A NEW WILT DISEASE OF *ACACIA NILOTICA* CAUSED BY *FUSARIUM OXYSPORUM*

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KAPOOR, S., HARSH, N. S. K. & SHARMA, S. K. 2004. A new wilt disease of *Acacia nilotica* caused by *Fusarium oxysporum*. This paper reports a new vascular wilt disease of *Acacia nilotica* seedlings caused by *Fusarium oxysporum*. The affected seedlings exhibited varied symptoms such as drooping of leaves at the tip or side twigs, top dying, mortality and in some cases recovery in the form of new shoots. Browning of vascular tissue and clogging of xylem vessels by mycelium and spores of fungus as well as gums were associated with diseased plants. The disease caused more mortality in seedlings raised in polythene bags (33.7%) than those in root trainers (16.9%). The source of infection was found to be soil borne and not seed borne. The pathogenicity of *F. oxysporum* was confirmed in the laboratory by artificial inoculation. Of the seven fungicides tested, Bavistin and Benlate inhibited pathogen growth completely in culture at 0.1% concentration. Soil drenching with Thiram (0.05%), followed by seed dressing with Bavistin (0.05%), gave the best growth performance of seedlings as well as inhibition of disease in root trainers.

Key words: Vascular wilt – symptom syndrome – soil borne infection – fungicidal control

KAPOOR, S., HARSH, N. S. K. & SHARMA, S. K. 2004. Penyakit layu baharu *Acacia nilotica* diakibatkan oleh *Fusarium oxysporum*. Kertas ini melaporkan penyakit layu baharu anak benih *Acacia nilotica* diakibatkan oleh *Fusarium oxysporum*. Anak benih yang dijangkiti menunjukkan pelbagai gejala seperti daun terlentok pada hujung atau pada dahan sisi, mati atas, kematian dan kadang-kadang pemulihan dengan terbentuknya pucuk baharu. Keperangan tisu vaskular dan penyumbatan vesel xilem oleh miselium dan spora kulat serta oleh gum berkait dengan tumbuhan berpenyakit. Penyakit ini mengakibatkan lebih kematian dalam anak benih yang dibesarkan di dalam beg politena (33.7%) berbanding dengan yang dibesarkan di dalam tabung pembentuk akar (16.9%). Punca jangkitan datang daripada tanah dan bukan daripada biji benih. Kepatogenan *F. oxysporum* disahkan di makmal melalui penginokunatan tiruan. Antara tujuh racun kulat yang dikaji, Bavistin dan Benlate merencat pertumbuhan kulat sepenuhnya pada kepekatan 0.1%. Pelencunan tanah dengan Thiram (0.05%) diikuti oleh rawatan biji benih dengan Bavistin (0.05%) memberi pertumbuhan terbaik apabila ditanam di dalam tabung pembentuk akar. Perencatan penyakit juga terbaik menggunakan rawatan sedemikian.

Introduction

Acacia nilotica is a small or medium size tree, which grows in dry fallow fields and field bunds in India. It is a multi-purpose tree, which yields fuel and gum. The

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leaves, young twigs and young pods are very much liked by milch cattle. The wood is also used for making agricultural implements and tent pegs. The wood makes excellent fuel and charcoal, while the bark extract is one of the most used tanning materials. It has attained an important place in agroforestry due to its multiple uses (Dwivedi 1993).

Due to its demand in plantations, agroforestry systems and homestead plantations, large quantity of planting material is being raised in nurseries. The seedlings are susceptible to insect pests and diseases in nurseries. The major nursery diseases of *A. nilotica* are seedling blight by *Colletotrichum acaciae* (Ito & Shibukawa 1956), wilt by *Fusarium* sp. (Srivastava *et al.* 1989), web blight by *Rhizoctonia solani* (Mehrotra 1989), charcoal root rot by *Rhizoctonia bataticola* (Srivastava & Kalyani 1990) and collar rot by *F. solani* (Singh & Jamaluddin 2000).

A wilt disease was noticed in *A. nilotica* seedlings raised in the glasshouse of the Forest Research Institute, Dehradun, both in root trainers and in polythene bags. The disease was investigated in detail.

Materials and methods

Disease symptoms and histopathology

Samples of the diseased plants were brought to the laboratory for examination of root systems and isolation of pathogen. Roots of diseased seedlings were carefully uprooted and washed with water. Thin sections of the root and collar region were cut and slides were prepared in cotton blue stain for microscopic observations. Isolations were made onto potato dextrose agar (PDA) medium from the root and collar region of diseased plants after surface washing with 0.1% mercuric chloride solution and incubation at 25 ± 2 °C.

A wad of filter papers was placed in a sterilised Petri dish and moistened with sterilised water. Some dead roots and longitudinal sections of the collar region of diseased plants were placed in this moist chamber and kept at room temperature for observation.

Identification of pathogen

The growth of fungal colonies developed on PDA was observed under microscope. Slides were prepared in water drops and cotton blue. The fungus was identified with the help of the standard monograph by Booth (1971) and matching with isolates in the National Type Culture Collection at the Forest Pathology Division, Forest Research Institute, Dehradun, India.

Seed testing

To determine whether infection was seed borne or soil borne, whole and broken seeds were surface sterilised with 0.1% mercuric chloride solution, washed with

sterilised water and placed on Petri plates containing PDA. The plates were incubated at 25 ± 2 °C and regularly observed for four weeks.

Pathogenicity tests

To prove Koch's postulates and determine the role of the isolated fungus in disease development, pathogenicity trials were conducted. A mixture of sand and soil in a 1:1 ratio was prepared and 10 culture tubes (15 × 2.5 cm), half filled and plugged with cotton were sterilised in an autoclave at 1.05 kg cm^{-2} for 30 min. Spore suspension of the isolated fungus was prepared in sterilised water from 7-day-old colony growing on PDA to give 10^5 spores per ml. Of the 10 tubes, 6 tubes were each inoculated with 10 ml spore suspension of the pathogen and *A. nilotica* seeds introduced, while the remaining tubes contained only *A. nilotica* seeds, which served as control.

Evaluation of fungicides

Seven fungicides (a.i. @ 0.1% concentration) were evaluated for their efficacy against the pathogen, using the poisoned-food technique (Carpenter 1942). Weighed quantity of fungicide was mixed in 50 ml of PDA in 150 ml conical flasks and autoclaved. After cooling to room temperature, the medium was poured into three Petri dishes. Control dishes were prepared without fungicide. The test fungus was centrally inoculated as a 5 mm diameter disc cut from the periphery of 7-day-old culture in PDA. The plates were incubated at 25 ± 2 °C and regularly observed for 10 days. The colony diameter was measured and per cent growth inhibition of test fungus was calculated.

Fungicide applications

Inoculum preparation

A trial was laid down in root trainers (250 ml) washed with water and then with 2% formalin (40%) for partial sterilisation. When formalin had completely evaporated, a sterilised (by tyndallisation) soil-sand mixture (1:1) was used to fill the root trainer, which was covered with polythene sheet. A spore suspension of the pathogen was prepared from 7-day-old culture in modified Bilay's medium (Booth 1971) in which 50 ml of the medium were poured in 10 flasks, sterilised, inoculated with the pathogen when cooled and incubated at 25 ± 2 °C for 12 days. The fungal suspension was taken out from all the flasks and final volume was made up to 1000 ml by adding sterilised water. Twenty-five ml of this spore suspension were added in each cone of root trainers.

Treatments

Seed dressing and soil drenching treatments with four fungicides (Bavistin, Benlate, Captaf and Thiram) were applied in four replications. The experiment was laid out

in a randomised block design. In the dry seed treatment, the required quantity of four fungicides was mixed with the seeds by shaking (@0.2% a.i. by weight of seeds). The seeds were mechanically scarified before treatment. Treated seeds (12 seeds per replicate) were sown in root trainers as per randomised block design. Soil drench with selected fungicides (@ 0.05% a.i., in water) was given in root trainers as per randomised block design (12 cones per replicate). Twenty ml of each fungicidal solution were introduced into each cone and seeds were sown. The trays were kept inside a germination chamber (25±2°C) to enhance germination. After germination, the trays were placed in natural day-night conditions. Regular observations were made from the day germination started. Plant height measurement and mortality were taken for 30 days. Data were subjected to analysis of variance.

Results

Observations (Table 1) on the damage of plants in the glasshouse after five months of seed sowing showed that the wilt disease was more severe in seedlings raised in polythene bags (33.7%) than those raised in root trainers (16.9%).

Symptoms of disease

The following symptoms were noticed:

- Initial wilting—some leaves at the top or side twigs showing flaccid appearance or drooping (Figure 1).
- Top drying and defoliation—drying and dying of tips of seedlings after defoliation (Figure 1).
- Recovery—in some cases new recovery shoots develop below the dead top.
- Mortality—completely dead seedlings seen as dried brown seedlings among the lot.
- Discoloration, darkening and mortality of feeder roots. The taproot was found completely dead and in some cases brownish (Figure 2).
- After the death of the main taproot, adventitious roots might arise.

Table 1 Disease percentage in *Acacia nilotica* seedlings

Category	No. of plants	% Disease
Root trainers		
Total plants evaluated	1742	16.86
Fully damaged	186	10.68
Partially infected	25	1.44
Plants showing recovery	71	4.75
Total infected plants	282	16.86
Polythene bags		
Total plants evaluated	2169	33.70
Fully damaged	600	27.67
Partially infected	14	0.64
Plants showing recovery	115	5.30
Total infected plants	729	33.70

Figure 1 Wilt disease symptoms in *Acacia nilotica* seedlings (healthy seedling extreme left)

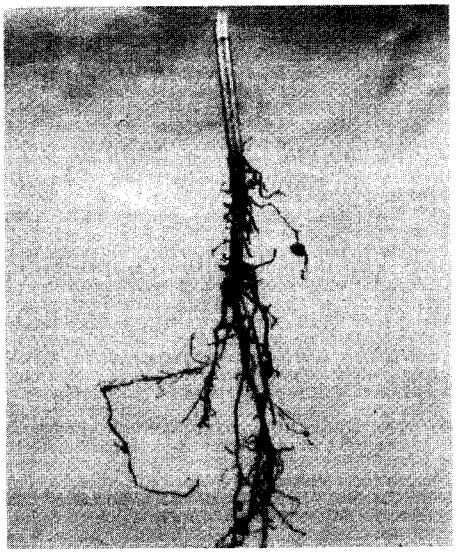

Figure 2 Dead root

Histopathology

Browning of tissue was observed. Mycelium and spores of the fungus were seen in the xylem vessels in the transverse sections of the collar region. Some vessels were clogged with mycelium, spores and gums (Figure 3). Hyphae of the fungus traversing the blackened tissue of the affected parts were noticed in the cross sections of the roots. Xylem vessels were plugged by an aggregate of hyphae, which extend to the adjoining tissue and also grew along the discoloured region (Figure 4). Discoloration of conducting tissue was noticed in longitudinal sections of the collar and root.

Figures 3 & 4 Clogging of xylem vessels by the pathogen

Identity of pathogen

Cottony white to peach coloured colonies which turned brown with age developed on PDA. The white cottony growth also appeared on infected tissue placed in moist chambers (Figure 5). The fungus was identified as *F. oxysporum.*

Mycelium (Figure 6) composed of hyaline, septate, branched hyphae. Conidia borne on simple phialides arising laterally on the hyphae or from short sparsely branched conidiophores. Conidia of two types: microconidia and macroconidia. Microconidia generally abundant, variable, oval to ellipsoid, cylindrical, straight to curved, aseptate, 5.70–11.40 × 2.85–4.27 μm; macroconidia borne on conidiophores usually 3-septate, fusiform to falcate, dorsiventral, straight or curved measuring 15.10–25.65 × 4.27–4.37 μm (1 septate), 22.80–45.60 × 4.27–5.70 μm (2 septate) and 28.50–37.05 × 4.27–5.70 μm (3 septate). Chlamydospores both smooth and rough walled, terminal as well as intercallary, 5.7–9.97 × 4.27–5.7 μm.

Figure 5 Fungal growth from collar and root tissues in moist chamber

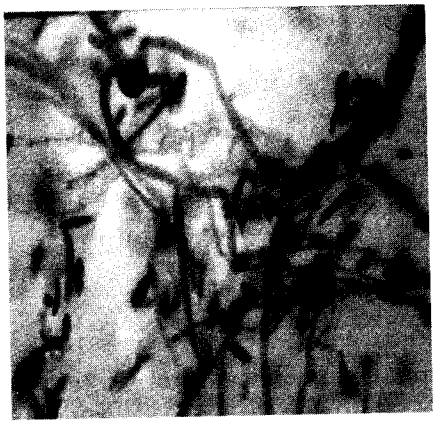

Figure 6 *Fusarium oxysporum*

Source of infection

Observations were made after one week of plating seeds on PDA plates and continued for a period of four weeks but *Fusarium* could not be isolated from the seeds.

Pathogenicity test

After 28 days, the effect of *F. oxysporum* on seed germination was noticed. Of the six inoculated tubes, germination only occurred in one but was later killed by *F. oxysporum*. Fungus mycelium was seen in the collar region as well as on stems of germinated seedlings. In the remaining five tubes, seeds failed to germinate and were fully colonised by the fungus. *Fusarium oxysporum* was successfully reisolated from infected seeds and seedlings, thus confirming the Koch's postulates. In the controls (4 tubes), germination occurred and seedlings grew healthily (Figure 7).

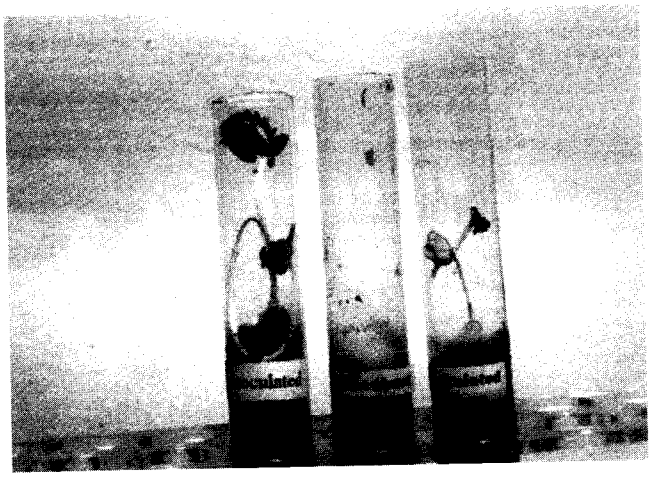

Figure 7 Pathogenicity test (uninoculated seedling extreme left)

Evaluation of fungicides

Bavistin and Benlate gave 100% (Table 2) control against *F. oxysporum*, followed by Captaf (71.7%), Thiram (66.7%) and Blitox (56.1%), while Topsin-M was found the least effective (Table 2). All treatments significantly inhibited growth of *F. oxysporum*. However, there were no significant differences between Bavistin and Benlate, Captaf and Thiram, Blitox and Thiram, and Blitox and Indofil-M.

Fungicide application

For seed dressing, Benlate and Thiram produced significantly superior growth of seedlings over the control, whereas for soil drenching, Bavistin and Thiram gave significantly better growth. Maximum growth of seedlings was observed in soil-drenching treatment with Thiram (8.7 cm) yielding 70.6% gain in seedling growth over control, followed by seed dressing with Benlate (7.8 cm) yielding 52.9% gain in seedling growth over control. Bavistin and Thiram gave better seedling growth when applied as soil drenching than seed dressing, whereas Benlate and Captaf performed better as seed dresser. It was also observed that mortality due to wilt disease was maximum in control (25%), followed by treatment with seed dressing with Thiram (22.9%) and soil drenching with Benlate (20.8%).

Table 2 Evaluation of fungicides against *Fusarium oxysporum in vitro*

Fungicide	% Growth inhibition	
Bavistin (carbendazim)	100	(89.96) a
Benlate (benomyl)	100	(89.96) a
Blitox (copper oxychloride)	56.06	(48.49) cd
Captaf (captan)	71.72	(57.53) b
Indofil-M (mancozeb)	47.72	(43.68) d
Thiram (thiram)	66.67	(54.72) bc
Topsin-M (thiophanate methyl)	15.91	(22.43) e
Control	0	(0.00) f
CD (LSD) at 5%	6.42	

Values in parentheses are arc sine transformation. Values with the same letter are not significantly different at the 0.05 probability level.

Table 3 Effect of fungicides on the growth of seedlings and mortality in *A. nilotica*

Fungicide	Seedling growth (cm)		Mortality	
	Seed dressing	Soil drenching	Seed dressing	Soil drenching
Bavistin	6.0 ab	7.2 abc	0	0
Benlate	7.8 b	6.1 bd	0	20.83
Captaf	6.7 ab	5.9 bd	0	0
Thiram	7.4 b	8.7 c	22.92	0
Control	5.1 a	5.1 d	25.00	25.00
CD (LSD) at 5%	1.89	1.89	–	–

Values are means of 25 seedlings. Values with the same letter are not significantly different at the 0.05 probability level.

Discussion

Fusarium oxysporum is a common root disease-causing fungus reported in many forest tree seedlings (Gibson 1975, Harsh & Gupta 1993). *Fusarium oxysporum* reported in *A. nilotica* seedlings in the present study is a new disease record from India (Bilgrami *et al.* 1991). The wilt disease caused by *F. oxysporum* in *A. nilotica* seedlings caused considerable damage both in root trainers and polythene bags, with nearly one-third of seedlings killed due to disease. Harsh *et al.* (1992) reported up to 75% mortality due to *F. oxysporum* wilt in *Dalbergia sissoo* seedlings. The vascular wilt diseases of forest tree species have been reported to cause mortality of seedlings or poor vigour (Manion 1981, Harsh & Gupta 1993). Manion (1981) reported losses due to damping-off disease in seedlings to 5% or more.

The potting mix in polythene bags became more compact; in root trainers, the trays being put on stands for aerial pruning of roots did not show compactness. The compactness of potting mix may have caused poor aeration of roots and thus acted as a predisposing factor for wilt disease. In root trainers, root development was more abundant than that in polythene bags, which may have provided better chances of escape and recovery from disease in the former. The recovery of the host could be correlated with new root development after the death of the main tap-root.

The symptoms exhibited in the present study were typical of a vascular wilt disease such as that caused by *Fusarium* spp. as reported by Snyder and Smith (1981). The diverse symptom complex exhibited by the disease qualified as wilt disease syndrome (Green 1981). *Fusarium oxysporum* isolated from *A. nilotica* seedlings was able to cause disease in germinated seedlings as well as inhibit germination of seeds, thus establishing its pathogenic nature. This was also confirmed by the fungus growth obtained on roots kept in moist chamber.

The source of infection could be identified in the potting mix as seed infection was ruled out in agar plate test with seeds. Diseases caused by *Fusarium* spp. have been reported to be soil borne in nurseries (Garrett 1944, Gibson 1975, Green 1981). However, seed borne nature of infection by *Fusarium* spp. has also been documented in forest tree species (Mehrotra & Sharma 1989, Harsh *et al.* 1994, Jamaluddin *et al.* 1997). Harsh *et al.* (1992) reported wilt disease of *D. sissoo* seedlings caused by *F. oxysporum* as soil borne.

Bavistin and Benlate were found to be the best control against *F. oxysporum* in this study. Sharma *et al.* (1985) and Harsh (1993) have previously reported Bavistin as an effective soil drenching and seed dressing fungicide against *Fusarium* diseases in nurseries. Paul and Bhardwaj (1987) found Captaf and Thiram effective against seed borne fungi of *A. catechu.* However, Topsin-M, which was reported by Harsh (1993) to be effective against *Fusarium* diseases was not observed effective in this study.

Fusarium affects the growth of seedlings adversely and this is the reason why control seedlings had lesser growth compared with treated seedlings. McNew (1960) reported that root disease by *Fusarium* species caused destruction of seedlings or impairment of their growth. Ojha (2000) reported the adverse effect of *Fusarium* species on growth of nursery seedlings of some multipurpose tree species.

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