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Research Article

The Decay Resistance and Hyphae Penetration of Bamboo *Gigantochloa scortechinii* Decayed by White and Brown Rot Fungi

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The decay resistance and hyphae penetration of bamboo *Gigantochloa scortechinii* decayed by white and brown rot fungi were investigated using scanning electron microscope (SEM). The bamboo grown in natural stand from three different age classes of 0.5, 3.5, and 6.5 years was harvested, oven dried, sterilised, and exposed to agar media containing 4% malt extract and 2% agar technical (no. 3) under laboratory condition for 8 weeks. The deterioration was expressed as percentage of weight loss, and the decay resistance classes were measured according to ASTM D 2017-81 (1986). This study found that the percentage weight loss was greatly reduced with the bamboo ageing. Regardless of age, the *G. scortechinii* was classified as highly resistant to decay by white and brown rot fungi. The scanning electron microscope (SEM) observation showed that the fungi hyphae mostly penetrated into the vessel and proceeded to the pit and parenchyma cells.

1. Introduction

Bamboos are one of the natural bioresources available in tropic and subtropic countries. They are considered as a renewable plant because the new shoot will be emerged from soil and grows to become mature bamboo replacing any former harvested culm used for utilisation [1]. Bamboo has attracted much attention due to its short maturation age compared to wood. The age influenced the properties and utilisation of bamboo [2]. The relation between bamboo ageing and maturation was discussed by [3–10]. These researchers made a general agreement that bamboo culm matures at two to three years, reaching its maximum strength. In *Guadua angustifolia*, [11] found that the bamboo reached a maximum strength and remained almost constant at the age between three to four years. The characterisation of *Gigantochloa scortechinii* in relation to its anatomical, physical, and chemical properties by [12], suggested that the bamboo culms can be classified as growing, development, and maturation phases at age of 0.5, 1.5, and 3.5 years, respectively. The *G. scortechinii* aged 3.5 years found to be suitable for any purposes. However this general agreement did not include the decay resistance.

The biological resistance is an important factor for quality control during bamboo processing and utilisation, as the bamboo is considered as susceptible to fungi and insect attacks [13]. In the laboratory test, [14] found that the weight loss of Indonesian bamboo decayed by white rot, brown rot, and soft rot fungi was not significantly different by the culm portions either bottom, middle, or top. However in the further study, [15] found that the bamboo culm aged 6 months decayed more than the older but there were no

Table 1: Basic characteristics of the sixth internode bamboo culm.

Basic characteristic	Age (years)		
	0.5	3.5	6.5
Internode length (cm)	32.9	35.2	40
Internode diameter (cm)	14.7	10.5	16
Culm wall thickness (mm)	8	7	7

significant difference between bamboo aged 1 and 3 years. However, these studies did not report the mode of attack by fungi in the bamboo culm.

The *G. Scortechinii* is the most abundant bamboo species in Peninsular Malaysia, and it was estimated that the Peninsular Malaysia itself had about 1.4 billion bamboo culms (calculated in 6 meter length, [16]). This abundant bamboo resource has a great potential to be exported to other countries as a commodity; therefore, the assessment of its decay resistance is important. The investigation of hyphae penetration in decayed bamboo using scanning electron microscope (SEM) is very important to understand the mode of fungus attack, and the information is useful in terms of selecting the preservation methods and biodegradation control during material storage and processing.

2. Materials and Methods

2.1. Sources of Materials. *G. scortechinii*, ages ranging from 0.5, 3.5, and 6.5 years old, grown in natural stand in Nami, Kedah (northern part of Peninsular Malaysia) was selected. The age of culms was recognized from the emerging shoot that first came out on the first day. From one clump (one culm per age), they were harvested and immediately coated with wax before being transferred to the laboratory. The basic characteristics of the culms are given in Table 1.

2.2. Preparation of Specimens. Only the sixth internode above ground level was used, and this is considered as diameter at breast height. The culm was cut to disc size of 3 cm thickness and trimmed to the final size of 1 cm. The green density and chemical contents were determined by using the water displacement method and the following standards (Table 2).

The specimens were oven dried at 103 ± 2°C, weighed, and sterilized with propylene oxide for two days prior to exposure to white rot (*Coriolus versicolor* L. ex Fr.) culture and brown rot (*Coniophora puteana* Scum. ex Fr.) growing on agar media in Petri dishes. The propylene oxide (30% concentration), used due to its inflammability, can completely evaporate from the specimen after treatment and was found suitable to sterilize bamboo. A 4% malt extract and 2% agar technical (no. 3) medium were used for these two basidiomycetes and incubated at 22°C (65–70% relative humidity) for eight weeks.

2.3. Determination of Deterioration. After eight weeks of exposure to fungi, the adhering mycelium was cleaned softly with a brush, and the specimens were weighed and then dried

Table 2: Standard used for chemical analysis.

Property	Method
Alcohol/toluene extractive	TAPPI T6m-59 [17]
Holocellulose	[18]
α-cellulose	TAPPI T9m-54 [17]
Lignin	TAPPI T13m-54 [17]
Ash	TAPPI T211om-85 [17]
Silica	TAPPI T244os-77 [17]
Starch	[19]

Table 3: Decay resistance class (ASTM D2017-81, 1986 [20]).

Average weight loss (%)	Resistance class
0 to 10	Highly resistant
11 to 24	Resistant
25 to 44	Moderately resistant
45 or above	Slightly resistant or nonresistant

to measure their loss in dry weight by comparing it with the preexposure value. The decay resistance class was determined according to ASTM D2017-81 [20] as shown in Table 3. The average percentage weight loss is indicated by the resistance classes to specific test fungi that ranged from highly resistant to slightly resistant or nonresistant.

2.4. Scanning Electron Microscope (SEM) Observation. Specimen exposed to fungi was sliced and fixed with glutaraldehyde solution (5%), ringed with phosphate buffer (ph 7.2), and osmium tetraoxide solution (1%) in accordance with that described by [21–23].

3. Results and Discussion

3.1. Weight Loss. The percentage weight loss of bamboo culms at different age groups against decay fungi in relation to the decay resistance classes is given in Table 4. The percentage weight losses were differed between the culm age and the decay fungi. In general, the *G. scortechinii* culms are more susceptible to brown rot than white rot fungi, and the percentage weight losses were ranged between 8.90 to 9.95 and 5.30 to 9.90, respectively. For comparison, the percentage weight losses of *Bambusa maculata*, *G. Atroviolacea*, and *Phyllostachys pubescens* decayed by brown rot (*Coniophora puteana* 167) after one-year exposure were ranged 2.8 to 4.2, 5.2 to 5.9, and 4.6 to 4.8, respectively [15]. The percentage weight losses of *B. vulgaris*, *Dendrocalamus asper*, *G. apus*, *G. atroviolacea*, and *G. pseudoarundinacea* decayed by white rot (*Coriolus versicolor* FRI Japan-1030) after 12 weeks exposure were 7.2, 15.2, 4.8, 7.7, and 20.7 [14]. This indicates that the variability of decay resistance is mostly influenced by bamboo species and fungus origin. Therefore, decay resistance test for eight-week exposure period of *G. Scortechinii* used in this study was considered reliable to investigate the effect of bamboo ageing on the decay resistance.

Table 4: Percentage weight loss and decay resistance class for white and brown rot fungi.

Age (yrs)	Percentage weight loss and decay resistance class			
	White rot	Resistance class	Brown rot	Resistance class
0.5	9.90 (3.58)	HR	9.95 (2.47)	HR
3.5	9.24 (3.65)	HR	9.49 (1.80)	HR
6.5	5.30 (2.45)	HR	8.90 (1.86)	HR

HR: Highly resistant. The values in parenthesis are standard deviation.

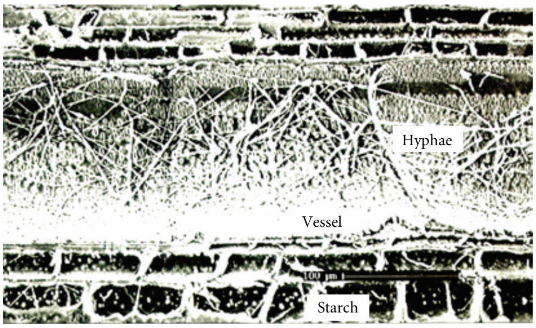

Figure 1: White rot hyphae penetration in vessel lumen (longitudinal section, 650x).

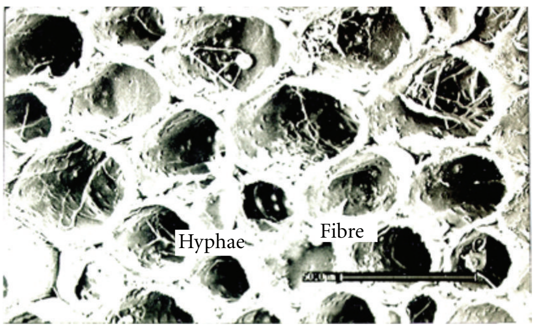

Figure 2: White rot hyphae penetration in fibre lumen (cross section, 618x).

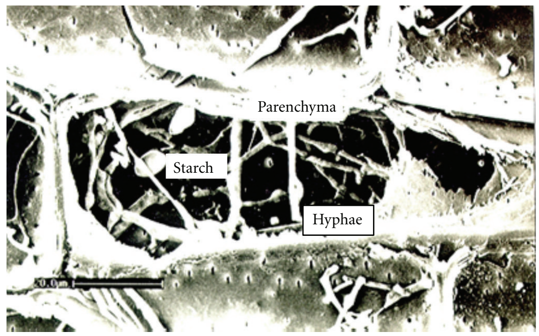

Figure 3: Starch granules utilized by white rot hyphae in Parenchyma cell (longitudinal, 1000x).

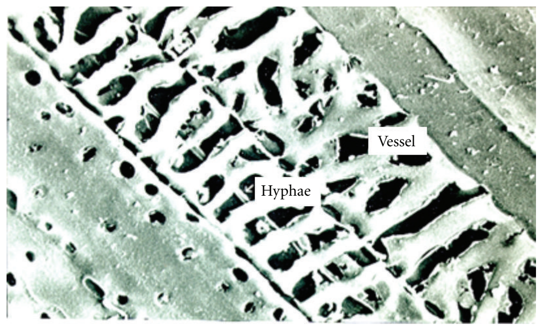

Figure 4: Brown rot hyphae penetration in vessel lumen (longitudinal section, 1670x).

Table 5: ANOVA Results on culms percentage weight loss.

Factors	Df	F value and statistical significance
Age	2	3.94**
Fungi	1	9.30***
Age X fungi	2	3.41**

** $P < 0.05$, *** $P < 0.01$, Df: degree of freedom, F: F distribution.

The percentage weight loss of decayed *G. scortechinii* decreased with ageing. This was clearly seen by a significant difference of percentage weight loss for different bamboo ages (Table 5). The oldest culm (6.5 years) had lowest percentage weight loss, followed by 3.5 years and the youngest ones. All the age groups were highly resistant to white and brown rot fungi. A higher lignin, silica contents as well as density may probably be the three major factors which reduced the deterioration in the oldest culm (Table 6). The incomplete lignification process of fibres and part of the ground parenchyma tissue within the youngest culm (0.5 years) could have attributed to the higher deterioration [24]. The amount of ethanol/toluene extractive content in *G. scortechinii* which ranged from 5.8% (0.5 years), 5.3% (3.5 years), and 5.6% (6.5 years) did not give any advantage for decay resistance.

3.2. Hyphae Penetration. The cavities formed by white and brown rot fungi as observed through the SEM are shown in Figures 1 to 6. It was found that the hyphae penetrated mostly into the metaxylem vessel rather than the fibre lumen's cell for both fungi (Figures 1 and 2). It was also observed that, from the lumen, the hyphae further penetrated into the parenchyma cell, where starch was mostly deposited (Figure 3, white rot). Larger space available in metaxylem vessel lumen, generally, may provide better movement for the penetration (Figures 4 and 5, brown rot).

Table 6: Mean chemical contents of *G. Scortechinii*.

Properties	Age (years)		
	0.5	3.5	6.5
Oven dry density (g/m^3)	0.53 (0.04)	0.61 (0.03)	0.68 (0.07)
Alcohole/toluene extractive (%)	5.8 (1.5)	5.3 (0.4)	5.6 (0.2)
Holocellulose (%)	78.6 (0.3)	80.6 (2.3)	82.3 (1.8)
α-cellulose (%)	64.6 (0.6)	64.6 (0.3)	64.6 (0.2)
Lignin (%)	23.4 (0.3)	27.8 (1.9)	29.0 (0.4)
Ash (%)	1.9 (0.02)	2.8 (0.10)	3.5 (0.02)
Silica (%)	0.6 (0.2)	1.7 (0.02)	2.0 (0.2)

The values in parenthesis are standard deviation.

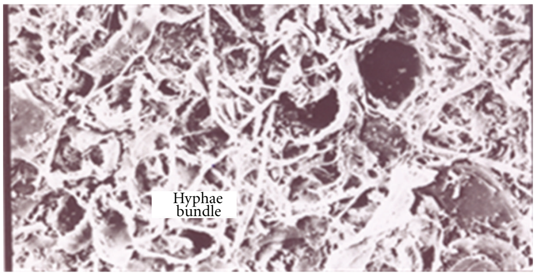

Figure 5: Brown rot hyphae bundle surrounding the vessel lumen (cross section, 600x).

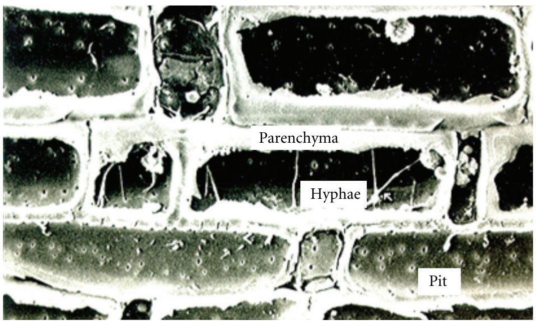

Figure 6: Brown rot hyphae penetration in parenchyma cell through the pits (longitudinal section, 650x).

The average metaxylem vessel diameters were 0.51 mm (0.5 years), 0.57 mm (3.5 years), and 0.50 mm (6.5 years); meanwhile the average fibre lumen diameters were 10 μm (0.5 years), 8 μm (3.5 years) and 9 μm (6.5 years), respectively [12]. The hyphae also used the pits present in the vessel walls as a pathway to penetrate the neighbouring cells (Figure 6, brown rot).

4. Conclusions

The susceptibility of *G. scortechinii* to white and brown rot fungi, measure by percentage weight loss, is greatly reduced with the bamboo ageing. The mode of fungi attacks is by penetrating the hyphae into the large metaxylem vessel cell and then proceeds to the parenchyma cell where starch is abundantly deposited. All bamboo culms of different age groups are highly resistant to decay by white and brown rot fungi.

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