

Control of Anthracnose Disease in *Swietenia macrophylla* using *Trichoderma viride* as Biocontrol Agent

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Abstract: The biocontrol potential of *Trichoderma viride* against anthracnose pathogen *Colletotrichum alienum* was studied *in vitro* and under greenhouse conditions. *In vitro* antagonism test carried out between *T. viride* and *C. alienum* showed a radial growth inhibition of the pathogen by 75% at 35°C. The greenhouse studies involved inoculation of healthy *Swietenia macrophylla* seedlings with suspensions of *T. viride* and fungicide (Carbendiazim) followed by addition of the pathogen, *C. alienum* inoculum after three days. After inoculation the infected leaf area was measured weekly and Area under the Disease Progress Curve (AUDPC) was calculated and compared among the treatments. The results showed that *T. viride* (AUDPC = 120) and fungicide (AUDPC = 93) significantly ($p < 0.05$) reduced the disease compared to the untreated control (AUDPC = 1200). *T. viride* significantly controlled the pathogen on par with fungicide treatment. This study revealed that the application of *T. viride* has good potential in controlling the anthracnose disease of *S. macrophylla*.

Key words: *Swietenia macrophylla*, *Trichoderma viride*, *Colletotrichum alienum*, anthracnose, biocontrol

INTRODUCTION

Swietenia macrophylla is a native tree species to Indian that usually attain the height of over 30 m. *S. macrophylla* is a promising tree species for industrial plantations and afforestation. The wood is used for plywood, furniture and ornamental purpose. As far as diseases concerned only one major disease was reported in *S. macrophylla* that is bark rot (Surianegara and Lemmens, 1993). However, we found anthracnose disease at nursery stage of *S. macrophylla* caused by *Colletotrichum alienum*. Generally *Colletotrichum* spp. cause significant economic damage to cereals, legumes, ornamentals, vegetables and fruit trees (Freeman *et al.*, 1998; Melanie *et al.*, 2004; Gregori *et al.*, 2010). Traditional methods used to protect crops from diseases have been largely based on the use of chemicals but chemical methods are not economical in the long run because they pollute the atmosphere, damage the environment, leave harmful residues and can lead to the development of resistant strains among the target organisms with repeated use (Naseby *et al.*, 2000). A reduction or elimination of synthetic chemicals applications in agriculture and forestry is highly desirable. One of the most promising means to achieve this goal is by the use of new tools based on biocontrol agents (Chet and Inbar, 1994; Harman, 2006). The potential biocontrol agent

T. viride is used to control the anthracnose disease in *S. macrophylla* in this study. *T. viride* is a soil borne mycoparasitic fungus has been shown effective against many soil borne plant pathogens (Papavizas, 1985; Pan *et al.*, 2001; Karthikeyan *et al.*, 2003; Jash and Pan, 2004). This study will be a model to control the disease by application of biocontrol agents like *T. viride* rather than chemical fungicides.

MATERIALS AND METHODS

Collection of diseased specimens: This study was carried out in the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, India from February to March 2014. The diseased samples (leaves) were collected from one month old *S. macrophylla* seedlings grown in research nursery at IFGTB, Coimbatore, Tamil Nadu, India. The diseased parts collected in sterile jars have brought to the laboratory, for further study.

Isolation and identification of pathogen: The pathogen was isolated using standard phytopathological isolation techniques (Sinclair and Dhingra, 1995). Infected *S. macrophylla* plant leaf were surface-sterilized with 96% ethyl alcohol, cut at the turn of diseased to healthy tissue and the leaf pieces were aseptically placed on sterile Potato Dextrose Agar medium (PDA) and incubated at

RESULTS

25°C for seven days. After seven days, the obtained mycelium was sub cultured to sterilized PDA medium to obtain a pure culture. Microscopic observations were conducted for further identification. The obtained pure cultures were incubated for three days on PDA slant at 25°C and afterwards kept in a refrigerator at 4°C until use.

Antagonistic assessment: The pure culture of *Trichoderma viride* was received from the culture bank forest pathology laboratory (IFGTB), Tamil Nadu, India and maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further use.

Dual culture method: About 5 day old culture, mycelial disc (5 mm) from a *T. viride* and test pathogen was placed on the plate opposite to each other equidistant from the periphery and were incubated in different temperatures 5, 15, 25, 35 and 45°C. After 6 days of the incubation period, radial growth of pathogen was recorded and percentage inhibition calculated in relation with control (Hajieghrari *et al.*, 2008).

Percentage of mycelial growth inhibition was calculated according to the equation:

$$L = \frac{C-T}{C} \times 100$$

where, L is inhibition of radial mycelial growth, C is radial growth measurement of pathogen in control, T is radial growth measurement of pathogen in the presence of antagonists.

Greenhouse experiment: Eighteen days old seedlings of *S. macrophylla* were planted in pots contains sterilized soil. The seedlings were inoculated with the 10 mL suspension (1×10^6 spores mL) of the pathogen *Colletotrichum alienum*. After three days the one set of seedlings (5 replicates) were inoculated with 10 mL of *T. viride* spore suspension (10^6 - 10^7 spores mL). The other set of seedlings (5 replicates) were inoculated with 10 mL of 0.1% of carbendazim fungicide. A set of controlled seedlings inoculated with *C. alienum* have been also maintained. All the seedlings were maintained in greenhouse conditions at 25°C with 70% of relative humidity.

Data collection and statistical analysis: The individual plants were rated visually on weekly intervals for percentage of leaf area with symptoms of anthracnose over the disease progress period. The average amount of disease developed over the disease progress period was expressed as the Area under the Disease Progress Curve (AUDPC) and estimated using the midpoint rule (Campbell and Madden, 1990). The data were analysed by Duncan's Multiple range test using SPSS software (ver.10).

Isolation and identification of pathogen: *C. alienum* was isolated from collected *S. macrophylla* plant leaves with anthracnose symptoms of brown dark lesions. The pathogen inoculated *S. macrophylla* seedlings showed anthracnose symptoms but the symptoms were not observed in the control seedlings. The fungus was re-isolated onto Potato Dextrose Agar Medium (PDA) from the lesions on the inoculated plants. The pathogen was isolated and again cultured in PDA. After seven days of incubation, the pathogen colonies resembles to those of the original isolates. *C. alienum* colony was identified with cottony, grey aerial mycelium with numerous dark-based acervuli and orange conidial ooze visible through the mycelium (Fig. 1 and 2).

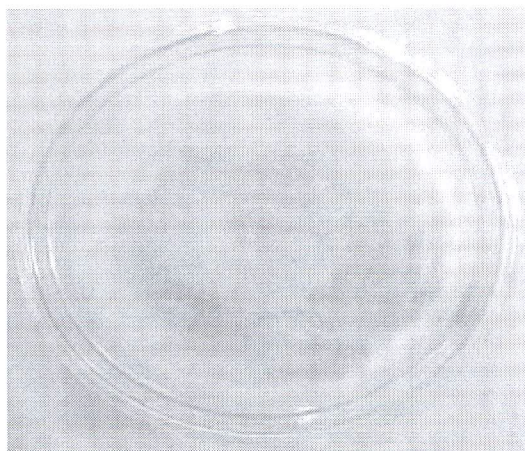


Fig. 1: *C. alienum* colony in PDA medium



Fig. 2: Microscopic view of *C. alienum* showing macroconidia

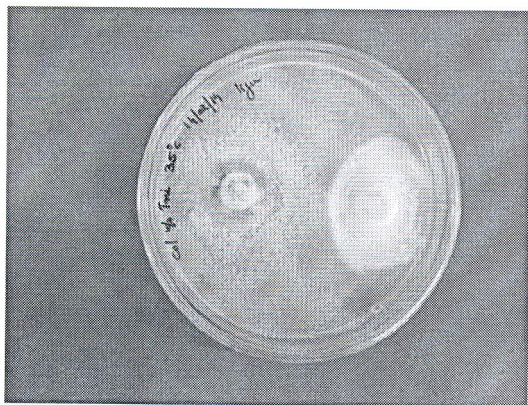


Fig. 3: Antagonistic test between *T. viride* (green colonies) and *C. alienum*

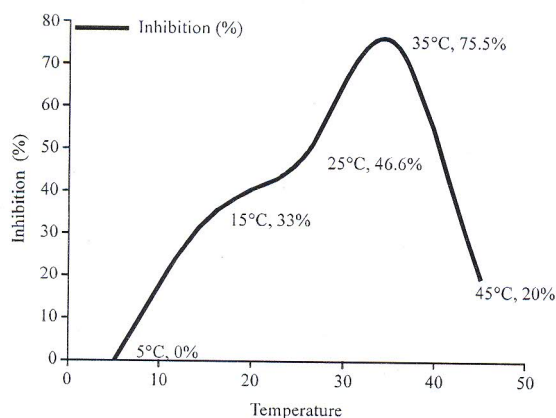


Fig. 4: Inhibition of *T. viride* at optimum temperature (35°C)

Antagonistic assessment: The antagonistic activity of *T. viride* showed that it can suppress the growth of the fungal pathogen *C. alienum* in dual culture technique. It was observed that *T. viride* reduced the growth of *C. alienum* by 75% at 35°C after the 10th day and it showing low level of antagonism at 5°C followed by 45°C. *T. viride* can also reduce the growth of the plant pathogen at 15 and 25°C. However, the maximum antagonism showed at 35°C (Fig. 3 and 4).

Greenhouse experiments: The average AUDPC values of the treatments are presented in Table 1. Each AUDPC value was calculated using the midpoint rule from three disease severity assessments taken weekly (Fig. 3). Plants inoculated with *T. viride* and the chemical fungicide had significantly ($p < 0.05$) reduced the disease severity

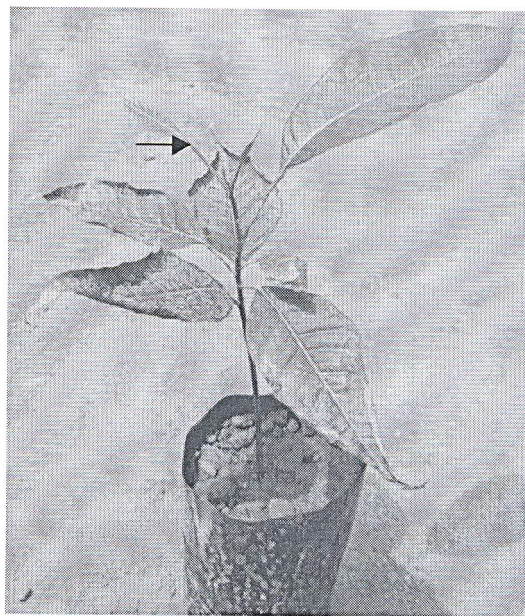


Fig. 5: *S. macrophylla* seedling showing recovery from the disease due to *T. viride*: Arrow mark indicates sprouting of new leaves

Table 1: Effects of application of *T. viride* on the progress of anthracnose disease of *S. macrophylla*

Treatments	Average AUDPC
<i>Trichoderma viride</i>	120.00 ^b
Fungicide (Carbendazim)	93.00 ^b
Negative control	1200.00 ^a
Positive control	0002.00 ^a

AUDPC means followed by the same letter are not significantly different by 5% level of DMRT ($p < 0.05$)

compared to the negative control (inoculated/untreated checks). The low AUDPC values (less disease severity) was recorded in chemical fungicide (AUDPC = 93) followed by *T. viride* treated plants (AUDPC = 120). Fungicide and *T. viride* treated were found significantly not different. It was also found that the diseased seedlings recovered and sprouting new leaves due to the treatment of *T. viride* (Fig. 5).

DISCUSSION

This results of the study showed that biological control is a promising tool to maintain current healthy planting stocks while reducing the release of polluting chemical fungicides to the environment (Akthar and Siddiqui, 2008). In this study *T. viride* has been found to retard the radial growth of *C. alienum*. This antagonistic mode of action of *T. viride* could be

attributed to the production of antibiotics and fungal cell wall degrading enzymes (Chutrakul *et al.*, 2008). The observed mycoparasitic action of *T. viride* in this study suggests that it has good potential in controlling *C. alienum* may be due to secretion of antibiotic metabolic. Rahman *et al.* (2007) also found controlling of *C. gleosporioides* by using the biocontrol agent *Pseudomonas aeruginosa* in papaya causing anthracnose disease. The results obtained under green house conditions indicated that the *T. viride* significantly reduced the severity of infection. The smallest area of leaf infection was observed in fungicide treated plants and the highest in the untreated checks (negative control). Even though fungicide treated plants showed the least infection, the difference with that of *T. viride* treated plants was not statistically significant. This indicated that the performance of *T. viride* was comparable to that of the chemical fungicide. It is possible that more than one antagonistic mechanism could have been involved in the reduction of the disease. However, it needs to be further examined as the relative importance of the mechanisms is dependent on the particular isolate, the target organism and also the ambient environmental conditions (Tronsmo, 1996). The use of *T. viride* based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to be done to develop stable, cost effective, easy to produce and easy to apply formulations.

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