ONLINE ISSN 2069-7430
ISSN-L 1841-4737

PRO LIGNO
www.proligno.ro

Vol. 19 N° 2 2023
pp. 29-37

RESISTANCE OF RUBBERWOOD (HEVEA BRASILIENSIS MULL. ARG.) TO BROWN-ROT FUNGUS (*SERPULA LACRYMANS*) AFTER TREATMENT WITH TEAK HEARTWOOD EXTRACTS

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Abstract:

This study was carried out to examine the effectiveness of wood extract Tectona grandis (Teak) as preservative material against brown rot fungi on rubberwood. This is with a view to making rubber wood more resistant to fungi using an environmentally friendly treatment method like the natural extractives from Teak wood. The heartwood samples of Tectona grandis were extracted in hot water and used in the treatment of rubber wood. Samples of rubberwood were prepared in dimensions of 20mm x 20mm x 9mm for fungal tests. Before the fungal tests, rubberwood samples were oven-dried at 105°C till constant weight was attained. Teak heartwood was extracted using hot water and some of the oven-dried rubberwood samples were soaked in the extract for 24 hours. The chemical constituents of leached extracts of Teak heartwood were analyzed using the Gas Chromatography-Mass Spectroscopy (GC-MS) method. The unextracted and extracted Teak samples (in the same dimension as the rubberwood samples for fungal tests), rubberwood samples soaked in Teak extracts and untreated rubberwood were all exposed to brown-rot fungus (Serpula lacrymans) for seven weeks. Visual ratings of the spread of the fungus mycelium on the samples were recorded weekly, while the weight loss due to fungal exposure was calculated after the exposure period. The density value of Tectona grandis wood samples (816.99kg/m^3) was higher than the value (573.80kg/m^3) reported for Hevea brasiliensis wood samples. Rubber wood was not dimensionally stabilized after treatment with Teak extracts (47%) as the water absorption values of rubber wood were not significantly different from those soaked in water (49%). However, rubber wood treated with Teak extracts showed higher resistance to brown-rot fungi (10.11%) compared to the untreated samples (24.89%).

This result indicated that the treatment of rubber wood with Teak extracts enhanced its resistance to brown-rot fungi, and the level of protection may have been conferred by the presence of some chemicals compounds such as saponin and tannin in the extracts of Teak heartwood wood. This study has shown that it is feasible to extract Teak heartwood as a bio-preservative to improve the resistance of other non-durable wood species.

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Key words: *Rubberwood, Tectona grandis, extracts, Serpula lacrymans, weight loss, bio-preservative.*

INTRODUCTION

Wood being a biological material is readily degraded by bacteria, fungi, and termites (Ebeling 1975). However, some wood species are naturally resistant to these degrading agents while others are very susceptible to deterioration (Sykacek *et al*. 2006). Natural resistance is the inherent ability of some wood species to resist the attack of bio-deteriorating agents without treatment with chemical preservatives (Owoyemi *et al.* 2014). An example of wood resistant to natural degradation is teak. Teak (*Tectona grandis*) is popularly known in the wood industry for its natural durability. Teak grows naturally throughout southeastern Asia (Bhat *et al.* 2001; Xie *et al.* 2001). It is one of the most valuable tropical hardwood species in the international market (Lourenco *et al*. 2015). Teak wood is priced for its beautiful texture, weather resistance, and high natural durability (Costal *et al*. 1958). This makes it suitable for different purposes including house construction, shipbuilding furniture making, poles, veneer, and cravings (Ozarska 1977; 1999). The high natural durability of teak is an important characteristic that has been attributed to various compounds identified in the extractives of teak (Rudman *et al*. 1958; Lukmandaru and Takahashi 2009).

In contrast to the natural durability of Teak wood, rubberwood, which is also a plantation species which has been reported to contain little or no extractive that contributes to either its durability. Hence, rubber wood logs or sawn timber are very susceptible to fungi and insects, such that when used, bio-deteriorating organisms will degrade the features that make it suitable for wood products. Examples of the features are strength properties, easy working quality (sawing, nailing, turning etc.) attractive colors etc. To make the wood last longer in service, its treatment with appropriate preservatives for protection against the attacks of bio-deteriorating organisms becomes necessary (Mohd *et al.* 1999). Olaniran *et al*. (2019) noted that to optimally use rubberwood in construction, applying treatments that enhance its durability will be an appropriate step.

The major objective of the preservative treatment is to extend the life of the material in service, thus decreasing the ultimate cost of the product and avoiding the need for frequent replacements (FAO 1986). Chemical preservatives such as Chromated copper acetate (CCA), zinc chloride, sodium borate, solignum, berganol, coaltar, creosote etc. are effective preservatives but can pose a serious health hazard to users. When treated with these preservatives, rubber wood can be very toxic to human health and the environment. In the quest to seek a natural and environmentally friendly alternative, extracts from teak wood is considered to be assessed for its effectiveness on the durability of rubberwood. Therefore, the objectives of this study are to assess the effect of treatment of rubberwood with teak heartwood extracts on its durability to a brown-rot fungus (*Serpula lacrymans*) and to analyze the leached extracts of Teak wood for possible chemical compounds that may enhance the durability of rubberwood after treatment.

Hillis and Laver (1978) observed that extractives from bark and wood have the potential to replace synthetic wood preservatives. Furthermore, Syofuna *et al.* (2000) noted that the content of extractives plays a key role in the prediction of the durability of wood. As the inherent disadvantages of most synthetic wood preservatives to man and the natural environment are quite alarming, the quest for developing eco-friendly wood preservatives as alternatives to conventional ones is imperative. Studies have shown that injecting wood extractives of durable species into non-durable specimens increases the wood samples' durability (Adegeye *et al.* 2009). For species like rubber wood without naturally toxic extractives, the focus is to use the extract from the heartwood of Teak to modify rubber wood for enhanced durability to brown-rot fungi.

MATERIALS AND METHODS

Rubberwood planks were procured from a sawmill within Akure metropolis and were cut into sample dimensions of 20mm × 20mm × 9mm for the durability test. The rubber wood samples were oven dried in an oven at 103±2°C until constant weight is attained and recorded as Wo. The samples were kept in a desiccator with silica gel to prevent further adsorption of moisture. The heartwood samples of *Tectona grandis* (Teak) were obtained from off cuts or residues at the sawmill at road block, Akure, Ondo state. Extraction of teak heartwood wood was done at the laboratory of the Department of Forestry and Wood Technology, FUTA, while culturing and inoculation of the fungi were done at the laboratory of the Department of Crop Soil and Pest Management in FUTA.

Determination of Density and Moisture content

Density and moisture content of Teak and Rubberwood were determined from samples with dimensions 20mm x 20mm x 9mm (Longitudinal x Radial x Tangential) prepared for the experiment. Twenty-five samples of Teak and Rubberwood samples were prepared. Density was calculated from oven-dried (at 103°C for 24 hours until constant weights were achieved) weights and dimensions of specimens, while moisture content was determined from initial weights and oven-dry weights of wood samples.

Extraction of Teak heartwood

Method of extraction of teak heartwood: Extraction of the teak heartwood was done using water hot water treatment. Wood samples were weighed and kept in a glass container with a lid. Distilled water (hot) was poured on the sample and left for about 20 - 30 minutes and brought down to cool (hot extraction). To prepare about 500ml of extract solution, 500ml of distilled water was added to 65.9g of wood.

Determination of chemical constituents of Teak extracts

Chemical composition of extracts of Teak heartwood extracts were determined using the gas chromatography-mass spectrometry (GC-MS) equipment (Agilent 7890A GC 5975 California USA). The details of the procedure include setting the column temperature at 80°C, and injection temperature at 250°C; pressure was set at 108kPa, while total flow was 6.2ml/min. Compounds that were present in Teak heartwood extracts were identified using molecular weight and compound formular as well as retention time. Compounds present were identified by comparing the values obtained and spectra data with compounds from the library data of corresponding compounds using automated Shimadzu software.

Treatment of Rubber wood with leached Teak Extract

Cold-soaking method of treatment was used. In this method, twenty-five samples of rubberwood were immersed in a container filled with teak extract. Soaking was done for 24 hours. The wood samples were removed from the container and drained of excess preservatives on a wire mesh.

To determine the percentage absorption of the extracts, the weight of the wood treated with extract was taken and recorded as W_1 and was calculated as follows:

$$\text{Absorption } \% = (W_1 - W_o)/W_1 \text{ X } 100 \quad (1)$$

where: W_1 = Treated weight (before exposure to fungi);
W_0 = Oven dried weight (before exposure to fungi).

Following the above calculation, the mean absorption of Teak heartwood extracts by rubberwood is 35.9%.

Culturing and Inoculation of wood samples with brown-rot fungi

The culture for pure Brown rot (*Serpula lacrymans)* was acquired from the International Institute for Tropical Agriculture (IITA). These fungi were subcultured into plates using potato Dextrose sugar agar (PDA) at 37°C for three days, and later inside bottle where the wood (treated and untreated) was inoculated at 20°C and 70% RH. The inoculation of brown rot was done by first disinfecting the container to be used, with ethanol. Furthermore, a solution of 39g of media in 100ml of distilled water was then heated to dissolve the medium completely. It was sterilized by autoclaving at 121°C under pressure for 15 minutes. Potato Dextrose Agar (PDA) commonly used for isolation and enumeration of yeast and molds from dairy and other food products was used. After which it was then shared 50ml into each container. The syringe used for the introduction of brown rot (*Serpula lacrymans)* was sterilized by fire before being used to lift the brown rot to avoid contamination.

Laboratory testing of resistance to decay fungi

Decay resistance was evaluated according to EN 305-1 (1994). One-hundred samples consisting of Teak samples extracted with hot water (25 samples), unextracted Teak (25 samples), rubberwood treated with extracts from Teak heartwood (25 samples), and untreated rubber wood (25 samples) were exposed to the brown-rot fungus. Visual rating was carried out for a period of 7 weeks on a scale of 0-5 (0-100%) to determine the cross-sectional area of each sample covered by brown rot fungus as shown in Table 1 (according to Carol and Vina 2007). At the end of seven weeks, % weight loss of the wood test sample was calculated to determine the relative decay susceptibility or resistance of the treated and untreated wood samples using the equation below and the summary of the result was analyzed using two-way ANOVA.

$$\text{Weight loss } (\%) = \frac{w_2 - w_3}{w_2} \times 100 \quad (2)$$

where: W2 = weight (g) of treated wood before exposure to fungi;
W3 = weight (g) of treated sample after exposure to decay fungi.

Table 1

Visual Rating for Assessment of Fungal Growth

Visual Rating	Fungal Growth percentage
0	Clean specimen (no growth on sample)
1	20% growth
2	40%
3	60%
4	80%
5	100% (Heavy mould growth on sample)

Data Analysis

The data obtained from this study were subjected to analysis of variance (ANOVA) for significant difference, using Statistical Package for the Social Science (SPSS) software.

RESULTS AND DISCUSSION

Density and moisture content of Rubber wood and Teak

The mean density values of oven dried *Hevea brasiliensis and Tectona grandis* wood samples is presented in Table 2. It can be observed that the mean density value of *Tectona grandis* wood samples (816.99kg/m^3) is higher than that of *Hevea brasiliensis* (573.80kg/m^3) wood samples. The results of analysis of variance (ANOVA) in densities of oven dried *Hevea brasiliensis and Tectona grandis* wood samples presented in Table 3 showed that the oven dried densities of *Hevea brasiliensis and Tectona grandis* wood samples are significantly different from each other ($p<0.05$). Density and moisture content are two important parameters that influence many properties of wood (Walker 2006). Although earlier studies have shown that the effect on density on wood decay is less pronounced, moisture plays a prominent role in fungal decay (Plaschkies *et al.* 2014; Thybring *et al*. 2018). For degradation by brown-rot fungi, moisture plays an important role since it is required in the diffusion of enzymes and growth inhibition (Thybring *et al*. 2018).

Table 2

Densities of Hevea brasiliensis and Tectona grandis wood samples.

Species	Density (kg/m^3)	Moisture content (%)
Hevea brasiliensis	573.80 ± 109.24	22.13 ± 9.33
Tectona grandis	816.99 ± 87.21	13.29 ± 4.28

Values are mean± standard error

Table 3

Analysis of Variance in densities of Hevea brasiliensis and Tectona grandis wood samples.

Sources of Variation	Sum of Squares	df	Mean Square	F	p-value
Species	739238.852	1	739238.85	75.66	0.000*
Error	468969.357	48	9770.20		
Total	1208208.209	49			

*means significant at $p\leq0.05$

Chemical composition of Teak extract

The result in Table 4 shows the Quantitative and Qualitative chemical constituents of *Tectona grandis*. From the table, nine (9) chemical compounds were observed in *Tectona grandis* which include tannin, saponin, alkaloids, steroids terpenoids, flavonoids, oxalate, phytate, and cardiac glycosides. It was observed that cardiac - qlycocides was absent. Teak extractives have been reported to be complex, with several compounds previously found in Teak to be over eighty (Yamamoto *et al*. 1998; de Castro *et al*. 2022). However, chemical compounds that mainly enhance the durability of Teak wood include tannins, quinones, and polyphenols; some of which are also present in Table 4.

Table 4

Chemical composition of Teak heartwood extracts

Chemical compounds Analyzed	Teak wood extract mg/g QA	Teak wood extract mg/g QTT
Tannin	+	0.02
Saponin	+++	3.64
Alkaloids	++	4.80
Steroids	+	3.80
Terpenoids	+	4.75
Flavonoids	++	2.14
Cardiac-glycocides	-	-
Oxalate	+	0.63
Phytate	+++	18.12

NOTE: = Absent/Not detected; + = Present, ++ = Moderately present, +++ = Abundantly present, QA = Qualitative Analysis, QTT = Quantitative Analysis

Durability of Rubberwood treated with Teak extract
Visual rating of the exposed samples to brown rot

The visual rating of *Hevea brasiliensis* and *Tectona grandis* samples exposed to brown rot fungi is presented in Fig 1. It can be observed that untreated rubber wood is more susceptible to brown rot fungi compared to rubber wood treated with teak extract, while extracted teak is lower compared to treated rubber this could be as a result of the presence of compounds such as tannin, saponin, alkaloids, steroids, terpenoids, flavonoids, oxalate, phytate which were present in teak and has been extracted and used to treat the rubber wood. The higher susceptibility of rubberwood to decay by the brown-rot fungus may be as a result of abundance of carbohydrates and sugars (Azizol and Rahim 1989), which serve as a major source of nourishment for its growth and survival in the wood.

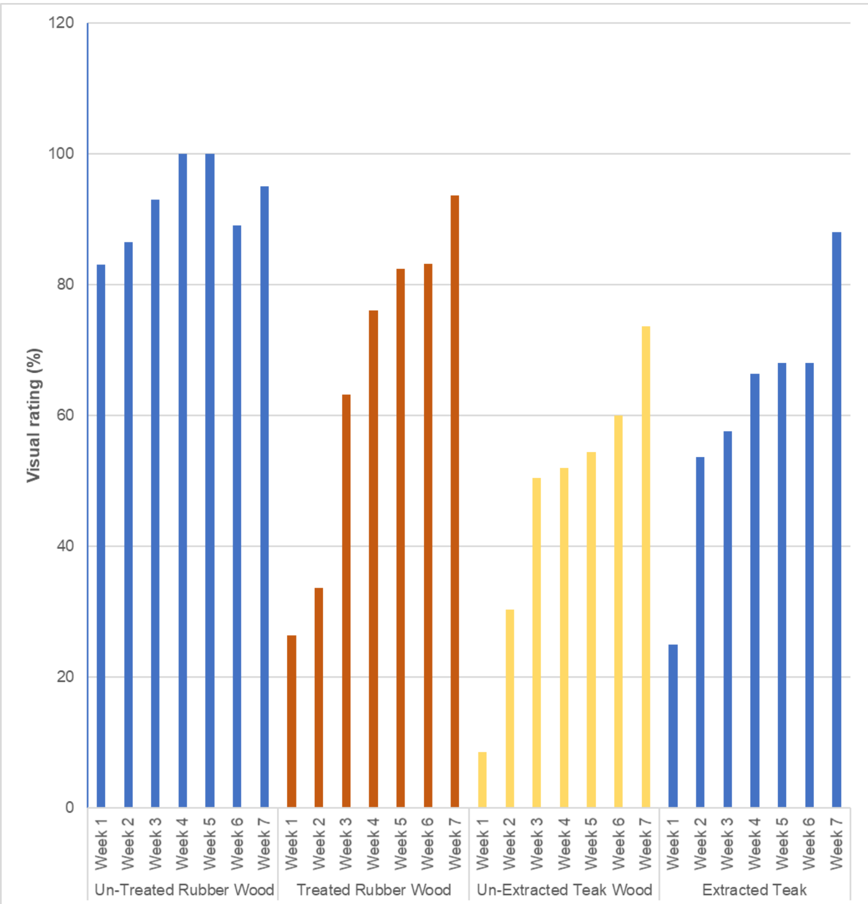

Fig.1.
Visual rating of Hevea brasiliensis and Tectona grandis exposed to brown rot fungi.

Percentage Weight loss to Fungi

The mean values of percentage weight loss to fungi attack of wood samples subjected to varying treatments were presented in Table 5. It can be observed that the percentage weight loss to fungi attack ranges from 8.25% to 24.89%. The result also revealed that the untreated *Hevea brasiliensis* wood samples had the highest mean percentage weight loss (24.89%), followed by treated *Hevea brasiliensis* wood samples with mean percentage weight loss of 10.11%, while the unextracted *Tectona grandis* wood samples had the least observed mean percentage weight loss (8.25%).

The results of the Duncan's multiple range tests that were performed to discern the differences in the mean values of percentage weight loss of wood samples subjected to varying treatments as depicted with a superscript in Table 5 revealed that the percentage weight loss of untreated *Hevea brasiliensis*, treated *Hevea brasiliensis* and extracted *Tectona grandis* are not significantly different ($p<0.05$) from each other. However, the result in Table 4 showed that the percentage weight loss of unextracted *Tectona grandis* significantly differs ($p<0.05$) from all other wood samples subjected to various treatments.

The results of analysis of variance (ANOVA) in percentage weight loss of wood samples obtained from wood samples subjected to various treatments were presented in Table 6. The result revealed that the varying treatment had significant effect ($p<0.05$) on percentage weight loss of wood samples.

Table 5

Percentage weight loss of un-treated and treated Rubberwood, and Tectona grandis exposed to brown-rot fungi (Serpula lacrymans)

Treatment	Weight loss (%)	Durability Class (EN 305-1) cited by Van Acker *et al 2003*
Rubberwood treated with extracts from Teak heartwood	10.11 ± 2.47^{b}	Durable
Untreated *Hevea brasiliensis*	24.89 ± 7.79^{a}	Slightly Durable
Tectona grandis samples extracted with hot water	8.90 ± 12.09^{b}	Durable
Unextracted *Tectona grandis*	8.25 ± 3.43^{b}	Durable

Values with the same superscript are not significantly different at $p\leq0.05$.

Table 6

Analysis of Variance in percentage weight loss of Hevea brasiliensis and Tectona grandis exposed to brown-rot fungi

Sources of Variation	Sum of Squares	df	Mean Square	F	p-value
Treatment	4729.146	3	1576.382	28.054	0.000*
Error	5394.290	96	56.191		
Total	10123.436	99			

* means significant at $p\leq0.05$

Resistance of rubberwood treated with extracts from Teak heartwood to brown-rot fungi

The analysis of variance indicated significant differences in all tested situations during the analysis of weight loss values. In the comparison of the average values of weight loss (Table 4), a reduction trend was observed for each of the tested treatments and both species. Unextracted teak showed a significantly lower value in weight loss when subjected to brown rot fungi, compared with untreated *Hevea brasiliensis* wood. For the *Hevea brasiliensis* wood, samples treated with teak extractives had significantly lower values of percentage weight loss to fungi compared to the untreated *Hevea brasiliensis* wood samples. This implies that the treatment of *Hevea brasiliensis* with teak extract has significant influence on resistance of wood against *Serpula lacrymans*.

Even though the weight loss values of unextracted and extracted *Tectona grandis* wood samples did not significantly differ from each other, the weight loss of unextracted *Tectona grandis* was lower. The lowest weight loss values recorded for the unextracted *Tectona grandis* wood samples means that they were highly durable (Van Acker *et al.* 2003). The findings of this study confirmed the assertions of Lukmandaru (2013), that some teak wood extractives have shown satisfactory inhibitory effects on the growth of brown and white-rot fungi. Brocco *et al*. (2017) and Motta *et al*. (2013) obtained similar results for the natural resistance of external teak heartwood *to P. placenta*. According to Moya and Berrocal (2010), the resistance of teak is complex and can vary between the internal and external portions of the sapwood. In addition, other factors, such as age, location, and growing conditions, can affect the natural resistance (Bhat *et al*. 2005). Although the origin and growth characteristics of wood were not considered in this study, the extractives and chemicals, which provide durability to teak wood, can be found in varying amounts in sapwood and heartwood (Lukmandaru and Takahashi 2009; Moya and Berrocal 2010). According to these authors, tectoquinone, a component that provides durability to teak heartwood, can be found in variable levels in teak, which may affect its biological resistance. The brown rot fungus caused the greatest mass loss to the untreated *Hevea brasiliensis* wood samples (Table 4). This may be attributed to high sugar content, and low density (as shown in Table 1). The fungus is attracted by the high sugar content, and its hyphae were able to penetrate the wood mass.

CONCLUSION

The outcome of this study has shown that extracts from Teak heartwood did not confer improved dimensional stability on rubber wood. It showed further that untreated *Hevea brasiliensis* wood is not naturally resistant to brown rot fungi. However, its resistance to brown rot fungi was improved after treatment with extracts from Teak heartwood. This study also further confirmed the natural resistance of *Tectona grandis* against brown rot fungi even after its heartwood was extracted. Results of this study have shown that it is feasible to extract from Teak heartwood as a bio-preservative to improve the resistance of other non-durable wood species.

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