae, (b) Pestalotia theae, (c) Calonectria theae, (d) Rhizoctonia solani, (e) Phomopsis theae, (f) Macrophoma theicola*)

The percentages of inhibition calculated for each *Trichoderma* isolate against six fungal pathogens are given in the Table 1.

Table 1: Effect of *Trichoderma* isolates on the radial growth of each fungal pathogen

Pathogen	Antagonist	Inhibition (%)
Colletotrichum camelliae	A	79.76
	B	88.88
	C	80.16
Pestalotia theae	A	69.89
	B	80.64
	C	73.11

Vol. 4, No. 01; 2019

ISSN: 2456-8643

Calonectria theae	A	66.23
	B	75.75
	C	74.88
Rhizoctonia solani	A	63.41
	B	81.70
	C	82.51
Phomopsis theae	A	71.66
	B	80.55
	C	73.88
Macrophoma theicola	A	64.64
	B	82.31
	C	72.25

The results obtained from the dual culture assay indicated that the colony diameters of all six fungal pathogens were significantly ($p<0.05$) affected by all three *Trichoderma* isolates as shown in the Fig 3 below.

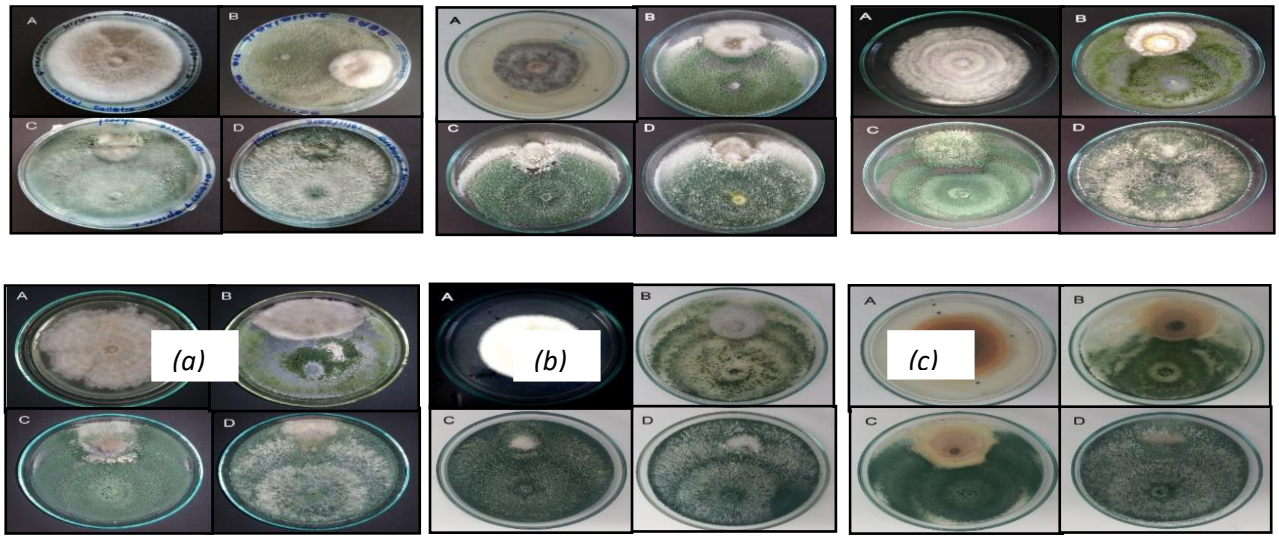

Figure 3: Growth inhibitions of dual culture plates of *(a) Colletotrichum camelliae, (b)Pestalotia theae, (c) Calonectria theae, (d) Rhizoctonia solani, (e) Phomopsis theae, (f)*

Vol. 4, No. 01; 2019

ISSN: 2456-8643

***Macrophoma theicola*) after 7 days of inoculation. A- Control Plate, B, C and D - Dual culture plates**

Discussion

Radial growth of the fungal strains was considerably hindered by all the test antagonists under the conditions of this study. A microbial biological control agent may express different mechanisms against pathogens during their antagonistic activity; by weakening, 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 of all the above mechanisms. Further studies have to be carried out to find the exact reason for the antagonism effect of *Tricoderma* spp. against the tested pathogens.

4.CONCLUSION

The pathogen cultures; *Colletotrichum camelliae, Pestalotia theae, Calonectria theae, Rhizoctonia solani, Phomopsis theae, Macrophoma theicola* were identified at their species level by using some macroscopic and microscopic features. The results obtained from dual culture tests showed that all the three *Trichoderma* strains effectively inhibit the growth of the fungal strains caused disease of Tea. *Trichoderma viride* was the best antagonist against all tested pathogens. It has exhibited 75.75 % to 88.88% inhibition rate over the test pathogen while *Trichoderma harzianum* and *Trichoderma asperellum* shown 63.41 %- 79.76% and 73.11% - 82.51% inhibition rates respectively. In conclusion, all the tested *Trichoderma* species showed antagonistic effect on the all pathogens under investigation. Therefore, the study reveals that the *Trichoderma* strains can be used as biological control agent (BCA) to control fungal pathogens of Tea as an effective disease control method.

List of Abbreviations

PDA – Potato Dextrose Agar, CRD – Completely Randomized Design, BCA – Biological Control Agent, ^{o}C – Celsius, cm^2 – Square centimeters, ml – Milliliters

Funding

This one year research project could able to secured LKR 500000.00 funds from the University Capital Grant in the year 2016 under the research grant no. UWU/RG/2016/07 and the title of "Antagonistic Effect of some Native *Trichoderma* Isolates on Economically Important Foliar Pathogens of Tea" and the Research Committee of the Uva Wellassa University of Sri Lanka expected to published the research works carried out with its financial supports in well recognized journals.

5. Acknowledgements

Authors should acknowledge Uva Wellassa University of Sri Lanka for providing sufficient funds and required laboratory facilities throughout the research period, Tea Research Institute, Thalawakele, Sri Lanka and Horticultural Research and Development Institute, Gannoruwa, Sri Lanka for providing their expertize to identify the tested antagonists and pathogens and Balangoda Plantation PLC, Sri Lanka for giving the opportunity to collect required samples for the study from their Tea Estates around Passara, Sri Lanka.

Vol. 4, No. 01; 2019

ISSN: 2456-8643

REFERENCES

Ahmad, I., Obaidullah, M., Hossain, M. A., Ali, M., & Islam, M. S. (2013). In-vitro biological control of branch canker (*Macrophoma theiocola*) disease of tea. International Journal of Phytopathology, 2(3), 163-169.

Arulpragasam P. V., 2008. Common Disease of Tea and their Management: Handbook on Tea. (1st Ed) A. K. N. Zoysa. Tea Research Institute of Sri Lanka, Thalawakelle. 173-209

Balasuriya A., 2003. 75 years of research and development in plant pathology. In: Twentieth Century Tea Reseach in Sri Lanka. (Ed) W. W. D. Modder. Tea Research Institute of Sri Lanka, Thalawakelle. 165-203

Brimner T, A. and Boland G, J. (2003). A review of the non-target effects of fungi used to biologically control plant diseases. Agriculture Ecosystem and Environment, (100), pp.316.

Chapela, I. H. (1991). Spore size revisited: analysis of spore populations using automated particle size. *Sydowia*, *43*, 1-14.

Cook, R., J. and Baker, K., F. (1983). The Nature and Practice of Biological Control of Plant.

Damm, U., Woudenberg, J. H. C., Cannon, P. F., & Crous, P. W. (2009). *Colletotrichum* species with curved conidia from herbaceous hosts. Fungal Diversity, *39*, 45.

Dennis C, Webster J (1971). Antagonistic properties of species groups of *Trichoderma* II. Production of non-volatile antibiotics. Transactions of the British Mycological Society. 57(1): 41-48.

Dennis, C., and Webster, J., (1971). Antagonistic properties of species-groups of T*richoderma*. Hyphal interaction III. Transactions of the British Mycological Society 57, 363-369.

Fokkema, N, J. (1978). Fungal Antagonism in the phylosphere. *Ann. Appl. Biol*, 89, pp.115-117.

Holmes, K.A., Schroers, H.J., Thomas, S.E., Evans, H.C., Samuels, G.J., (2004). Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. Mycological Progress 3, 199–210.

Karmokar, B. C., Ullah, M. S., Siddiquee, M. K., & Alam, K. M. R. (2015). Tea Leaf Diseases Recognition using Neural Network Ensemble. International Journal of Computer Applications, 114(17).

Kumar, Gagan & Maharshi, Anupam & Patel, Jai & Mukherjee, Arpan & Singh, Harikesh & Sarma, Birinchi. (2017). Trichoderma: A Potential Fungal Antagonist to Control Plant Diseases. SATSA Mukhapatra - Annual Technical Issue. 206-218.

Loos, C. A. (1950). Calonectria theae n. sp.—the perfect stage of *Cercosporella theae* Petch. Transactions of the British Mycological Society, 33(1), 13-18.

Ozbay, N. and Newman, S.E, 2004. Biological Control with Trichoderma Spp. With Emphasis on T. harzianum. Pakistan Journal of Biological Sciences, 7, pp.78-84