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In vitro efficacy of *Trichoderma harzianum* against major fungal pathogens of Teak and Mahogony seedlings

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ABSTRACT

Trichoderma an asexually reproducing filamentous fungi commonly found in varied soil types in all climatic zones. Their ability to grow and multiply rapidly in various substrates makes it a good biocontrol agent. Six isolates of *Trichoderma harzianum* from rhizoplane regions of grasses *Cynodon dactylon* (CD-01, CD-02 and CD-03) and *Paspalum conjugatum* (PC-01, PC-02 and PC-03) were tested for their antagonistic activity against major fungal pathogens - *Fusarium oxysporum* and *F. solani* causing root rot and wilt, *Sclerotium rolfsii* causing damping off and *Colletotrichum gloeosporiodes, Curvularia lunata* and *Pestalotiopsis macqulens* causing foliar diseases of Teak and Mahogany seedlings from Central Nurseries of Kerala by dual culture method. All the isolates showed antagonistic activity against *Fusarium oxysporum* (53-70%), *F. solani* (60-72%), *Sclerotium rolfsii* (51-62%), *Colletotrichum gloeosporiodes* (52-75%), *Curvularia lunata* (47-70%) and *Pestalotiopsis macqulens* (61-67%). Among the *T. harzianum* isolates tested PC-03 was found to exhibit minimal antagonistic activity. However, *Trichoderma* – Pathogen interaction showed variations indicating the activity of *Trichoderma* isolates varied to a greater or lesser extent depending upon the pathogenic species.

Keywords: Fungal diseases, *Trichoderma harzianum*, Rhizoplane and Antagonism

INTRODUCTION

Teak (*Tectona grandis* L.) and Mahogony (*Swietenia macrophylla* King) are mainly known for their timber and a number of nurseries have been established for producing healthy plant stocks. A number of factors affect their successful out plantings. Among seedling diseases are the major ones of which fungi being the primary pathogenic agent (Bakshi, 1976; Bloomberg, 1985). Rots, Wilts, damping off and various foliar diseases are the major diseases found in seedlings. Chemical agents have been practised for their assured results but indiscriminate use has resulted in environmental pollution. Alternative strategy has been in search there by minimise pollution and other hazards caused by chemicals.

Biological control is being applied as an alternative and a number of microbes have been found to show potentiality as bio-controlling agents. Microbes can be found on the leaves, roots, soil adjacent to roots and even inside plant tissues as endophytes and their interactions provide a wide array of opportunities to explore the complexities in association as well as their interaction in the growth and development. Rhizosphere and Rhizoplane inhabiting micro-organisms competitiveness for water, nutrients and space plays an important role in the growth and ecological fitness of their host (Hartmann *et al*. 2009).

Trichoderma a filamentous fungi have been extensively studied for its potentiality as an antagonistic agent (Henis and Chet, 1975; Hadar *et al.,* 1979 and Elad *et al.,* 1980). Their ability to successively thrive in diverse environment and easy to isolate the species makes it an important biocontrol agent. Besides antagonising, their role in plant growth promotion and inducing defence mechanism have also been reported (Harman *et al.,* 2004 and Vinale *et al.,* 2009).

Grasses form an important component of ecosystem which keeps rejuvenating with each growing season. They produce fibrous roots which homes abundance of diverse microbes. A great diversity of rhizosphere and rhizoplane microorganisms have been described and also in many cases been used as bio-control agents. The present work has been carried out to study the antagonistic activity of rhizoplane fungi *Trichoderma* against Fungal pathogens of Teak and Mahogany seedling diseases from Central Nurseries of Kerala *in vitro*

MATERIALS AND METHODS

Isolation of pathogenic fungi

Disease survey have been carried out in Central nurseries of Kerala located at Cheruvanchery, Valluvassery, Chettikulam and Kulathupuzha. Infected samples were collected and were taken to the laboratory. The samples were washed thoroughly, blotted and were inoculated on antibiotic amended PDA medium. The pathogen was isolated and identified by referring to standard manuals (Arx, 1981; Barnett and Hunter, 1972; Domesch and Gams, 1972; Ellis and Ellis, 2001 and Gilman, 1994).

Isolation of *Trichoderma* sp. from rhizoplane region

Trichoderma sp. were isolated from roots of grass species *Cynodon dactylon* and *Paspalum conjugatum.* Root samples were collected from Northern Kerala parts of Western Ghats, washed in slow running tap water, blotted and were fragmented into 1 cm long segments. Root segments were inoculated on antibiotic amended PDA medium. Fungal colonies were isolated and identified by referring to standard manuals as described earlier.

***In vitro* antagonism by dual culture technique**

Pathogenic fungi isolated from Teak and Mahogany seedlings and test rhizoplane fungi from grasses *Cynodon dactylon* and *Paspalum conjugatum* were cultured on their respective medium under 12/12 hr light and dark cycle at 23±2°C for five days. Five mm diameter disc of selected fungi from grass and test pathogen were taken from the growing edge of a five-day-old pure culture using a cork borer. The control plates were inoculated with the pathogen and antagonists separately. Petri-dishes were incubated at 23±2°C and daily growth measurements of fungal colonies were recorded for seven days. The percentage inhibition of radial growth of the pathogen was calculated using a formula by Vincent (1947).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 – Test organism in Control
R_2 – Test organism in Dual culture

Statistical analysis

Antagonistic ability of *Trichoderma* isolates were statistically analysed and compared by Duncan's Multiple Range Test (DMRT) using SPSS (ver. 21) software developed by IBM Corporation.

Evaluation of Antagonism

The evaluation of antagonism between the *Trichoderma* and the test pathogen was scored 1-5 (Bell *et al*., 1982). The cultures were observed after seven days of incubation. The given isolate of *Trichoderma* was considered to be antagonist if the score was ≤ 2 and not highly antagonist if the score was ≥ 3.

Colony Interaction	Type of Antagonism
Complete overgrowth of the antagonist over the pathogen	1
75% overgrowth of the antagonist over the pathogen	2
Both the antagonist and the pathogen grow 50% and neither organism dominate	3
75% overgrowth of the pathogen and withstand antagonism	4
Complete overgrowth of the pathogen	5

RESULTS AND DISCUSSION

Disease survey conducted in the Central Nurseries and incubation of samples for the associated pathogens resulted in the isolation of *Fusarium oxysporum* and *F. solani* causing root rot and wilt, *Sclerotium rolfsii* causing damping off, *Colletotrichum gloeosporiodes* causing leaf spots and blights, *Curvularia lunata* causing leaf spots and *Pestalotiopsis macqulens* causing leaf spots to be major symptoms associated with Teak and Mahogany seedlings. *Trichoderma harzianum* isolated from grasses *Cynodon dactylon* (CD-01, CD-02 and CD-03) and *Paspalum conjugatum* (PC-01, PC-02 and PC-03) (Table-1) (Fig-1) were tested for their antagonistic activity against Teak and Mahogany fungal pathogens (Table 2) (Fig-2). The isolates showed inhibition against *Fusarium oxysporum* (53-70%), *F. solani* (60-72%), *Sclerotium rolfsii* (51-62%), *Colletotrichum gloeosporiodes* (52-75%), *Curvularia lunata* (47-70%) and *Pestalotiopsis macqulens* (61-67%). Among the isolates PC-03 was found to exhibit minimal inhibitory activity.

Table 1. Morphological characteristics of *Trichoderma harzianum* isolates isolated from rhizoplane regions of grasses

SL. no.	Grass species and *T. harzianum* Isolate No.	Culture characteristic
1	*Cynodon dactylon* CD-01	Colony initially white with 11mm growth per day later turning into yellow and finally to green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
2	*Cynodon dactylon* CD-02	Colony initially white with 10mm growth per day later turning into green. Reverse light coloured. Phialides (8-11 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
3	*Cynodon dactylon* CD-03	Colony initially white with 14mm growth per day later turning into green and finally to dark green. Reverse light coloured. Phialides (5-8 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
4	*Paspalum conjugatum* PC-01	Colony initially white with 10mm growth per day later turning into yellow and finally to light green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
5	*Paspalum conjugatum* PC-02	Colony initially white with 11mm growth per day later turning into green and finally to dark green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
6	*Paspalum conjugatum* PC-03	Colony initially white with 11mm growth per day later turning into light green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)

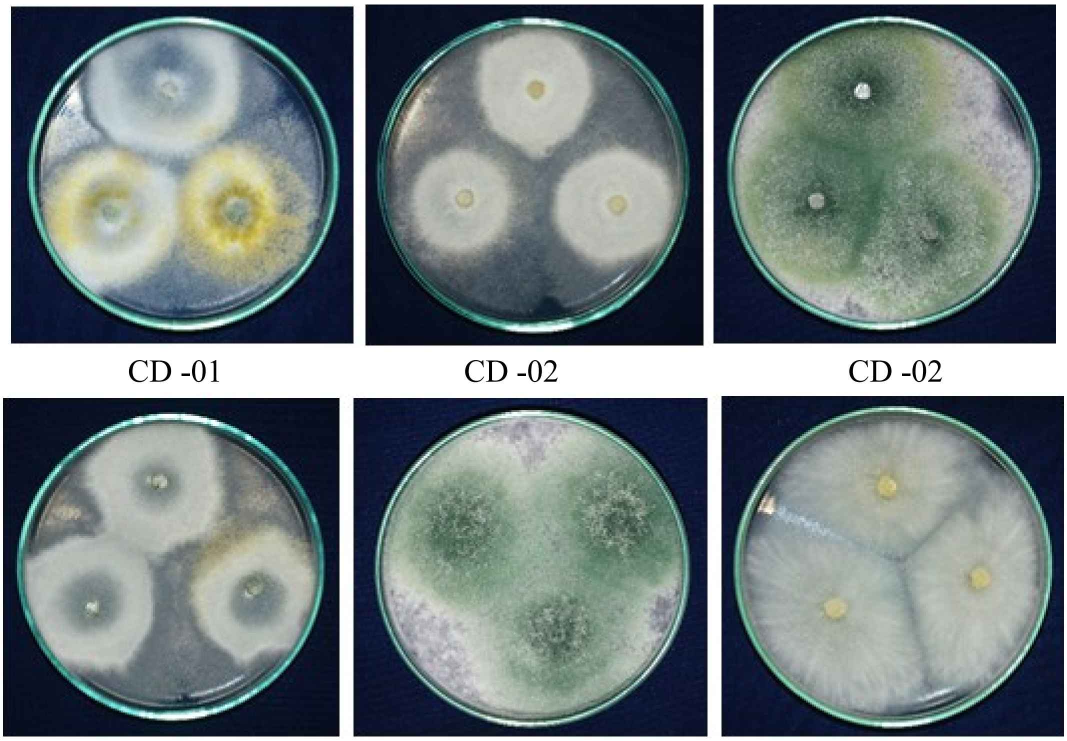

Fig 1. *Trichoderma harzianum* isolates at Five days of incubation

Table 2. Antagonistic activity of *Trichoderma* isolates and reaction types against fungal pathogens

Trichoderma harzianum isolates	Percent inhibition and colony interaction types against fungal pathogens											
	F O		*F S*		*S R*		*C G*		*C L*		*P M*	
	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT
CD-01	69.23 ± 0.48[1] e[2]	-	68.57 ± 0.78[1] b[2]	1	60.00 ± 0.23[1] c[2]	3	75.00 ± 0.54[1] c[2]	1	58.82 ± 0.78[1] b[2]	1	61.90 ± 0.36[1] a[2]	1
CD-02	57.69 ± 0.56[1] b[2]	-	60.00 ± 0.86[1] a[2]	1	60.00 ± 0.36[1] c[2]	3	75.00 ± 0.16[1] c[2]	2	64.70 ± 0.54[1] d[2]	1	66.60 ± 0.42[1] a b[2]	1
CD-03	65.38 ± 0.96[1] d[2]	-	68.57 ± 0.18[1] b[2]	1	62.20 ± 0.45[1] d[2]	3	75.00 ± 0.77[1] c[2]	1	70.50 ± 0.49[1] e[2]	1	64.28 ± 0.13[1] a b[2]	1
PC-01	61.53 ± 0.98[1] c[2]	-	71.43 ± 0.73[1] c[2]	1	62.20 ± 0.95[1] d[2]	3	72.50 ± 1.10[1] b[2]	1	64.70 ± 0.76[1] d[2]	1	64.28 ± 0.47[1] a b[2]	1
PC-02	57.69 ± 0.53[1] b[2]	-	71.43 ± 0.73[1] c[2]	1	51.10 ± 0.57[1] b[2]	3	72.50 ± 0.87[1] b[2]	1	61.76 ± 0.83[1] c[2]	1	64.28 ± 0.49[1] a b[2]	2
PC-03	53.84 ± 0.69[1] a[2]	-	60.00 ± 1.13[1] a[2]	2	-	5	52.50 ± 0.69[1] a[2]	2	47.05 ± 0.62[1] a[2]	3	64.28 ± 0.77[1] b[2]	3

Data is an average of three replicates

*RT - Reaction Type [1] Standard deviation

[2] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

F O- Fusraium oxysporum, F S- Fusarium solani, S R- Sclerotium rolfsii, C G- Colletotrichum gloeosporioides, C L- Curvularia lunata, P M- Pestalotiopsis macqulens

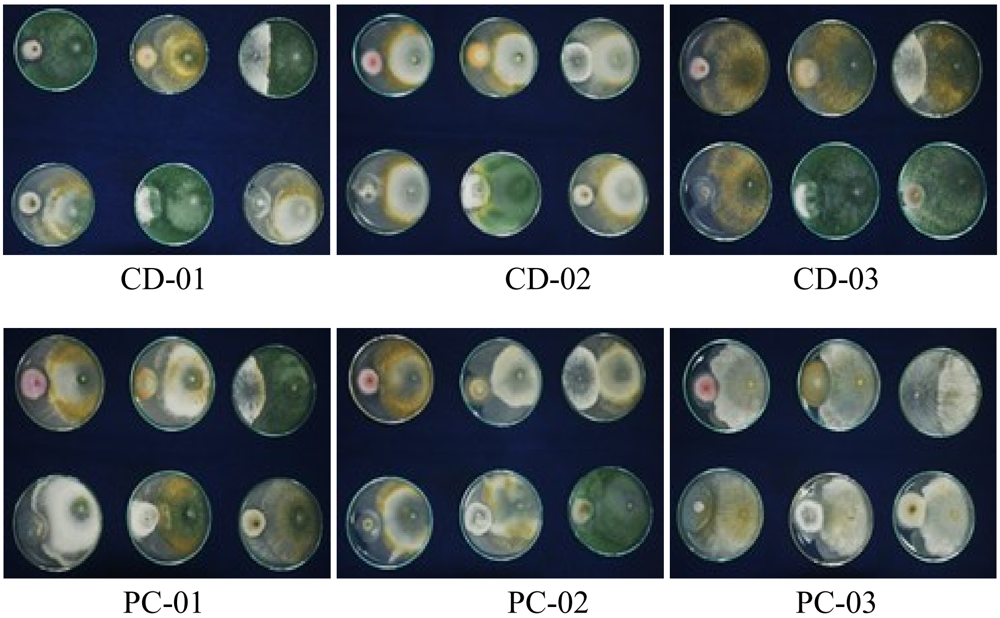

Fig 2. Antagonistic activity of *Trichoderma harzianum* isolates against fungal pathogens *Fusraium oxysporum, Fusarium solani, Sclerotium rolfsii, Colletotrichum gloeosporioides, Pestalotiopsis macqulens and Curvularia lunata*

Various species of *Trichoderma* namely *T. koningii*, *T. harzianum* and *T. viride*, respectively have been studied for their antagonistic activity *in vitro* (Mathew and Gupta, 1998; Prasad *et a*l., 1999; Bunker and Mathur, 2001; Pandey *et al*., 2005; Grosch *et al*., 2007). *Trichoderma* isolates also varied in their reaction types, this was evident in the case of *Sclerotium rolfsii*. Among the root pathogens *F. solani* was more susceptible to the antagonist and this was also evident with the interaction type as the antagonists were able to completely overgrow the pathogen. *Sclerotium rolfsii* exhibited an interaction type where both the pathogen and the antagonist grew 50% and neither dominated on each other except for PC-03 where the pathogen was able to over grow the antagonist. In case of *F. oxysporum* zone of inhibition was observed. In case of foliar pathogens all the species were susceptible to the antagonist. This was also evident with the reaction type as all the antagonists were able to completely overgrow the pathogens. A vast variety of microbes have the ability to be potentially used as biocontrol agent but the selection of an appropriate isolate forms an important aspect for its success in field application. The present work showed the potentiality of rhizoplane mycoflora and its efficacy against various forest plant pathogens and can be further analysed for its use as an alternative to chemical fungicides.

CONCLUSION

Trichoderma harzianum exerted good antagonistic activity against all the pathogens studied and makes this species as a biocontrol agent which can be used as an alternative to chemicals. The variations among the isolates stressed on the selection of effective isolate and needs a series of steps in their appropriate application for their infield success (Ravensberg, 2011). Rhizoplane regions of grasses homes diverse fungal organisms and can be used as biological weapons against various plant pathogens.

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Conflicts of interest: The authors stated that no conflicts of interest.

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