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A new culm rot disease of bamboo in India and its management

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Abstract—A new culm rot disease in bamboo caused by *Pterulicium xylogenum* is reported for the first time. The disease was recorded on three species of bamboo, i.e., *Bambusa vulgaris* var. *waminii, Dendrocalamus giganteus* and *Gigantochloa* sp. Nearly 45% of culms of *B. vulgaris* var. *waminii* and 36% of culms of *Gigantochloa* were found to be dead or dying due to the disease. The pathogenicity of the fungus was tested and proved. Bioassays were conducted with five fungicides: except Captan all were found effective in inhibiting the growth of *P. xylogenum*, even at 0.025%. The disease can be controlled to an appreciable level by spraying and drenching with a mixture of 0.05% copper oxychloride and carbendazim (a.i. by weight).

Key words: Bamboo; culm rot; fungicidal control; *Pterulicium xylogenum*.

INTRODUCTION

Bamboo is integral to the culture and economy of south-east Asia and is a preferred planting material for aforestation activities as an alternative to timber in meeting the industrial and rural requirements, checking erosion and conserving soil and moisture [1].

At the New Forest campus of the Forest Research Institute (FRI, Dehradun, India; altitude 640 m asl; latitude 30°20′40″N; longitude 77°52′12″E), different species of bamboo have been maintained in a bambusetum and a botanical garden as a germplasm collection, as well as reference material. An interesting disease was noticed in June 2000, infecting the young emerging culms and resulting in their deformity and death. Since then, detailed studies have been carried out on

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its symptomatology, losses, pathogenicity, laboratory bioassay of fungicides and management of the disease in the field.

MATERIALS AND METHODS

Collection of diseased material and isolation of the pathogen

The disease was first noticed in the bambusetum and the botanical garden of FRI in June 2000 and symptoms of the disease were recorded. The infected culms and fruit bodies were collected and brought to the laboratory in clean polythene bags for microscopic, anatomical and cultural studies. The specimens were also compared with the herbarium specimens available at Department of Biosciences, University of Minnesota (BPIUS 0333117). Microscopic slides were prepared from the infected culms and fruit bodies. Isolations were made on Potato Dextrose Agar (PDA) medium (15%, HiMedia Laboratories, Mumbai, India) from the diseased tissues of the culms after surface disinfection with 0.01% mercuric chloride solution and also from hyphal tissues of fresh fruit bodies of the fungus and maintained as pure cultures of the pathogen at 5°C in a refrigerator.

Pathogenicity tests

Pathogenicity tests were conducted under glasshouse conditions. Apparently healthy young culms (1 month old) were collected from the field and planted in a plastic pot filled with sterilized garden soil. The culms were inoculated at the nodes, internodes and sheaths using 5-mm-diameter mycelial discs collected from 7-day-old cultures grown on PDA. Before inoculation, the plant surface was washed with sterilized water and wiped with rectified spirit. The fungus was inoculated directly, as well as after making 2–3-cm-long fine superficial incisions (<1 mm deep) with a sterilized scalpel. As a control, blank discs of PDA were used to inoculate the healthy culms. Three replications were maintained for each treatment. The inoculated culms were then covered with polythene bags for 48 h to maintain high humidity and later the covering was removed. After pathogenicity tests, reisolations were made from the diseased culms for comparison.

Fungicide test

Studies were conducted with five fungicides, namely Bavistin (Carbendazim), Captan (Captaf), Dithane M-45 (Mancozeb), Tagcop-50 (Copper oxychloride) and Topsin-M (Thiophanate methyl) using the poison food technique [2]. Three doses of the fungicides, i.e., 0.025, 0.050 and 0.075% (a.i. by weight) were tested by mixing with PDA. Five-mm discs of actively growing test fungus were inoculated in the centre of Petri plates (9 cm diameter). Control plates were inoculated with fungal discs on PDA without fungicides. Three replicates were made for each treatment. The inoculated plates were incubated at 25 ± 2°C for 7 days. Linear growth was

recorded by measuring diameter of the colony of the test fungus radially in two directions and taking average value. Fungicidal efficacy was then analyzed for percent growth inhibition of the test fungus.

Damage assessment and field trial for disease management

Damage due to disease was assessed in one clump each of *B. vulgaris* var. *waminii* (Brandis) Wen and *Gigantochloa* sp., which exhibited severe disease, by counting the healthy and infected/dead culms in from 2002 to 2004.

Field trial for the management of the disease was carried out by treating these clumps with the fungicides (Tagcop and Bavistin) in July 2003, after cleaning and removing the dead culms and litter. Similarly, a patch (4 m diameter) of diseased culms was marked with nylon rope in the affected clump of *B. vulgaris* var. *wamini* and treated. The solutions of the desired concentration of the fungicides were prepared in water by taking into account their active ingredient (a.i.). The first spraying was done with 0.05% a.i. Tagcop on July 2003. After 15 days, a second spraying was done with 0.1% a.i. Bavistin. Soil drenching of the clumps with 0.1% Bavistin (1.5 l/m^2) was also done with the second spraying. Effect of treatments was analyzed by observing the fungal growth/fruit bodies and counting the new healthy culms at the end of September 2003.

During 2004, fungicidal treatment was given again to the two species. The first drenching, with a mixture of 0.05% Tagcop and 0.05% Bavistin, was given on 22 July 2004; the second and third drenching followed at 15-day intervals. On application of second and third drenching, healthy and dead/diseased culms were counted in new culms only, and a final count was taken in December 2004.

RESULTS

The disease was observed in three species of bamboo, i.e., *B. vulgaris* var. *waminii, Dendrocalamus gigantea* and *Gigantochloa* sp.

Pathogen

Fruit bodies arise from a white to pale subiculum appressed to the culm surface. They are much branched and bushy, up to 6 cm in height, pinkish buff to creamy white, drying light brown to buff brown, with a disc up to 2 mm in dia. present at the base. The stem is distinct, short, and slender; branching is dichotomous with branches 0.5–1 mm wide, slender, terete, and attenuate to finely subulate tips (Figs 1 and 2).

Growth rate of the fungus on PDA was 5 mm per day. Colonies are creamy white; the marginal hyphae appressed, more or less dense, and outline of the colony even. Aerial mycelium is silky; the reverse darkened. Hyphae from the advancing zone and aerial mycelium possess clamps, are up to 3 μm wide with multiple and

Figure 1. Mycelial growth at the base of the clump.

Figure 2. Subiculum with fruiting bodies having disc-like base.

sprouting clamps; oidia (3.0–11)×(2–3) μm are observed; hyphae are dextrinoid; terminal swellings are present in aerial mycelium. Hyphae in submerged mycelium are much branched, have clamps and are up to 2.5 μm wide.

This species has been identified as *Pterulicium xylogenum* (B. & Br.) Corner, based on microscopic characters produced by fruiting bodies formed in culture on quarter-strength Potato Dextrose Yeast-extract Agar (PDY/4) [3] and collected in nature. The fruiting body context contains skeletal hyphae that are thick-walled, lack clamps and have few septa or branches, and generative hyphae that are thin-walled and clamped. Other characters include clavate basidia (((24.5–39.5)–45.8)×((7.1–8.7)–10.7) μm to (35–(65–70))×(10–13) μm in size [4]) producing amygdaloid, smooth, non-amyloid basidiospores (((9.5–9.9)–(10.3–10.7))×(5.5–(6.3–6.5)) μm to (11–13)×(6–7.5) μm in size [4]) with a prominent hilar appendix, and probable cystidia, which Corner [4] interpreted as possible basidioles (((26.9–31.6)–36.3)×(6.3–7.9) μm in size) with subacute apices. The basidiospores are usually thin walled and contain many granules and a lipid droplet. With bright-field and phase microscopy the spores give a suggestion of ornamentation, but they are clearly smooth when viewed with differential interference contrast microscopy. The distinguishing feature of this dimitic genus is the corticioid patch, which is reported to produce a fertile hymenium in Malaysian material when facing downward [4]. The corticioid patch or subiculum on collected fruiting bodies lacks skeletal hyphae and in cultured material contains scattered hymenium-like elements 10–13 μm wide, similar to those in a collection from the Phillipines (Los Banos, on *Bambusa*, September 1920, O.A. Reinking, BPI US0333317), which were 6–8.3 μm wide. The absence of a well-developed hymenium on the corticioid patch was also occasionally noted by Corner [4] in other collections.

The specimens of the fruiting bodies and culture of the fungus have been deposited in the Herbarium and National Type Culture Collection of Forest Pathology Division, FRI, Dehradun, under Nos 8655 and 1153, respectively.

Symptoms

Initially profuse white mycelial growth at the base of clumps over leaf litter was noticed at the start of rainy season (Fig. 1). Soon circular to fan shaped white mycelial patches (subiculum) developed over the culm sheath (Fig. 2). Needle-shaped white to dirty white-branched fruiting bodies arising from the subiculum with a disc-like attachment at the base from the affected culm surface were noticed (Figs 2 and 3). Necrotic lesions also appeared on the culms (Fig. 4). Curling of internodes and shortening of nodes resulting into deformity were the prominent features of the disease (Fig. 3). Mortality of the culms started with drying of the culms from tip backwards. Young culms emerging during the season attacked and killed by the pathogen. The curling of tips of culms at later stages of growth arresting their further development suggested that the infection is carried along with the growing tips. The fungal mycelium was observed growing inside the pith and cavities of affected culms (Fig. 5). The fungal mycelium traversing the tissues of the culm and degradation of fibre wall were apparent in the microscopic sections.

Figure 3. Subiculum with fruiting bodies having disc-like base. Curling and shortening of internodes.

Pathogenicity tests

The inoculated culms exhibited disease symptoms with profuse mycelial growth all over the culm surface (Fig. 6). Initiation of fruiting bodies of the fungus was also noticed. Isolations made from the inoculated culms yielded the fungus similar to that was inoculated, thus confirming Koch's postulates. The culture characters also matched with that of *Pterulicium xylogenum*.

Fungicide tests

Except Captan, all the three concentrations of fungicides completely inhibited the growth of the fungus. It was 30% at 0.075% for Captan.

Figure 4. Necrotic lesions on culms.

Damage assessment and management

The assessment of damage in two bamboos is shown in Table 1. It is evident that the *B. vulgaris* var. *waminii* clump has more disease than *Gigantochloa* as almost 45% of the culms were either dead or dying in the former in comparison to about 36% in the latter. Moreover, the emergence of new culms was also higher in *Gigantochloa* (10.6%) than in *B. vulgaris* var. *waminii* (6.8%).

The treatments were found effective in arresting the growth of the fungus and enhancing emergence of new culms, which was 12 times for *B. vulgaris* var. *waminii* and nearly 1.5 times for *Gigantochloa* sp., of the number of healthy culms before treatment (Table 2), whereas the new culms emerging without treatment were only 6.8 to 10.6% in 2002 (Table 1). However, in *B. vulgaris* var. *waminii*, out of 24 new culms, 13 (54%) exhibited disease symptoms.

Figure 5. Mycelium growing inside the culm.

Table 1.
Assessment of damage due to culm rot disease in 2002

Host	Total no. of culms	No. of dead culms	No. of dying culms	No. of new culms
B. vulgaris var. *waminii*	117	21	32	8
Gigantochloa sp.	132	19	28	14

Table 3 shows the results of fungicidal treatment done in 2004 in new culms emerged during the season after the fungicidal treatments. The treatments were

Figure 6. Dead culm with profuse mycelium after pathogenicity test.

Table 2.
Effect of fungicidal treatment on culm rot disease in bamboo in 2003

Host	Before treatment		No. of new culms after treatment
	No. of healthy culms	No. of dead/dying culms	
B. vulgaris var. *waminii*	2	21	24 (11*)
Gigantochloa sp.	110	6	46

* Remained healthy.

effective in arresting the disease as nearly 66% of new culms of *B. vulgaris* var. *waminii* and 53% of new culms of *Gigantochloa* remained healthy. More new culms emerged after treatment, for example, nearly 27% in the *B. vulgaris* var *waminii* (Fig. 7) and 24% in *Gigantochloa*.

Table 3.
Effect of fungicidal treatment on culm rot disease in bamboo in 2004

Host	After treatment					
	No. of new healthy culms			No. of dead/dying new culms		
	06.8.2004*	21.8.2004**	21.12.2004***	06.8.2004	21.8.2004	21.12.2004
B. vulgaris var. *waminii*	31	30	27	13	14	16
Gigantochloa sp.	34	47	25	Nil	Nil	22

*Observations 15 days after first treatment; **15 days after 2nd treatment; ***final observations after 4 months.

Figure 7. New growth after fungicidal treatment.

DISCUSSION

This is a new disease in bamboo, as such a disease with typical symptoms of deformity and rotting of culms is not known from any part of the world [5, 6]. The disease seems to be spreading to different species of bamboo as three species were found affected. The clumps of the three species were far apart (at least 100–300 m aerial distance), suggesting aerial spread of the disease through airborne spores. The spread of fungus on leaf litter at the base of culms can be considered as a means of its survival and lateral spread. The old infected culms also acted in similar fashion during the favourable season. The disease is favoured by the rainy season (mid-June to September) when high relative humidity (69–88%) and temperature (mean 21–28°C) prevail.

It is important to note that the disease attacked the bamboo raised in the bambusetum and botanical garden. So far, the disease was not observed in natural bamboo growing areas, although the fungus has been reported occurring on dead bamboo culms and leaf sheaths from the countries around India [4, 7]. Changed environmental (macro- and micro-climatic) conditions are known to affect the association between plant pathogens and their hosts, which greatly influence the virulence of the native pathogens, the susceptibility of the host or both [8]. Exposure of the hosts to new locations has been known to increase the risk of attack by indigenous pathogens and/or development of a relatively non-specific pathogens becoming aggressive to introduced or native hosts [9]. It may be conjectured here that *P. xylogenum* is basically a saprotroph, which under favourable conditions has acted as a necrotrophic parasite and thus fits in the role of a facultative parasite, the latter being an opportunist pathogen [10].

The morphological characters of the fruit body closely resemble those reported by Corner [4]. *Pterulicium xylogenum* has been placed in Pterulaceae [11]. Molecular analysis has shown that coral fungi are members of the Euagarics clade [12, 13]. This is the first pathogenic report for *Pterulicium xylogenum* on bamboo. Corner [4] reported this fungus on dead bamboo culms, dead leaf sheaths and trunks of various palms from Sri Lanka (Ceylon), Malayasia (Malaya), Phillipines and Uganda. Zhishu *et al.* [7] reported this fungus from China on rotting bamboo culms. The only other genus of coral fungi known to be plant pathogenic is *Typhula* [14].

Rot of emerging culms by *Fusarium moniliforme* var. *intermedium* has been reported in various bamboo species in Bangladesh [15], in India [5, 6] and in Pakistan [16]. Rot of growing culms by *Fusarium equiseti* has been reported in bamboo plantations and natural stands in India [6]. The deformity and culm rot reported in the present study is altogether different in symptoms, causal organism and damage pattern to the above-mentioned diseases.

Emergence of new culms and reduction of disease is a clear indication of effectiveness of fungicidal treatments. A two-pronged approach targeting the fungus on the surface of culms through prophylactic treatment with copper oxychloride (Tagcop) and in the soil and culms with a systemic fungicide carbendazim (Bavistin) helped in managing the disease. The treatment is found effective for the culm rot disease of the bamboo raised under special conditions of bambusetum and botanical garden but for management of the disease in natural system may require a different approach.

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