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INTEGRATED DISEASE MANAGEMENT TO CONTROL SHISHAM (*DALBERGIA SISSOO* ROXB.) DECLINE IN PAKISTAN

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Abstract

Surveys of different areas of Punjab conducted during 2003-2005, revealed that shisham (*Dalbergia sissoo* Roxb.) plants are severely suffering from wilt and dieback diseases. Maximum mortality of up to 80% was observed along the canal bank followed by 20–40% along the highways and roadsides. The least disease incidence and mortality rate was observed in plants growing on agricultural and well managed lands. Generally older plants were found to be more susceptible to dieback attack than the younger ones. However, wilt was common both in young and old plants. Stressed conditions especially drought and high soil moisture content were found to be responsible for attack of pathogen and severity of the disease. *Fusarium solani* (Mart.) Appel & Wr. was isolated from the roots of shisham plants suffering from wilt disease. From roots of dying back trees *Fusarium oxysporum* (Schlechtend.): Fr., and *Phytophthora cinamomi* Rands were isolated. Shisham decline can be controlled by adopting integrated disease management (IDM) approach. Benomyl was effective in controlling wilt in 6-8 years old shisham plants. *Trichoderma harzianum* Rifai, *T. viride* Pers. ex Gray and *T. aureoviride* Rifai can be used as biological control agent to reduce the inoculum of *F. solani*. Eighteen phenotypically different varieties of Shisham have been identified from Punjab University, Quaid-e-Azam Lahore, Pakistan and adjacent areas. Resistant shisham varieties should be planted on well drained sandy loam soils to control wilt and dieback. The severely infected and dead plants should be uprooted followed by disinfection of the soil to avoid further spread of the pathogenic inoculum.

Introduction

Shisham has been inflicted with two severely damaging diseases viz., wilt and dieback, in Pakistan in the recent years and incidence is also reported in the Tarai tract of Nepal, believed to be its home. In wilt disease effects produced on trees are more or less of the same type as those produced by drought or frost. The disease was first observed by Bakhshi (1954) both in natural forests and plantations in UP, India. The disease is systematic in that the entire tree shows symptoms of the attack. In the early stage, an affected tree is characterized by drooping leaves and branches, due to loss of turgor. The leaflets turn yellow, dry up and eventually drop off rendering the branches bare. The entire tree becomes thin in contrast to the adjoining dense green trees. Death of the affected tree is rapid and occurs within 1–6 months after the crown shows the symptoms of wilt. *Fusarium solani* is suggested to be the cause of this disease (Bakhshi, 1954; Manandhar & Shrestha, 2000; Bajwa *et al.*, 2003). The pathogen is mostly restricted to roots. The fungal hyphae and jelly like substances plug the vessels resulting in wilt symptoms (Bakhsai & Singh, 1959).

The dieback disease has more specialized symptoms than wilt. The symptoms are thinning of leaves and crown, drying up of end branches, yellowing of whole plant in

certain cases, table topped conditions and stag-headness in extreme cases. Small dry twigs kept on falling continuously and the tree looked like a blunt stub containing thick branches. The dieback takes place in successive stages and is characterized by progressive death of shoots and roots starting at the tip. There are controversial reports regarding the causal agent of dieback. The fungus *Phellinus gilvus* has also been isolated from the roots of dying back shisham trees (Bakshi, 1974). Another fungus *Ganoderma lucidum* is considered to be the primary cause of this disease. This pathogen is root inhabiting and infects the roots through intact as well as injured surfaces. Lateral spread of the disease in plantations is through root contact (Sharma *et al*., 2000). According to Gill *et al.,* (2001) the primary cause of shisham dieback is *Phytophthora cinamomi*. Our research group in Punjab University Lahore has isolated *F. oxysporum* and *P. cinamomi* from dying back shisham trees.

In integrated management all available pertinent information regarding the plant, its pathogens, history of disease in previous years, varietal resistance to diseases, the environmental conditions expected to prevail, locality, availability of materials, land, labour and cost is taken into account in developing the control program (Agrios, 2005). The present study describes the integrated management of shisham decline through chemical, biological and cultural methods as well as by cultivating resistant varieties of the shisham.

Materials and Methods

Survey of different areas of Punjab: Different areas of the province Punjab viz. Sialkot, Gujranwala, Lahore, Jehlem, Rawalpindi, Khoshab, Bahawalpur, Multan and Sargodha were surveyed from 2003-2005, to study the effect of factors like pH, drought and high soil moisture on disease severity. In each district survey was carried out on agriculture lands, along the highways and roadsides, and along the roadside of canals.

Chemical control of *F. solani* – the cause of wilt: I*n vitro* toxicity of three fungicides viz., Ridomil Gold, Benomyl and Aliette were tested against *F. solani* by the poisoned food technique (Nene & Thapliyal, 1979). Each fungicide was mixed separately in autoclaved melted PDA medium to obtain required concentration i.e., 10, 20, 30, 40 and 50 ppm. Twenty ml of poisoned melted PDA medium was poured into each sterilized plate and allowed to solidify. PDA medium without fungicides served as control. After solidification of medium, 3 mm agar plugs of the fungus on PDA were transferred in the center of the plates. Each treatment was replicated thrice. All the plates were incubated at 25±2°C. Growth inhibition rate was recorded after 8 days of incubation. Percent inhibition in fungal growth was calculated according to Vincent (1957). Data was analyzed by applying t-test.

The fungicide (Benomyl) found most effective in inhibiting the mycelial growth of *F. solani* in poisoned food technique was further evaluated in field for the control of shisham wilt by soil drenching with fungicidal solution of 200 ppm concentration. Three shisham plants of 6-8 years old, showing clear symptoms of wilt disease were selected in Punjab University, Lahore. Fifty litres of 200 ppm suspension of Benomyl were drenched in the soil around each selected tree. The disease incidence was recorded 45 days after treatment.

***In vitro* biological control of *Fusarium solani*:** Cultures of *F. solani*, isolated from roots of diseased (wilted) Shisham were maintained on malt extract agar (MEA) medium.

Cultures of various antagonistic fungi viz., *Trichoderma viride, T. harzianum, T. koningii, T. areoviride* and *T. pseudokoningii* were obtained from First Fungal Culture Bank, Department of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan and maintained on MEA medium. To study the antagonistic effect of various test fungal species in inhibiting the growth of *F. solani*, 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 having MEA medium. After inoculation plates were incubated at 25±2ºC. Petri plates with only *F. solani* served as control. Each treatment was replicated thrice. Data on mycelial growth of the pathogenic fungus was taken after 5 days of inoculation. Data was analyzed by applying Duncan's Multiple Range Test (Steel &Torrie, 1980).

Identification of resistant Shisham varieties: A thorough survey of Quaid-e-Azam Campus of Punjab University Lahore and adjacent areas was carried out during July-August, 2003. Different varieties of Shisham were identified on the basis of physical appearance, branching pattern, leaf and leaflet size and shape, leaf and branching density, and pod and stem surface characteristics. The dieback incidence in various varieties was recorded. Surveys of Lahore, Sialkot, and Gujranwala districts were undertaken afterwards to study the disease resistance potential in the identified varieties under different environmental and edaphic conditions.

Results and Discussion

Survey of different areas of Punjab: The highest percentage of mortality of shisham, ranging from 75–80%, was recorded along the canal banks indicating that high soil moisture is one of the major causes of disease severity. The mortality rate along the road sides especially along highways was also fairly high ranging from 20–40%. The least mortality and disease incidence of 10% or below was recorded on the shisham trees growing on agricultural lands. The incidence was also fairly high at drought stressed localities.

Chemical control of *F. solani*: Among the three fungicides evaluated against *F. solani in vitro*, Benomyl was found to be highly effective causing a significant reduction in mycelial growth of the test fungus even in very low concentration of 10 ppm. Ridomil was effective in higher concentration while Aliette failed to affect the growth of this fungus significantly even at 50 ppm concentration level (Fig. 1). Some other fungicides such as Vitavax, Dithane M-45, Bavistin and Benlate are also known to have significant suppressive effect on growth of *F. solani* (Ahmad *et al.*, 1996). Benomyl, the most effective fungicide *In vitro* trial was also found very effective *In vivo* experiment. All the three shisham plants, which were likely to be dead by wilting during next few weeks, managed to recover themselves from disease after treatment with Benomyl. The treatment with this fungicide may prove highly beneficial to save the shisham trees from the menace of wilting. However, there is need to study the effectiveness of this fungicide against the wilt attack in older trees. A benomyl derived fungus toxicant MBC (Methyl-2-benzi midazol carbamate) is also known to be effective against wilting. It is a stable fungicide suitable for injection into the trees (Mcwain & Gregory, 1973).

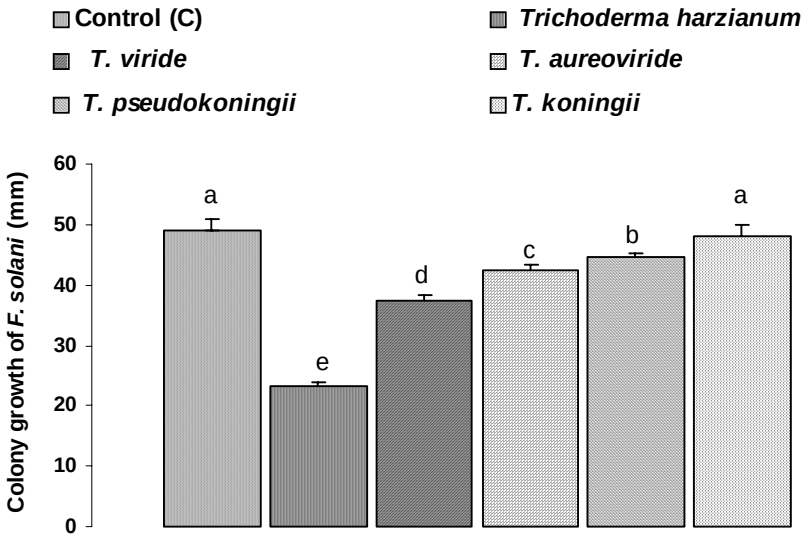

Fig. 1. Colony growth of *Fusarium solani* in control and in the presence of various antagonistic fungi. Bars with different letters at their top show significant (p = 0.05) difference as determined by Duncan's Multiple Range Test.

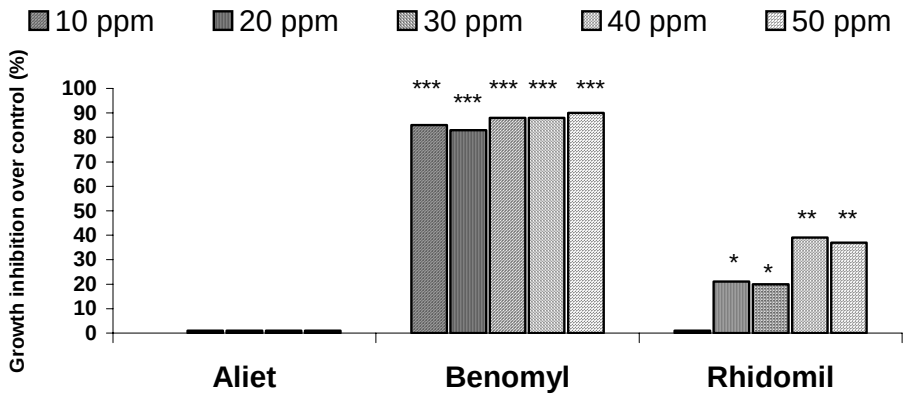

Fig. 2. Percentage reduction in *In vitro Fusarium solani* growth due to three fungicides as compared to control.
*, **, ***, show significant difference from control at 5, 1 and 0.1% level of significance as determined by t-test.

***In vitro* biological control of *Fusarium solani*:** The effect of five species of *Trichoderma* on *in vitro* growth of *F. solani* is shown in Fig. 2. *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. This antagonistic fungus suppressed the colony growth of pathogen by 52.4%. *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. areoviride, 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. 2). Earlier Dennis & 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.

Identification of Shisham varieties: Eighteen shisham varieties were identified from Quaid-e-Azam Campus of Punjab University Lahore and adjacent area. On the basis of their disease resistant potential they were named as Resistant 1 and Resistant 2, Susceptible 1 to Susceptible 4, and Unspecified 1 to Unspecified 12. The unspecified varieties 1 – 12 were named so because plants of these varieties did not show any disease symptoms but their number was not large enough to decide their resistant potential.

On the basis of the results of the present studies, following integrated management strategies are recommended to control the shisham decline in Pakistan.

- The dead, wilted and dying back plants should be uprooted so that the breeding places of the pathogens are destroyed to stop further spread of the disease.
- New plants should be planted in suitable sites i.e. on well-drained sandy loam soils. Plantations on clayey and waterlogged soils should be avoided.
- Mixed cropping is recommended rather than monoculture to avoid dieback.
- New shisham nurseries should be raised from the seeds and cuttings of the resistant variety and the susceptible varieties should be gradually replaced with the Resistant.
- Watering is beneficial during relatively dry autumns and during dry summer months.
- If dieback symptoms appear, and no specific cause can be determined, try fertilization and watering.
- Use Benomyl to cure wilting in young Shisham plants.
- Add organic materials such as farmyard manure along with some antagonistic fungi to reduce the spread of the pathogen.

References

Agrios, G.N. 2005. *Plant Pathology.* Elsevier Academic Press. pp. 348.

Ahmad, M., M.A. Khan, F. Ahmad and S.M. Khan. 1996. Effectiveness of some fungicides on the colony growth of *Fusarium oxysporum* and *F. solani* associated with potato wilt. *Pak. J. Phytopathol.,* 8: 159-161.

Bajwa, R., A. Javaid, J.H. Mirza and N. Akhtar. 2003. Chemical control of wilt in Shisham (*Dalbergia sissoo* Roxb.). *Mycopath*, 1(2): 111-113.

Bakhsai, B.K. and S.L. Singh. 1959. Root disease of Shisahm (*Dalbergia sissoo*). VIII: Inoculation study on wilt. *Indian forester*, 85: 415-421.

Bakhshi, B.K. 1954. Wilt of shisham (*Dalbergia sissoo*) due to *Fusarium solani. Nature*, 174: 278-291.

Bakshi, B.K. 1974. Control of root disease in plantation in reforested stands. *Indian Forester*, 100: 77-78.

Brasier, C.M. 1975. Stimulation of sex organ formation of *Phytophthora* by antagonistic species of *Trichoderma.* I. The effect *in vitro*. *New phytopathol.*, 74: 183-194.

Dennis, C. and J. Webster. 1971. Antagonistic properties of species groups of *Trichoderma* II. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.*, 57: 41-48.

Elad, Y., I. Chet and J. Katan. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzeanum. Can. J. Microbiol.*, 28: 719-725.

Gill, M.A., I. Ahmad, A. Khan, M. Aslam and T. Mahmood. 2001. *Phytophthora cinamomi* – a cause of shisham decline in the Punjab. In: *Proc. of National Seminar on Shisham Dieback, Oct. 27, 2001, Punjab Forestry Research Institute Faisalabad*. pp. 21.

Lorito, M., G.E. Harman, C.K. Hayes and A. Dipietro. 1993. Chitinolytic enzymes produced by *Trichoderma harzeanum*: Antifungal activity of purified endochitinase and chitobiodase. *Phytopathology*, 83: 302-307.

Manandhar, G. and S.K. Shrestha. 2000. Fungi associated with dieback of sissoo. In: *Proc. of the sub-regional seminar on dieback of sissoo (Dalbergia sissoo), Katmandu, Nepal, 25-28 April 2000*. pp. 27-30.

Mcwain, P. and G.F.A Gregory. 1973. Benomyl derived fungitoxicant for tree wilt disease control. USDA *For. Serv. Res. Note, N.E. Forest Expt. Sta*. No. 162. RPP. 53(2): 689 Abst.

Nene, H.L. and P.N. Thakliyar. 1979. *Fungicides in plant disease control.* Oxford and IBH Publishing company, New Delhi, pp.507.

Sharma, M.K., R.M. Singaland and T.C. Pokhriyal. 2000. *Dalbergia sisso* in India. In: *Proc. of the sub-regional seminar on dieback of sissoo (Dalbergia sissoo), Katmandu, Nepal, 25-28 April 2000.* pp. 5-16.

Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of statistics.* A Biometrical Approach. 2nd edition. McGraw Hill Book Co.Inc. New York, USA.

Vincent, J.M. 1957. Distribution of fungal hyphae in the presence of certain inhibitors. *Nature*, 159: 850.

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