

IN-VITRO DECAY RESISTANCE OF 12 MALAYSIAN BROADLEAF HARDWOOD TREES AS A FUNCTION OF WOOD DENSITY AND EXTRACTIVES COMPOUNDS

K Roszaini¹*, MD Hale² & U Salmiah¹

¹Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia

²School of Environment, Natural Resources and Geography, Bangor University, Bangor LL57 2DG, United Kingdom

*roszaini@frim.gov.my

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ROSZAINI K, HALE MD & SALMIAH U. 2016. In-vitro decay resistance of 12 Malaysian broadleaf hardwood trees as a function of wood density and extractives compounds. This study investigated the factors contributing to the natural decay resistance of 12 Malaysian hardwood species. A 16-week decay test was performed using wood blocks (19 mm × 19 mm × 19 mm) in test jars against three white-rot fungi (*Pycnoporus sanguineus*, *Trametes versicolor* and *Lentinus sajor-caju*). Huge variations in weight loss occurred between wood species. Average weight loss of wood for all species was 0.20–44.80% against *P. sanguineus*, 0.09–52.51% against *T. versicolor* and 0.16–33.17% against *L. sajor-caju*. The corresponding basic densities were between 391 and 1020 kg m⁻³. When mass loss was compared against chemical characteristics and wood density, it was concluded that contribution of wood extractives in improving the durability of wood was highly significant compared with its density.

Keywords: White-rot fungi, natural durability, extractives contents

INTRODUCTION

Information on wood durability is very useful in order to determine its utilisation. Besides insects or termites, decay resistance against fungi also affects the durability of wood in service. Losses caused by these deteriorating influences are enormous and run into millions of dollars each year.

White-rot fungi are the only known microorganisms that can completely break down woody cell wall including lignin, cellulose and hemicellulose to carbon dioxide and water (ten Have & Teunissen 2001). Brown-rot fungi prefer hemicellulose and cellulose, leaving the lignin undigested (Blanchette 1995). Durability of wood can be assessed by graveyard stake (field) test or accelerated laboratory test. Laboratory testing of decay is more preferred compared with field test because it is faster, more scientific and could be conducted in the laboratory using several apparatus (Eaton & Hale 1993).

Many studies have been conducted on natural durability of Malaysian timbers. Unfortunately, these studies do not explain the factors that determine the resistance of wood

against fungi. Thus, damage by these agents on Malaysian hardwood has not been fully investigated. Each timber species has different properties due to structural and non-structural components in the wood. For example, wood extractives content varies depending on the timber species, position of the samples taken and age of the trees. It will also determine which pests will attack the timber. Therefore, knowledge of wood-destroying fungi that are related to wood extractives and density is needed to help define more precisely the dangers that they present.

The 12 timber species chosen for this study were commercial timber species widely used in construction and furniture industry. *Neobalanocarpus heimii*, *Madhuca utilis*, *Cotylelobium lanceolatum*, *Dialium kunstleri* var *trifoliolatum*, *Dipterocarpus grandiflorus* and *Fagraea fragrans*, are widely used for heavy construction such as bridges, piling, wharves, beams, railway sleepers, telegraph and power line posts, flooring and furniture. *Pometia pinnata* and *Khaya ivorensis* are used to make beams, posts, joists, door and window frames, kitchen furniture, spools,

gymnasium equipment and heavy duty flooring. Meanwhile, the other four species (*Shorea curtisii*, *Alstonia angustifolia*, *Cinnamomum scortechinii* and *Hevea brasiliensis*) are widely used in manufacturing furniture, core block board, plywood chests, toys, decorative work and coffins (Ani et al. 2002).

Available data on Malaysian timbers are insufficient to predict variations on tropical timbers. Thus, the aim of this study was to determine the durability of 12 Malaysian wood species samples. For this, in-vitro laboratory decay tests were conducted to estimate the ability of three white-rot fungi (*Pycnoporus sanguineus*, *Trametes versicolor* and *Lentinus sajor-caju*) in degrading Malaysian hardwood.

MATERIALS AND METHODS

Raw materials

Five wood specimen blocks measuring 19 mm × 19 mm × 19 mm were cut from the heartwood of each timber species except for *H. brasiliensis* where the sapwood was used because of the difficulty to define between its sapwood and heartwood regions. The timbers, aged between 15 and 20 years old, are listed in Table 1. Moisture contents of wood samples were measured at different times and the wood samples were placed in desiccators until the time to be introduced into the test jars.

Decay fungi test

Natural durability tests of the 12 timber species were carried out according to the standards (CEN 1994, 1997). Three white-rot fungi, namely, *P. sanguineus* (culture collection KUM 70117), *T. versicolor* (CTB 863A) and *L. sajor-caju* (KUM 70097) were used in this study. Malt extract agar mixture (8 g of malt and 4 g agar dissolved in 200 mL distilled water) was prepared as culture medium. After sterilisation, the culture medium was poured into 9-cm testing jars before introducing the fungal strain. All jars were placed in a conditioning room at 22 °C and 65% relative humidity for 2 weeks for the fungi to grow.

Sterilised fabric paper mesh was laid on the surface of the agar medium and four wood blocks (one control and three were replicates for each timber species) were placed on top of the fabric paper mesh. All test jars were maintained at 25 to 27 °C and 70% RH for 16 weeks. Weekly observations were made to ensure that there was no contamination.

The degree of fungal attack was estimated by determining the weight loss of wood block after 16 weeks. All test blocks were taken out and the mycelium was removed from the test blocks prior to drying to a constant weight (nearest 0.01 g) at 103 ± 5 °C for 24 hours and reconditioned at 27 °C and 70% relative humidity. The test blocks were weighed before and after oven drying and average percentage of weight loss was

Table 1 Twelve Malaysian timber species tested for decay under laboratory conditions

Timber species	Trade name	Family
<i>Neobalanocarpus heimii</i>	Cengal	Dipterocarpaceae
<i>Cotylelobium lanceolatum</i>	Resak	Dipterocarpaceae
<i>Madhuca utilis</i>	Bitis	Sapotaceae
<i>Pometia pinnata</i>	Kasai	Sapindaceae
<i>Dipterocarpus grandiflorus</i>	Keruing	Dipterocarpaceae
<i>Dialium kunstleri</i> var <i>trifoliolatum</i>	KerANJI	Leguminosae
<i>Khaya ivorensis</i>	Khaya	Meliaceae
<i>Fagraea fragrans</i>	Tembusu	Loganiaceae
<i>Shorea curtisii</i>	Seraya	Dipterocarpaceae
<i>Alstonia angustifolia</i>	Pulai	Apocynaceae
<i>Cinnamomum scortechinii</i>	Medang	Lauraceae
<i>Hevea brasiliensis</i>	Rubberwood	Euphorbiaceae

calculated for each individual timber species and test fungus. Moisture contents of the test blocks were determined based on oven-dry weights at the end of the test.

Determination of wood density

Basic density of samples (19 mm × 19 mm × 19 mm, radial × tangential × longitudinal) was determined according to ASTM standard D143 (ASTM 2009). For each timber species, 10 replicates were taken giving a total of 120 wood blocks.

Extraction process

For each wood species, five air-dried milled wood samples (2 g based on oven dry weight) were extracted with toluene:industrial methylated spirit 2:1 using Soxhlet apparatus for 6 hours following ASTM standard D1105 (ASTM 1996). The solvent was removed by vacuum rotary evaporator, and the extracts were weighed. Extraction yield was expressed as percentage of the wood dry weight.

Statistical analysis

An analysis of variance (ANOVA) was carried out using the MINITAB 15 software to test differences in fungi resistance between the species. Correlation analysis was carried out on the raw data to assess the importance of wood density and extractives contents on the durability against decay fungi.

RESULTS AND DISCUSSION

Wood density and extractives contents

The differences in wood densities between the 12 Malaysian hardwood species were clearly significant (Table 2) and these reflected the differences in weight loss against all fungus tested. Wood densities of samples ranged between 396 and 1020 kg m⁻³ but the highest mean value was observed in *D. grandiflorus* (908 kg m⁻³) and the lowest, in *A. angustifolia* (441 kg m⁻³). Differences in wood densities between species may be due to genetic, physiological, silvicultural treatments, growth, mortality, tree age and

location in the tree where samples are taken. Since many of these factors act in combination, it is quite impossible to find a distinct causal effect for the differences (Koch 1985, Muller-Landau 2004).

Wood density had highly negative correlation with weight loss against *T. versicolor* (Table 3). However, this correlation was not significant against *P. sanguineus* and *L. sajor-caju*. *Neobalanocarpus heimii* (813 kg m⁻³) was the only wood species free of fungal attack. *Dipterocarpus grandiflorus*, which had the highest wood density (908 kg m⁻³), showed no decay by *P. sanguineus* but slightly by *L. sajor-caju*. Similar results were observed for *C. lanceolatum*, except against *L. sajor-caju*. On the other hand, *S. curtisii* (586 kg m⁻³) showed no decay by *P. sanguineus* but slight decay against *T. versicolor* and *L. sajor-caju*. The rest of the wood species showed moderate decay by the three fungi. This meant that wood density alone could not be a single indicator of decay because density was largely related to the presence of extractives. Instead, it could be considered as one of the factors that contributed to the durability of wood against fungus.

Extractives contents were generally significantly different between wood species (Table 2). Average extractives contents were between 2.50 and 15.0%. The highest value was found in *N. heimii* (14.8%) which classified the species as durable while the lowest, *H. brasiliensis* (2.8%) was in non-durable class. Variations in wood extractives among tropical hardwood species have been widely reported (e.g. Yamamoto & Hong 1989, Kawamura et al. 2010). Extractives contents and concentrations may vary widely between timber species and within the tree itself (Schultz et al. 2008) and contribute substantially in durability. Table 3 showed that the correlation was highly negatively significant ($p < 0.001$) against all fungi tested. Durability of wood is attributed to its soluble extractives which act as natural preservatives against white- or brown-rot fungi (Celimene et al. 1999). Durability of wood also varies with various biological agents due to variations in the contents of wood extractives. Natural decay of wood depends on the concentration of toxic extractable substances which are formed during the formation of heartwood (Alfenas 1982).

Table 2 Average extractive content and wood density of 12 Malaysian wood species against three white-rot fungi

Species	Density (kg m ⁻³)	Extractive content (%)
<i>Neobalanocarpus heimii</i>	813 c	14.79 a
<i>Cotylelobium lanceolatum</i>	854 b	9.34 b
<i>Madhuca utilis</i>	749 d	9.02 c
<i>Pometia pinnata</i>	776 cd	9.14 bc
<i>Dipterocarpus grandiflorus</i>	908 a	4.51 g
<i>Dialium kunstleri</i> var <i>trifoliolatum</i>	780 cd	5.02 f
<i>Khaya ivorensis</i>	494 g	8.39 d
<i>Fagraea fragrans</i>	680 e	8.81 cd
<i>Shorea curtisii</i>	586 f	7.58 e
<i>Alstonia angustifolia</i>	441 h	4.56 fg
<i>Cinnamomum scortechinii</i>	538 g	4.51 g
<i>Hevea brasiliensis</i>	594 f	2.81 h

Means followed by the same letter are not significantly different in the same group at the 0.001 level of probability

Table 3 Correlation coefficients on properties of 12 Malaysian timbers

Property	Weight loss (%)	Density (kg m ⁻³)	Extractives (%)
<i>Pycnoporus sanguineus</i>			
Density (g cm ⁻³)	-	-	0.388**
Extractives (%)	-	-	-
Weight loss (%)	-	-0.245 ns	-0.575***
<i>Trametes versicolor</i>			
Density (g cm ⁻³)	-	-	0.310*
Extractives (%)	-	-	-
Weight loss (%)	-	-0.527***	-0.514***
<i>Lentinus sajor-caju</i>			
Density (g cm ⁻³)	-	-	0.419**
Extractives (%)	-	-	-
Weight loss (%)	-	-0.102 ns	-0.610***

* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significant, n = 6

Weight loss

After one week exposure, almost all wood samples were covered with fungus although growth of *P. sanguineus* was slow compared with *T. versicolor* and *L. sajor-caju*. About 20% of *H. brasiliensis* wood blocks were covered by actively growing *T. versicolor* and *L. sajor-caju*. Weight losses caused by the three fungi are presented in Table 4. Different fungi species dominate different stages of wood degradation (Käärik

1975). They also act differently on wood tissues (Liese 1970). Slower decay rate for many white-rot fungi is due to lack of nutrients, particularly nitrogen (Butcher & Drysdale 1974). Between the three fungi tested, *T. versicolor* (except in *C. lanceolatum*, *D. kunstleri* var *trifoliolatum* and *N. heimii*) was the most aggressive. Average weight loss of samples ranged from 0.56 to 30.45% for *P. sanguineus*, 0.34 to 36.95% for *T. versicolor* and 0.57 to 25.35% for *L. sajor-caju* (Table 4). *Neobalanocarpus heimii*

Table 4 Average weight loss of 12 Malaysian wood species against three white-rot fungi

Species	Average weight loss (%)		
	<i>Pycnoporus sanguineus</i>	<i>Trametes versicolor</i>	<i>Lentinus sajor-caju</i>
<i>Neobalanocarpus heimii</i>	0.56 (0.08) e	0.34 (0.16) g	0.57 (0.16) f
<i>Cotylelobium lanceolatum</i>	1.21 (1.23) d	5.24 (3.08) e	4.23 (1.12) d
<i>Madhuca utilis</i>	0.72 (0.21) d	7.22 (2.63) e	3.58 (0.82) d
<i>Pometia pinnata</i>	4.38 (2.70) c	20.89 (7.88) c	4.00 (0.72) d
<i>Dipterocarpus grandiflorus</i>	0.69 (0.58) de	6.48 (1.92) e	1.03 (0.54) e
<i>Dialium kunstleri</i> var <i>trifoliolatum</i>	29.98 (4.50) a	14.08 (11.45) cd	11.81 (10.19) b
<i>Khaya ivorensis</i>	3.90 (6.24) c	27.86 (7.11) b	3.67 (2.68) d
<i>Fagraea fragrans</i>	5.21 (1.77) c	12.57 (2.71) d	6.52 (1.52) c
<i>Shorea curtisii</i>	0.26 (0.17) f	1.65 (0.57) f	1.27 (0.35) e
<i>Alstonia angustifolia</i>	28.97 (10.54) a	35.19 (7.54) a	15.32 (2.34) b
<i>Cinnamomum scortechinii</i>	6.99 (0.52) b	10.08 (2.06) d	7.11 (0.53) c
<i>Hevea brasiliensis</i>	30.45 (14.78) a	36.95 (6.23) a	25.35 (7.78) a

Means followed by the same letter are not significantly different in the same group at the 0.05 level of probability, values in brackets are standard deviations

showed very high level of resistance against all three fungi and was largely free of fungal attack at the end of the test period. *Madhuca utilis*, *D. grandiflorus* and *S. curtisii* had low weight losses too but they were not consistent between fungus and varied from 1 to 8% for *T. versicolor* and *L. sajor-caju*.

Pycnoporus sanguineus showed more decay on *D. kunstleri* var *trifoliolatum*, *A. angustifolia* and *H. brasiliensis* compared with *T. versicolor* and *L. sajor-caju*. Average weight loss of 0–10% is considered as highly resistant, 11–24% resistant, 25–44% moderately resistant and above 45%, slightly or non-resistant (ASTM 1993). Based on this classification of resistance, *N. heimii*, *M. utilis*, *D. grandiflorus*, *S. curtisii*, *P. pinnata*, *K. ivorensis*, *C. scortechinii*, *C. lanceolatum* and *F. fragrans* can be categorised as highly resistant while *D. kunstleri*, *A. angustifolia* and *H. brasiliensis* as moderately resistant against *P. sanguineus*. This showed that there were variations in the amount of decay (weight loss) for each timber species depending on the fungus.

Generally, *P. sanguineus*, *T. versicolor* and *L. sajor-caju* caused an average weight loss of more than 10%. Weight loss of 2% is the threshold for decay, and wood-decay fungi causing weight loss less than this level may be functioning as mycoparasites or scavengers (Worrall et al. 1997). Variations in average weight loss were only significant for two timber species against

P. sanguineus and *T. versicolor* and three species against *L. sajor-caju* (Table 5). *Alstonia angustifolia* (64.47%) and *H. brasiliensis* (68.85%) had huge weight losses variations against *P. sanguineus*, while *D. grandiflorus* (76.09%) and *K. ivorensis* (55.94%) against *T. versicolor*. *Alstonia angustifolia* (61.09%), *H. brasiliensis* (64.31%) and *F. fragrans* (68.04%) showed huge variations in average weight loss against *L. sajor-caju*. All of them showed significant differences in weight losses. The rest of the timber species (range 2–50%) showed no significant difference in weight loss.

Only *A. angustifolia* and *H. brasiliensis* had significant differences in weight losses compared with the rest of the samples against *P. sanguineus*. Weight losses of *D. grandiflorus* and *K. ivorensis* were significantly different against *T. versicolor*. *Alstonia angustifolia*, *H. brasiliensis* and *F. fragrans* showed significant variations in average weight losses against *L. sajor-caju*.

Variations of decay resistance within species are correlated with distribution and nature of toxic extractives (Amusant et al. 2004). Variation in durability between timber species may be ascribed to certain gums, waxes, resins, tannins and other phenolic substances, essential oils, terpenes and possibly other materials which become infiltrated into the cell walls and are offensive to the fungi. Basal and the outer layers of heartwood are the most durable parts of a tree and they are rich in extractives contents. Besides

Table 5 ANOVA results showing the proportion of total variation in average weight loss and moisture content accounted for samples within wood species

Species	Average weight loss			Average moisture content		
	PS	TV	LSC	PS	TV	LSC
<i>Madhuca utilis</i>	31.33 ns	46.41 ns	38.27 ns	31.59 ns	11.93 ns	87.90**
<i>Neobalanocarpus heimii</i>	36.11 ns	9.06 ns	38.44 ns	67.54*	21.41 ns	27.76 ns
<i>Pometia pinnata</i>	20.80 ns	12.74 ns	35.25 ns	91.53***	16.63 ns	56.76*
<i>Dialium kunstleri</i> var <i>trifoliolatum</i>	36.12 ns	25.13 ns	12.10 ns	67.48*	14.04 ns	60.03*
<i>Dipterocarpus</i> <i>grandiflorus</i>	29.69 ns	76.09**	45.30 ns	41.28 ns	58.77*	53.92*
<i>Khaya ivorensis</i>	8.86 ns	55.94*	2.44 ns	21.43 ns	40.18 ns	14.65 ns
<i>Cinnamomum</i> <i>scortechinii</i>	26.21 ns	43.10 ns	31.67 ns	77.56**	25.90 ns	56.25*
<i>Alstonia angustifolia</i>	64.47*	23.66 ns	61.09*	59.60*	37.13 ns	89.09***
<i>Cotylelobium lanceolatum</i>	23.14 ns	2.19 ns	11.57 ns	13.87 ns	15.83 ns	86.30**
<i>Hevea brasiliensis</i>	68.85*	40.30 ns	64.31*	38.75 ns	50.90 ns	6.95 ns
<i>Shorea curtisii</i>	23.68 ns	9.42 ns	31.11 ns	74.38**	9.45 ns	32.78 ns
<i>Fagraea fragrans</i>	48.52 ns	7.27 ns	68.04*	63.88*	25.79 ns	65.16*

PS = *Pycnoporus sanguineus*, TV = *Trametes versicolor*, LSC = *Lentinus sajor-caju*; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, ns = not significant

extractives content, other factors which may contribute to variations in decay are the lignin and starch contents of heartwood (Scheffer & Cowling 1966).

Moisture content of the wood

Water is an important factor that affects wood decay. In the current study, moisture content increases with weight loss (Table 6). Timber species with lower range of moisture contents had lower weight losses while those with higher range of moisture contents had higher weight losses. All timber species tested had higher moisture contents when exposed to *T. versicolor* (range 25.68–274.38%) followed by *L. sajor-caju* (21.12–92.86%) and *P. sanguineus* (19.89–145.83%). Among all test blocks exposed to the three fungi, highest moisture contents were observed in *H. brasiliensis* (110.81, 66.70 and 74.20% for *T. versicolor*, *L. sajor-caju* and *P. sanguineus* respectively) while the lowest were in *N. heimii* (28.48, 23.66 and 22.39% respectively). This proves that moisture is absolutely necessary for decay and intensity of wood degradation by white-rot fungi increases as moisture increases. (De Groot 1975). Moisture must be present before deterioration is initiated (Scheffer &

Cowling 1966). Decay by fungal attack only occurs when the wood has enough moisture to saturate the wood fibre (exceeding 20% of oven-dry weight) and exposed to air.

CONCLUSIONS

The natural durability of 12 Malaysian woods species against three white-rot fungi was determined and the roles of wood density and extractives in natural durability of wood were discussed. Dense and extractive-rich species were more resistant to decay. Of the 12 hardwoods species used in the present study, four timber species (*N. heimii*, *C. lanceolatum*, *M. utilis* and *S. curtisii*) had far less decay than the rest of the species. Slower decaying rates of these four timber species by *P. sanguineus*, *T. versicolor* and *L. sajor-caju* might be due to extractives contents in the wood.

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Table 6 Average moisture content of 12 Malaysian wood species against three white-rot fungi

Species	Average moisture content (%)		
	<i>Pycnoporus sanguineus</i>	<i>Trametes versicolor</i>	<i>Lentinus sajor-caju</i>
<i>Neobalanocarpus heimii</i>	22.39 (1.47) g	28.48 (1.67) g	23.66 (1.64) f
<i>Cotylelobium lanceolatum</i>	30.85 (2.31) d	43.58 (9.04) ef	33.81 (5.96) e
<i>Madhuca utilis</i>	37.63 (7.56) d	62.32 (5.63) c	41.54 (4.41) cd
<i>Pometia pinnata</i>	48.01 (11.80) b	68.91 (9.83) b	39.04 (4.29) d
<i>Dipterocarpus grandiflorus</i>	25.80 (1.33) f	42.23 (2.65) ef	31.25 (2.36) e
<i>Dialium kunstleri</i> var <i>trifoliolatum</i>	48.00 (18.77) bc	57.53 (16.47) cd	47.86 (7.84) b
<i>Khaya ivorensis</i>	37.86 (13.94) cd	99.20 (24.41) a	44.13 (5.62) bc
<i>Fagraea fragrans</i>	34.34 (7.35) d	71.25 (10.15) b	40.71 (7.50) cd
<i>Shorea curtisii</i>	26.85 (1.64) e	48.52 (8.22) de	34.77 (4.64) e
<i>Alstonia angustifolia</i>	52.17 (15.48) b	85.11 (40.37) ab	57.30 (15.62) a
<i>Cinnamomum scortechinii</i>	31.98 (4.61) d	60.71 (11.15) c	33.56 (2.92) f
<i>Hevea brasiliensis</i>	74.20 (37.37) a	110.81 (62.08) a	66.70 (12.61) a

Means followed by the same letter are not significantly different in the same group at the 0.05 level of probability, values in brackets are standard deviations

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