

***In vitro* biological control of *Fusarium solani* – cause of wilt in *Dalbergia sissoo* Roxb.**

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Abstract

Five species of *Trichoderma* viz. *Trichoderma viride* Pers. Ex Gray, *T. harzianum* Rifai, *T. koningii* Oudem, *T. aureoviride* Rifai and *T. pseudokoningii* Rifai, and three species of *Aspergillus* viz. *Aspergillus fumigatus* Fresenius, *A. glaucus* Link and *A. oryzae* (Ahlb.) Cohn were evaluated for their *in vitro* antagonistic potential against *Fusarium solani* (Mart.) Sacc., the cause of wilt disease in Shisham (*Dalbergia sissoo* Roxb.). Among the *Trichoderma* species *T. harzianum* showed the best performance followed by *T. viride*, *T. aureoviride*, *T. koningii* and *T. pseudokoningii*, respectively, resulting in 52.4, 24, 13.7, 9 and 2% reduction in colony growth of the test pathogenic fungus. Similarly there was 23, 20 and 7.5% reduction in colony growth of *F. solani* due to antagonistic effects of *A. fumigatus*, *A. glaucus* and *A. oryzae*, respectively.

Introduction

Shisham tree in Pakistan have been inflicted with dieback and wilt diseases during the last ten years. The incidence of these diseases is also reported in Bangladesh, India and Nepal (Baksha and Basak, 2000; Joshi and Baral, 2000; Sharma *et al.*, 2000). These diseases have caused a huge damage to this precious tree species. Incidence of shisham dieback and wilt diseases particularly in central Punjab has been a matter of serious concern to foresters and farmers since its rapid proliferation in recent past. Survey of different districts of Punjab (Bajwa *et al.*, 2003a) has revealed up to 70% shisham mortality along the canal sides. Mycologists have recorded some 62 pathogenic species of fungi on shisham. Most of the fungi have been studied from mycological point of view and a little has been mentioned on pathological aspects (Khan, 1989). Few pathological studies have been carried out in recent past. Gill *et al.*, (2001) have suggested *Phytophthora cinamomi* as the causal agent of dieback disease. Bajwa *et al.*, (2003b) isolated *Fusarium solani* from wilting shisham plants and Benomyl was reported as most effective chemical control agent in shisham saplings. However, in Shisham strands where pathogen has established itself, the chemical only provides temporary relief. Besides that long-term use of chemicals is not recommended because of the risks involved. Use of environmentally safe bio-control agents can affectively control diversity of soil borne pathogens (Nam *et al.*, 1988; Park and Kim, 1989; Saleem *et al.*, 2000). In last couple of decades

several studies have confirmed the biocontrol potential of *Trichoderma* species against phytopathogenic fungi (Hadar *et al.*, 1984; Grondona *et al.*, 1997; Hanson and Howell, 2004). The present study, therefore, was carried out to investigate the bio-control potential of *Trichoderma* and *Aspergillus* species against mycelial growth of *F. solani*, the cause of wilt in shisham.

Materials and Methods

Cultures of *F. solani*, isolated from roots of diseased (wilted) shisham trees in previously (Bajwa *et al.*, 2003), were maintained on malt extract agar (MEA) medium. Cultures of various antagonistic fungi vi. *Trichoderma viride*, *T. harzianum*, *T. koningii*, *T. aureoviride*, *T. pseudokoningii*, *Aspergillus fumigatus*, *A. glaucus* and *A. oryzae* were obtained from First Fungal Culture Bank of Pakistan, Department of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan and maintained on MEA medium.

The antagonistic effect of various test fungal species in inhibiting the growth of *F. solani* was studied by following the method given by Jahnson *et al.*, (1959). For this purpose 6 mm diameter plugs of *F. solani* and various antagonists were taken with the help of sterilized cork bore and placed at the opposite sides of the petri plates of 9cm diameter having MEA medium. After inoculation plates were incubated at 25±2°C. Petriplates with only *F. solani* served as control. Each treatment was replicated thrice. Data on

mycelial growth in terms of colony diameter of the pathogenic fungus were taken after 5 days of inoculation. Data were analyzed by applying Duncan's Multiple Range Test (Steel and Torrie, 1980).

Results and Discussion

The effect of five species of *Trichoderma* and three species of *Aspergillus* on *in vitro* growth of *F. solani* is shown in Fig. 1 & 2. Data recorded after five days of inoculations revealed variable influence of antagonistic fungi influenced the colony growth of *F. solani* differently. Among the five species of *Trichoderma*, *T. harzianum* exhibited the best performance where the colony growth of the test pathogenic fungus was 23 mm as compared to 49 mm in control treatment (Fig. 1). This antagonistic fungus suppressed the colony growth of pathogen by 52.4% (Fig. 2). *T. harzianum* is also known to cause 91.4% reduction in colony growth of *Phytophthora capsici*, a collar rot causing fungus in chillies (Saleem *et al.*, 2000). *T. viride* followed by *T. harzianum* in performance and decreased colony growth of *F. solani* by 24%. The other three species of *Trichoderma* viz. *T. aureoviride*, *T. pseudokoningii* and *T. koningii*, reduced the colony growth of *F. solani* by 13.7, 9 and 2%, respectively. Effect of all the tested *Trichoderma* species except *T. koningii* was statistically significant against the test pathogenic fungal species (Fig. 1 & 2). In contrast to the

present study, Saleem *et al.*, (2000) observed 83.4% suppression in colony growth of *P. capsici* due to antagonistic activity of *T. koningii*. It reveals that the antagonistic fungi are specific in their antagonistic activity. Dennis and Webster (1971) and Elad *et al.*, (1982) reported that *Trichoderma* spp. are capable of producing either antibiotics or extracellular lytic enzymes or both and these are responsible for antagonism. According to Brasier (1975), volatiles released by *Trichoderma* spp. reduced the growth of *Phytophthora* spp. followed by a vacuolation of its cell contents that eventually resulted in lysis of hyphal tips. Lorito *et al.*, (1993) studied the antifungal activity of *T. harzianum* and found that chitinolytic enzymes produced by *T. harzianum* inhibit the growth of a variety of plant pathogenic fungi.

Effect of all the three species of *Aspergillus* in suppressing the *in vitro* growth of *F. solani* was significant. There were 23, 20 and 7.5% reduction in colony growth of *F. solani* due to *A. fumigatus*, *A. glaucus* and *A. oryzae*, respectively (Fig. 1 & 2).

The present study concludes that species of both *Trichoderma* and *Aspergillus* have the potential to suppress the colony growth of the wilt causing pathogen of shisham. Further studies are required to study the *in vivo* effect of these fungi against the pathogen.

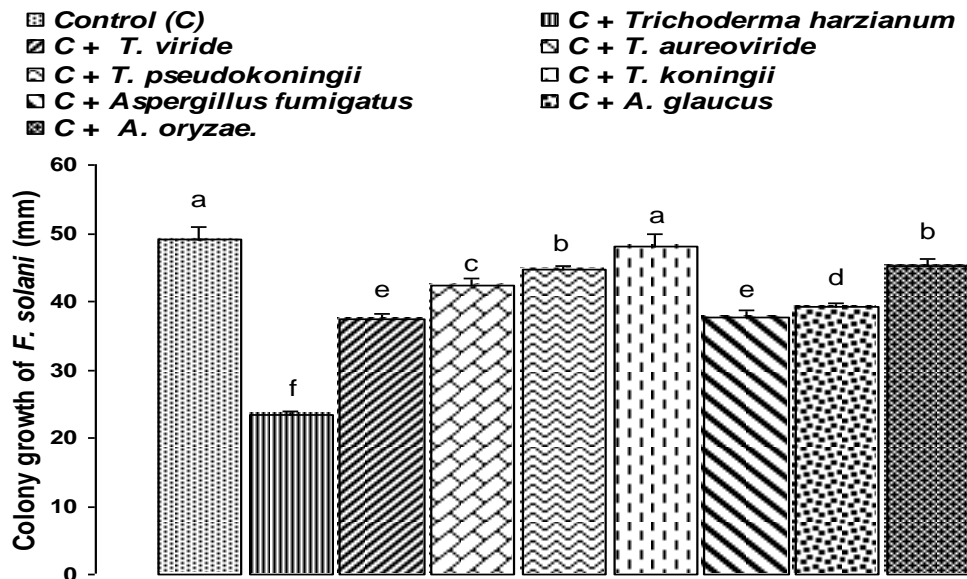


Fig. 1: Colony growth of *F. solani* in control and in the presence of various antagonistic fungi. Bars with different letters at their tops show significant ($P = 0.05$) difference as determined by DMR Test.

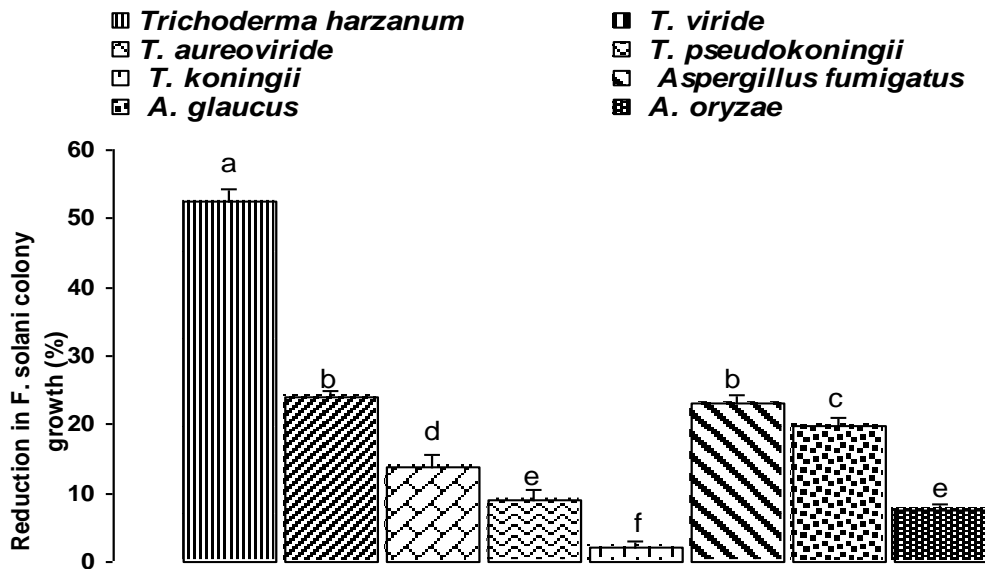


Fig. 2: Percentage reduction in colony growth of *F. solani* due to various antagonistic fungi. Bars with different letters at their tops show significant ($P = 0.05$) difference as determined by DMR Test.

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