Faculty of Resource Science and Technology

RELATIVE SAPSTAIN (BLUE-STAIN) AND MOULD SUSCEPTIBILITY OF SELECTED LIGHT COLOURED MALAYSIAN TIMBERS.

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Relative sapstain (blue-stain) and mould susceptibility of selected light coloured Malaysian timbers.

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ABSTRACT

Sapstain and mould fungal infection rates were studied on rubberwood (*Hevea brasiliensis*), kembang semangkok (*Scaphium* sp), jelutong (*Dyera costulata*) and sentang (*Azadirachta excelsa*). Infection rates of these woods by sapstain (*Botryodiplodia theobromae*), mould (*Trichoderma* sp) and natural innocula (unknown fungi) were also correlated with wood basic density, hot water extractive and cold-water extractive contents of wood. Two mycology tests were also compared: laboratory sapstain and mould test, and natural above-ground exposure at the field (unknown fungi). Rubberwood showed the highest mean basic density (0.704 g/cm^3) while jelutong was the lowest (0.426 g/cm^3). For the hot water extractive, jelutong show the highest mean extractive content (17.3 %) and the lowest was kembang semangkok (12.8 %). Sentang have the highest mean cold-water extractive content (13.3 %) while jelutong was the lowest (10.5 %). Rubberwood was the most susceptible wood against blue-stain, mould and natural fungal infection at field while sentang was least susceptible to colonization in the field. There was generally no relationship between wood properties and relative susceptibility to fungal infection, except that cold water extractive content was significantly negative correlated with sapstain infection rates.

Keyword: sapstain, mould, basic density, water extractive, wood durability

ABSTRAK

Jangkitan 'sapstain' dan 'mould' di kaji ke atas rubberwood (Hevea brasiliensis), kembang semangkok (Scaphium sp), jelutong (Dyera costulata) dan sentang (Azadirachta excelsa). Kadar jangkitan daripada 'sapstain' (Botryodiplodia theobromae), 'mould' (Trichoderma sp) dan pemencilan semulajadi (kulat yang tidak diketahui)juga dikaitkan dengan kepadatan asas kayu, kandungan ekstrak air panas dan kandungan ekstrak air sejuk.Dua ujian mikologi telah digunakan untuk membuat perbandingan: teknik kajian makmal terhadap 'sapstain' dan 'mould' dan dedahan terus di atas tanah di lapangan. Kayu getah menunjukkan kandungan kepadatan yang paling tinggi (0.704 g/cm^3) manakala jelutong adalah yang terendah (0.426 g/cm^3). Jelutong menunjukkan bacaan yang paling tinggi bagi min kandungan ekstrak air panas (17.3 %), dan yang terendah ialah kembang semangkok (12.8 %). Sentang pula mengandungi ekstrak air sejuk yang paling tinggi (13.3 %) manakala jelutong adalah yang terendah (10.5 %). Kayu getah merupakan kayu yang paling mudah dijangkiti oleh kulat yang menyebabkan 'blue-stain', 'mould' dan pada kulat di lapangan manakala sentang pula merupakan kayu yang kurang dijangkiti oleh kulat di lapangan. Secara umumnya, tidak terdapat sebarang hubungan di antara sifat umum kayu yang dikaji dengan tahap jangkitan kulat, kecuali bagi kandungan ekstrak air sejuk terdapat korelasi negatif yang bererti dengan kadar jangkitan 'sapstain'.

Kata kunci: 'sapstain', 'mould', kepadatan asas, kandungan ekstrak, kekuatan kayu

1.0 INTRODUCTION

1.1 Importance of timber industry and the need for controlling wood degradation.

The natural forest has contributed significantly to the socio-economic development of Malaysia, that timber products stands supreme in terms of export earnings (Krishnapillay & Abdul Razak 1998). The timber sector is poised to remain as an important factor in the export-driven economy of many countries that will be the mainstay of economic activities of timber producing countries (Anon. 1998). But the degradation of wood by fungal organisms, from fungal stains to fungal decay, has been a problem, which has plagued the timber industry for centuries (Seabright 1995). Fungal attack of wood substrates is an essential part of the ecosystem cycle in nature but it is a regarded as a problem to us (Wong & Salmiah 1994, Seabright 1995).

1.2 Fungi infection of wood and the impact of the quality of timber's value.

Fungi will live and survive either parasitically or saprophytically, breaking down complex structures of polysaccharide through enzymatic reaction (Seabright 1995). These microorganisms feed on proteins found in the parenchyma cells that breakdown of timber's biomass and will also make use of any nitrogen content that speeds up the process of decomposition. The timbers that have been affected will change in colour, loss in weight, softening of the surface and the prevalence of musty odour. The degradation can occur in a variety of ways depending on the strain of fungi (Zabel & Morrell 1992). However sapstain fungi is unable to decay wood most of the time, and therefore unlike decay fungi, is able to only cause considerable discoloration of

light hardwoods. Such severe discoloration affects the saleable value of Malaysian timber products manufactured from light hardwoods, such as rubberwood, ramin and kembang semangkok.

1.3 Types of fungal infection.

Fungi, which cause severe discoloration and disfigurement of wood in storage and in service, are generally described as staining fungi while those that grow superficially on wood are called moulds (Eaton & Hale 1993). Among the light hardwoods of Malaysia, sapstain is considered a major biological degradation problem, which affects the final monetary value and aesthetics of timber products made from light hardwoods species. The most frequently recorded sapstain fungus is *Botryodiplodia theobromae* that is particular problem on light-coloured hardwoods (Eaton & Hale 1993). Major sapstains are caused by fungi with pigmented hyphae that grow primarily in the parenchyma tissues of the sapwood (Zabel & Morrell 1992). It has long been recognized as a major pathological problem in freshly felled rubberwood and the light hardwood and softwood timbers in Malaysia [Hong & Wong 1994 (cited in Wong & Sabri 2000), Wong et al 1999].

According to Zabel & Morrell, 1992, moulds are particularly common on hardwoods when the wood is very wet, but some can develop on wood held for long periods at high relative humidity. *Trichoderma* sp is one of the most common moulds fungi on the surface of green timber which produce green colonies or minute cushions of spores (Eaton & Hale 1993, Zabel & Morrell,

1992), also can cause sapstaining. The development of stain in timber and the emergence of moulds on its surface are considered of great economic importance to the timber and wood preserving industries because of losses in commercial quality of the product (Eaton & Hale, 1993).

1.4 Natural durability of wood.

Sapstain and mould susceptibility of wood is partly connected with natural durability, because when the natural durability of wood is high, it will affect ease of the colonization by fungi. According to Eaton & Hale, 1993, the ability of the heartwood of any one wood species to resist decay is said to be its 'natural durability' or its 'decay resistance'. Sapwood is always regarded as having low natural durability although many species of wood may have little or indistinct sapwood. Natural durability varies within the tree, especially in species with very durable heartwood (Eaton & Hale 1993), the larger the tree the greater are the differences at the base.

1.5 Extractives content.

Naturally occurring inhibitory substances can usually be removed from wood by extraction with water or organic solvents, whence they are often described, together with some other substances, as extractives. Extracts from durable heartwood are more toxic than those from sapwood from the same tree. Durability is reduced by removal of extractives from wood. The relative toxicity

of extractives corresponds broadly with the durability of different timbers (Rayner & Boddy, 1988) and possibly also to the sapstain and mould severity of light coloured timbers.

1.6 Objectives

1.6.1 To examine which Malaysian timbers of light hardwood categories are naturally most or least susceptible to blue stain and mould infection represented by the fungi *Botryodiplodia theobromae* and *Trichoderma* sp respectively, and also to natural fungal infection at field.

1.6.2 To briefly determine which of the three major wood properties affect sapstain rates among the selected timber species.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Woods:

Wood materials were obtained from Associate Professor Dr. Andrew Wong. Each wood was labeled as shown below:

Wood material	Labeling name
Rubberwood (*Hevea brasiliensis*)	RW
Kembang Semangkok (*Scaphium* sp)	KSK
Jelutong (*Dyera costulata*)	JL
Sentang (*Azadirachta excelsa*)	SE

2.1.2 Fungi:

Stock cultures of fungi were obtained from mycology lab culture collection of Unimas. Each fungus was labeled as shown below:

Fungi	Labeling name
Botryodiplodia theobromae	Sapstain
Trichoderma sp	Mould

Natural fungal infection at field was labeled as 'Field'

2.1.3 Equipments and apparatus:

ALP Model AG-23 autoclave and HIRAYAMA HICLAVE HVE-50 autoclave were used to sterilize all the apparatus (120°C) that have been used in sterile technique method to against the contaminant during the tests. The equipment was also use to steam-sterilize (100°C) wood blocks.

NATIONAL Blender was used to blend the sapstain and mould fungal mycelium with Potato Dextrose Agar (PDA) media, while the sterile technique of mycology tests were done in the ESCO laminar flow while the petri dish with fungi and wood inside were incubated in the dark (25°C) the PROTECH Model GC-1050 growth chamber.

SL SHELL LAB 1350 FX oven were used in order to oven dry (105°C) the woods specimen. The measurement of the oven dry weight of wood blocks and mycological media were made using electronic balance, while LEICA camera-attached light microscope was used to prepare light micrographs of the infection by fungi under the microscope.

2.2 Methods

2.2.1 Mycology test method

2.2.1.1 Sterile technique (in the lab)

Five replicates for each fungi test of woods placed in glass Petri dish, with moisten filter paper base and netlon mesh, covered with aluminium foil. Then, steamed at 100°C for 1 hour, 3 days consecutively.

The fungi inoculated from the stock culture, 2 petri dishes each, using sterile technique. The growth of fungi took about 1 week. After that, the fungi re-inoculated to prepare the pure culture, 5 replicates each. The cultures were raised on PDA for 7-14 days. Mycelia fragments-cum-spore suspensions of each fungal stain were prepared separately by blending the colony agar in 400 ml sterile distilled water with sterile blender.

The steamed woods were dipped into the blended fungi and then placed in each glass petri dish prepared with double moisten filter paper base and netlon mesh under aseptic condition. The glass petri dish was placed in the black plastic bag together with a beaker of water, and incubated at 25°C inside the humidified chamber. The woods with different fungi placed separately from each other, 1 plastic bag for 1 fungus. The infection by fungi was measured by using visual rating method (**Table 1**) for 30 days consecutively, every day. The sterile distilled water will be added in the petri dish when it seems to dry, using sterile technique.

After 30 days of infection, the woods dipped in the Formalin-aceto-alcohol or FAA (90 ml of ethanol 70% + 5 ml of glacial acetic acid + 5 ml of formalin 40%). After a few weeks, slide preparation is begin. The woods were cut using microtome. After slice of wood was made, it was transferred into safranin for 1 hour. Then, it was transferred continuously for 2 minutes each into ethanol (10%, 30%, 50%, 70%, and 100%) and lastly into xylene. The slice of wood was placed on the slide and added with Canada balsam, then covered with slide cover. Microscopic picture were taken to observe the fungi attacked.

2.2.1.2 Natural exposure (at the field)

The exposed wood material (5 replicates of each species) placed on the netlon mesh and positioned on the land directly. The designed box that covered with aluminium foil used to place the netlon mesh with woods material (**Plate 1**). The box located under the table with plastic surrounding it to protect from sun and rain (**Plate 2**). The infections of fungi to the woods were measured by using visual rating method (**Table 1**) for 30 days consecutively.

Plate 1: Woods that place on netlon mesh exposed to the environment.

Plate 2: Modified field technique to place the woods at natural exposure.

Table 1: Visual Rating Method (according to Wong and Sabri 2000)

Condition of infected wood (%)	Visual Rating scale	Median surface mycelial coverage (%)
No fungal growth	0	0
1 – 5	1	3
6 – 20	2	13
21 – 35	3	28
36 – 50	4	42.5
51 – 75	5	63
76 – 100	6	88

2.2.2 Selected general wood properties

Both wood density and extractive content are among the main properties which help distinguish one wood species from another, therefore these two parameters were evaluated and compared

between wood species to try and detect similarities or differences in sapstain and mould infection rates among the selected light hardwoods.

2.2.2.1 Basic density

Five replicates of each species of woods, which are *Hevea brasiliensis, Azadirachta excelsa, Scaphium* sp and *Dyera costulata,* were oven dried for 24 hours at 100°C. Once took out from the oven, the woods were immediately put into dessicator with Silica gel inside. Once cooled, the weights were measured using the balancer. The volumes of the woods were being measured using the ruler. Formula used in the calculating of basic density was:

$$\text{Basic density} = \frac{\text{Oven dried weight (g)}}{\text{Volume (cm}^3\text{)}}$$

2.2.2.2 Hot water extractive content

The purpose of determination of hot water extractive content was to provide a measure of extraneous components such as tannins, gums, sugars, and colouring matter, and also starches. The experiment was performed in duplicate. About 1.5 g of air-dried grounded sample was weighed out on a filter paper. The grounded sample was transferred to a 250 ml Erlenmeyer flask and the top of the bottle was covered with aluminium foil, submerged in a boiling water bath;

100 ml of distilled water was added. The water level was held just above the solution level in the flask, three hours digestion. The heating was repeated on the next day for another 3 hours. The contents of the flask was filtered to a tare-filtering crucible, washed with hot water and dried to constant weight at 105°C, the hot water extractive content was calculated on oven dry basis.

Calculation for percentage of hot water extractive content:

Weight of air dried grounded sample	=	X
Weight of crucible	=	Y1
Weight of crucible + oven dried extract	=	Y2
Weight of oven dried extract	=	Y2 – Y1 = Y3
Percentage (%) of hot water extractive content	=	$\frac{(X - Y3)\ 100}{X}$

2.2.2.2 Cold water extractive content

The cold water extractive content provides the same measure as hot water extractive content in lignocelluloses materials. The analysis was carried out in duplicate. About 1.5 g of dried grounded sample was weighed out on a filter paper. The sample was transferred into a 400 ml beaker and 300 ml of distilled water was slowly added, to make sure that the grounded sample was well wetted initially to avoid tendency to float. The top of the beaker was covered with aluminium foil. The mixture was allowed to digest at room temperature for 48 hours. The material was transferred to a tare-filtering crucible, and washed with cold distilled water, and

dried to constant weight at 105°C. The cold water extractive content was calculated on oven dry basis.

Calculation for percentage of cold water extractive content:

Weight of air dried grounded sample	=	X
Weight of crucible	=	Y1
Weight of crucible + oven dried extract	=	Y2
Weight of oven dried extract	=	Y2 – Y1 = Y3
Percentage (%) of cold water extractive content	=	$\frac{(X - Y3)\ 100}{X}$

2.3 Statistical analysis

LSD is the basis for multiple comparison of mean values at the two-tailed 0.05 level of significance, where the LSD value was computed using the relevant Mean Square Errors from the relevant ANOVA Tables that were obtained from MINITAB, SPSS and EXCEL softwares. The formula for computing LSD as shown below:

$$LSD = \sqrt{\frac{2 \times MSE}{\text{Replicates}}} \times t_{df\ error}(0.025)$$

There were 20 paired x-y values were used in the regression study. The correlation between selected wood properties (basic density, hot water extractives content and cold water extractives content) and three types of fungi infection (sapstain, mould and natural infection at field). Simple correlation analysis ($P<0.05$) was done on paired variables as follows:

Basic density *versus* sapstain, mould and field

Hot water extractives content *versus* sapstain, mould and field

Cold water extractives content *versus* sapstain, mould and field.

3.0 RESULTS

3.1 Fungal infection susceptibilities

The infection on wood blocks through mycology test at field and in the lab can be seen on **plate 3** to **plate 14.** Susceptibility among the wood species was different among fungi at field and laboratory studies (**Figure 1 - Figure 3**), but rubberwood remains the most susceptible among the other woods throughout all the infection tests. The infection from *Botryodiplodia theobromae* (sapstain) during 30 days was almost the same on rubberwood and jelutong (**Figure 1**). For kembang semangkok, the infection slightly lower than the two woods species before. For rubberwood, kembang semangkok and jelutong, there were fully infected by the end of the day 30. Among the woods, the infection on sentang was much slower than the other woods species. **Plate 15** show the hyphae of the sapstain fungus, *Botryodiplodia theobromae*, that attacked the fiber cell of the rubberwood.

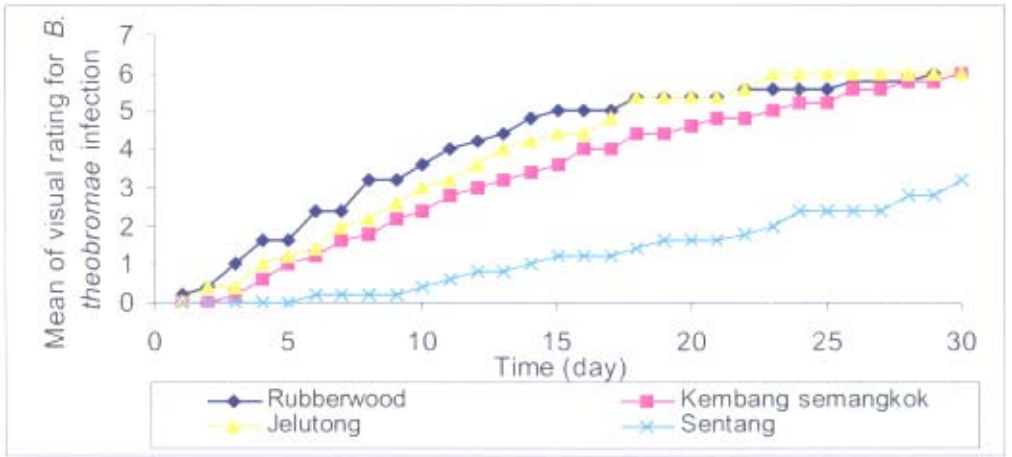

Figure 1: Mean of visual rating of wood infected by *Botryodiplodia theobromae*

Trichoderma sp infected more rapidly on rubberwood as infection by sapstain fungus before. After 21 days, rubberwood was already completely fully infected by the fungus (**Figure 2**). Sentang infected more rapidly by *Trichoderma* sp compared to the *Botryodiplodia theobromae* infection. For the first two weeks, jelutong infected faster than kembang semangkok, but after the two weeks later, kembang semangkok infected rapid than jelutong. Only rubberwood and sentang were fully infected by the end of day 30. Among the four species of woods, infection on jelutong was the slower by this fungus.

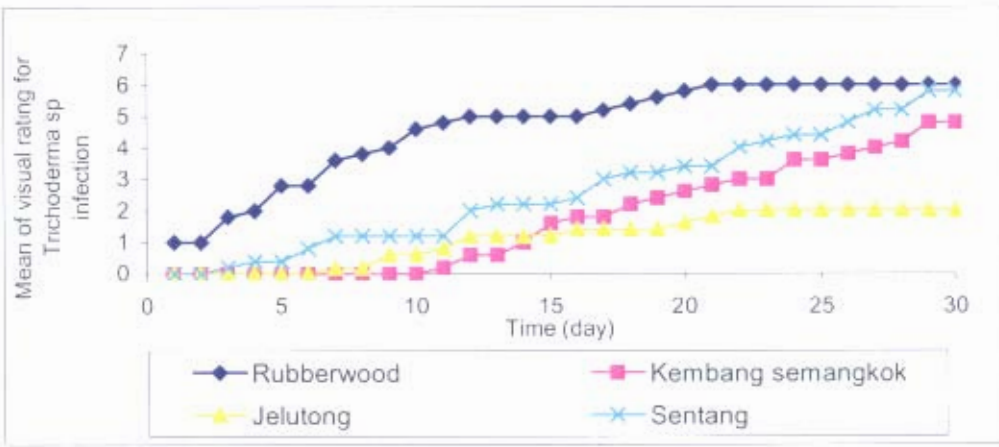

Figure 2: Mean of visual rating of wood infected by *Trichoderma* sp.

At natural exposure, the rubberwood was fully covered by fungi infection on day 16 (**Figure 3**), between 76% to 100% coverage (**Table 1**). Infections by fungi at field still rapid on rubberwood as infection by sapstain and mould. The fungi infection ranked followed by kembang semangkok and jelutong. All the three species were fully infected by the end of day 30. During the exposure day, any fungi did not infect on sentang.

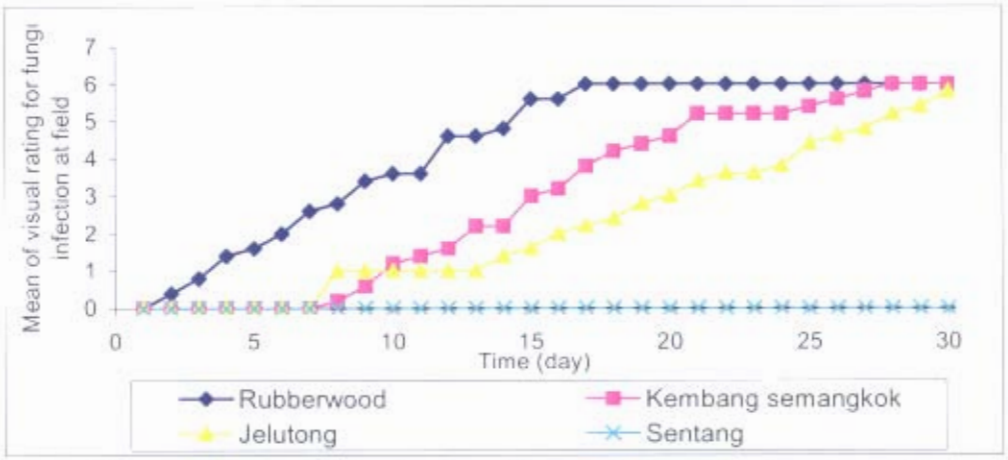

Figure 3: Mean of visual rating of woods infected by fungi at field

The visual rating of fungi coverage showed significant differences ($P<0.05$) in growth between all woods species through the two mycology test methods, shown by ANOVA tables (**Appendix G to I**). For sterile technique in the lab test, both of fungi show significant difference between the wood species. Infection from *Botryodiplodia theobromae* differed significantly between wood species ($P<0.05$). *Trichoderma* sp infection of the woods were differed significantly at ($P<0.05$). For fungi infection at field, the average visual rating of growth between wood species differed significantly ($P<0.05$), for the duration of 30 days.

3.2 Wood properties

3.2.1 Basic density

Rubberwoods have the highest mean basic density among the woods that is 0.704 g/cm^3 with standard deviation 0.049. With standard deviation 0.073, kembang semangkok was the second highest which have 0.661 g/cm^3 then followed by sentang and jelutong, 0.555 g/cm^3 and 0.426 g/cm^3 each, with standard deviation 0.015 and 0.053 each.

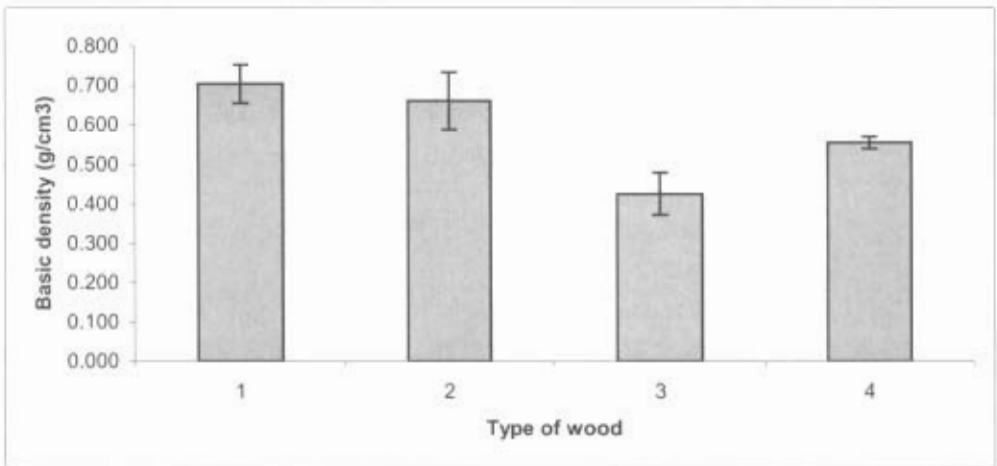

Figure 4: Basic density (g/cm^3) between four light hardwood species (1=Rubberwood, 2=Kembang semangkok, 3= Jelutong, 4=Sentang)