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Antifungal Activities of Heart Wood Extract (HWE) of Teak *Tectona grandis* Against Two White Rots in Woods of *Gmelina arborea* and *Triplochiton scleroxylon*

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Abstract: Wood samples of *Triplochiton scleroxylon* and *Gmelina arborea* obtained from Omo Forest Reserve, South West Nigeria were treated with Heart wood extract of Teak (HWE), cuprinol clear and kerosene. The wood blocks were inoculated with 2 white rots *Pleurotus squarrosullus* and *Lentinus subnundus* for 12 weeks and subjected to weight loss experiment. Significant variations were found among wood blocks treated with chemical preservatives < 0.05, with HWE performing best after 6 weeks after inoculation. Least weight loss was recorded at 6 weeks after inoculation. In particular, 4% HWE was most effective of all preservative investigated. HWE was more effective in *Gmelina arborea* than in *Triplochiton scleroxylon*. Heart wood Extract of Teak reduced weight loss caused by white rots in two hardwood species. A significant variation however exists between wood species in their response to attack by the white rots. Specifically, weight loss reduced from 20% to 4% in *T.scleroxylon* and from 15% to 5% in *G. arborea*.

Key words: Heart wood Extract · *Triplochiton scleroxylon* · *Gmelina arborea* · White rot · Weight loss

INTRODUCTION

Recent restrictions, internationally, are limiting the use of non-biodegradable, chemicals for wood preservation, primarily, due to increased disposal problems as treated wood is taken out of service.

The current trend is therefore to seek alternatives to synthetic chemicals with attention focused on the use of natural products of plant origin; which are not only effective, but also biodegradable. Wood extractives are extraneous components of the trees [1], which are natural chemicals that proffer resistance against decay in wood [2]. Studies have shown that injecting wood extractives of durable species into non-durable specimen increases the wood samples' durability [3]. It is therefore, pertinent to harness knowledge on the potent heartwood extractives of durable timbers for the development of environmental friendly wood protecting chemicals. Such intentions and pursuits not only have the ultimate aim of sustainable production of naturally durable species on the earth, but are also, reflective of growing concerns on the environmental impact of traditional wood preservatives [4]. There is great potential in the use of extractives as natural preservatives [5], as many components of extractives are toxic to micro-organisms, imparting decay resistance to wood [6]. In Developing countries especially that of West Africa, where sawmilling is predominantly practised in small scale, huge volume of wastes in form of saw dust are generated.The methods of disposal is usually through dumping into water bodies and burning creating serious environmental pollution. It is therefore important to device a better alternative way of disposal which will be environmental friendly. Extraction of chemical from sawdust of durable wood species to produce biodegradable chemical capable of prolonging service life of non-durable like *Triplochiton scleroxylon* and semi-durable species such as *Gmelina arborea* is one of such methods.

Triplochiton scleroxylon K. Schum is an important indigenous species in the Nigerian timber market, due to its versatility and huge volume supply. It is used in plywood production as core stock and extensively in the furniture industry. It is a very successful species under plantation management [7]. Unfortunately however, this species has low service life, as a result of its susceptibility to attack by agents of biodeterioration, but would last longer, when treated with preservatives. Gmelina plantations were established in Nigeria at various

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locations, mainly in the southern part of the country about 40 years ago to provide pulp wood for the pulp and paper Industries which are being established in the country at about the same time. The plantations which have now outgrown their usefulness for paper are being harvested for saw logs to complement the supply of timber from the natural stock. There is need for a sustainable method of treating these important species with preservatives in order to prolong their service life so as to reduce the drain from the forest. The heart wood of *Tectona grandis* has the ability to prolong the service life of Non-durable and semi-durable species, Ogunsanwo *et al.* [8].Using saw dusts from the species to produce biodegradable chemical preservatives will help in harnessing available resources to achieve environmental friendly technology which is capable of reducing poverty, while enhancing the scope of tropical forest products utilization.

This study is therefore aimed at evaluating the biological efficacy of heartwood extractive of *Tectona grandis* against wood decaying organism and comparing its effectiveness as a fungicide with traditional oil-based preservative (Cuprinol-clear) against two wood rotting organisms.

MATERIALS AND METHODS

Preparation of Wood Test Block: The wood samples of *Triplochiton scleroxylon and Gmelina arborea* were obtained from tree samples obtained form Omo Forest Reserve, South western Nigeria. Sapwood of good quality and straight grained stock were obtained from the wood samples. The wood species were sawn into sizes of 6cmx 2cm x 2cm.

The blocks were prepared for test by drying and sterilizing them in the oven for 18 hours at 103°C. The weight was recorded as initial dry weight for each of the blocks. Four hundred test blocks/ species were dried and labelled T1-400 (*Triplochiton scleroxylon*) and G1-400 for (*Gmelina arborea*).

Preparation of Extractive Chemicals: Heartwood portions of *Tectona grandis* were chipped, milled to fine particles, weighed and then collected in bags. 500g of the milled sample was weighed on the load weighing machine and then, transferred into the soxhlet apparatus and extracted in absolute ethanol in accordance with the T2 0403-76 standard [3]. Evaporation *In vacuo*: to separate the heartwood extractives (HWE) from the solvent the mixture was evaporated *in vacuo* using a rotary evaporator. The mixture of the absolute ethanol and the pasty HWE liquid in the 500ml round bottom flask was connected to the rotary evaporator. The temperature regulator was set at 78°C. The rotavapour was set at 7rpm; the absolute ethanol was collected in the 100ml round bottom flask while the oily HWE extractive remained in the 500 ml round bottom flask

Preparation of Test Fungicide: The volume-to-volume method was used to dissolve the HWE extractive in kerosene. This implies that, 1ml of HWE in 99ml of Kerosene (solvent) is equivalent to 1% dilution. Cuprinol-clear, an organic solvent type effective against fungi was also diluted using the same method. Its composition is as follows:

Zinc (as Naphthanate)	1.8%-1.9%
Pentachlorophenol	2.0%-2.1%
Gama-hexachlorocyclohexane	0.5%-0.55%

Concentration Levels: 4% and 8% were prepared for the two preservatives. Thus, the following solutions were prepared C_1-8% Cuprinol-clear solution, C_2-4% Cuprinol-clear, C_3-8% HWE, C_4-4% HWE, C_5-Kerosene only.

Treatment of Test Blocks: Dipping impregnation method [9, 10] was used for treatment of the wood test blocks with the preservatives. They were completely immersed in the fungicides for 10 minutes. The wooden blocks (6cm x 2cm x 2cm) prepared from the sapwood portions of Obeche and Gmelina were conditioned and treated with various concentrations of the HWE (4% and 8%) and Cuprinol-clear (4% and 8%) so as to obtain maximum absorption and retention.

Culture Medium: The inoculum of *Pleurotus squarrosullus* and *Lentinus subnudus* were obtained from the Department of Botany and Microbiology. A nutrient medium of Potato Dextrose Agar (PDA) in distilled water was prepared. First the PDA (35g) was mixed with 1liter of water in conical flask and then homogenized. After homogenizing, 40ml of PDA was poured into McCartney bottles and sterilized by autoclaving at 0.1 N/mm^2 (120°C) for a period of 20 minutes. After sterilization the flask were laid sideways so that the medium is retained in the neck of the bottle. The medium was inoculated with the test fungi within 6 days after preparation of the bottles [11, 12]. The bottles were then incubated at room temperature (27 + 2°C) in the laboratory.

Infection of Test Blocks: The blocks were exposed by placing them aseptically in the bottles in which there are actively growing cultures of the test fungi. The blocks were placed such that they came in contact with the aerial mycelium of the fungus and not the medium, itself, into which some of the preservative might otherwise leach out [12, 13]. Four test blocks treated at each dilution level, 1 block treated with Kerosene and 1 block as control were placed in bottles containing each of the two test fungi. The control test blocks were wrapped in aluminium foil and sterilized in the oven before introduction to the test fungi.

Incubation and Duration of Test: May 13, 2009May 13, 2009May 13, 2009May 13, 2009The bottles containing the test blocks were incubated at 27 + 2°C for 2, 4, 6, 8, 10 and 12 weeks. At the end of each incubation period, the blocks were removed from the culture bottles, cleaned of the adhering mycelium, taking care not to remove the splinters of wood and weighed immediately to determine moisture absorbed. Then, weighed samples were oven-dried at 103°C to constant dry wet [12, 13].

Moisture Content after Incubation: After each incubation period, the percentage moisture absorbed by the wood samples was determined. The wet weights of the blocks were calculated after which they were oven-dried for 18 hours at 103°C. The test blocks were allowed to cool before final weighing. The moisture content was thus calculated.

Weight Loss Determination: At the end of each incubation period, test blocks were careful removed, oven dried and reweighed to determine weight loss.

Experimental Design: A 2 x 2 x 6 x 6 factorial with completely randomized design experiment was used.

Where; 2 = Test blocks (Obeche A_1 and Gmelina A_2)

2 = Test fungi (*Lentinus subnudus* F_1 and *Pleurotus squarrosulus* F_2)
6 = Chemical levels
C_1 = 8% Cuprinol-clear,
C_2 = 4% Cuprinol-clear,
C_3 = 8% HWE,
C_4 = 4% HWE,
C_5 = Kerosene,
C_6 = Control
6 = Duration of inoculation. (2, 4, 6, 8, 10, 12 weeks)

RESULTS AND DISCUSSION

Effects of Preservative Treatment on Percentage Weight Loss in Obeche: There were significant variations in percentage weight loss obeche wood blocks exposed to *Lentinus subnudus*. It was observed that weight loss was lowest for all samples after 6 weeks of inoculation Fig. 1. Also, the untreated obeche blocks had percentage weight losses between 9% and 20%, while other blocks had weight loss not higher than 15%, with Obeche blocks treated with 8% HWE having the lowest weight loss, indicating the toxic effects of chemicals on the treated blocks.

Impregnated Obeche test blocks exposed to *Pleurotus squarrosullus,* showed similar patterns of weight loss for treated samples. The highest weight loss (28%) was recorded after 8 weeks for untreated blocks. The chemical preservatives reduced the weight loss to < 16% during the period of inoculation.

Effects of Preservative Treatment on Percentage Weight Loss in Gmelina: Gmelina test blocks inoculated with *Lentinus subnudus* reduced weight loss by 4-7% after 6 weeks, blocks treated with kerosene had the highest weight loss (15%), but also lowest weight loss after 6 weeks (3%). This is followed by 4% cuprinol and 4% HWE of teak. However, while the weight loss increased to about 12% and 9% respectively for kerosene-treated and cuprinol-treated blocks in week 8, blocks treated with 4% HWE increased slightly in weight loss to about 5%, showing that HWE has the ability to resist that attack of white rot better than other preservatives in Gmelina wood blocks.

Table 1: Results of follow up tests for mean values of percentage weight loss of treatment means (LSD)

Parameter	Mean weight loss (%)
Wood species	
Obeche	0.73a
Gmelina	0.59b
Weeks after inoculation	
Two	0.69a
Four	0.79a
Six	0.46b
Eight	0.74a
Ten	0.68a
Twelve	0.70a

Note: Means with the same letter are not significantly different at $\alpha = 0.05$

Table 2: Analysis of variance for percentage weight loss of treatment combinations after 12 weeks of inoculation (transformed values)

Source	DF	Sum of Square (SS)	Mean of square (MS)	f-value	p-level
Wood types	1	0.971	0.971	71.57	0.000*
Fungi types	1	0.002	0.002	0.15	0.699ns
Chemicals	5	0.022	0.004	0.33	0.897ns
WT x FT	1	0.002	0.002	0.12	0.734ns
WT x CHM	5	0.548	0.110	8.07	0.000*
FT x CHM	5	0.049	0.010	0.72	0.611ns
WT x FT x CHM	5	0.023	0.005	0.34	0.884ns
Error	96	1.303	0.014		
Total	119	2.919			

Note that '*' implies significant and 'ns' not significant at á = 0.05. Where:

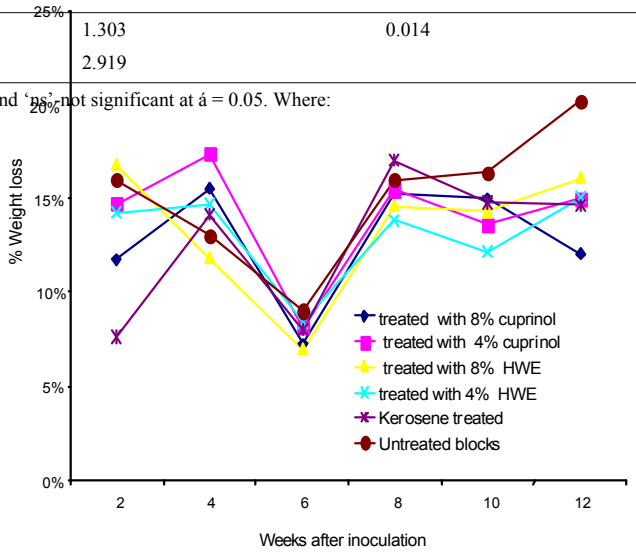
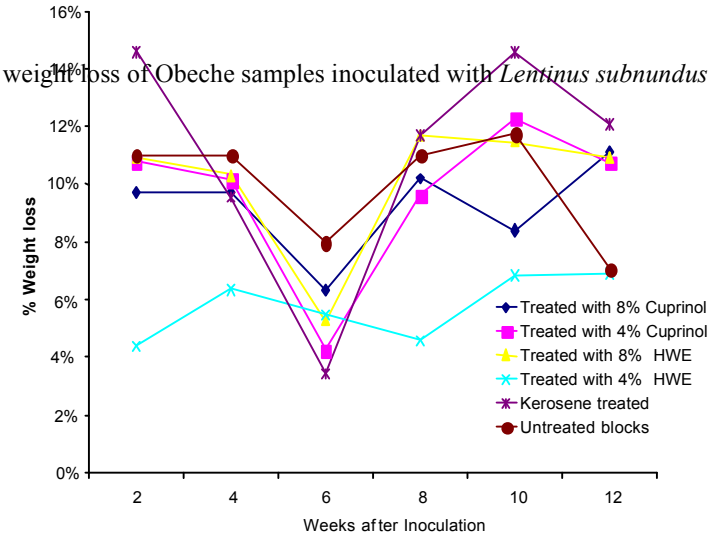


Fig. 1: Mean percentage weight loss of Obeche samples inoculated with *Lentinus subnundus*



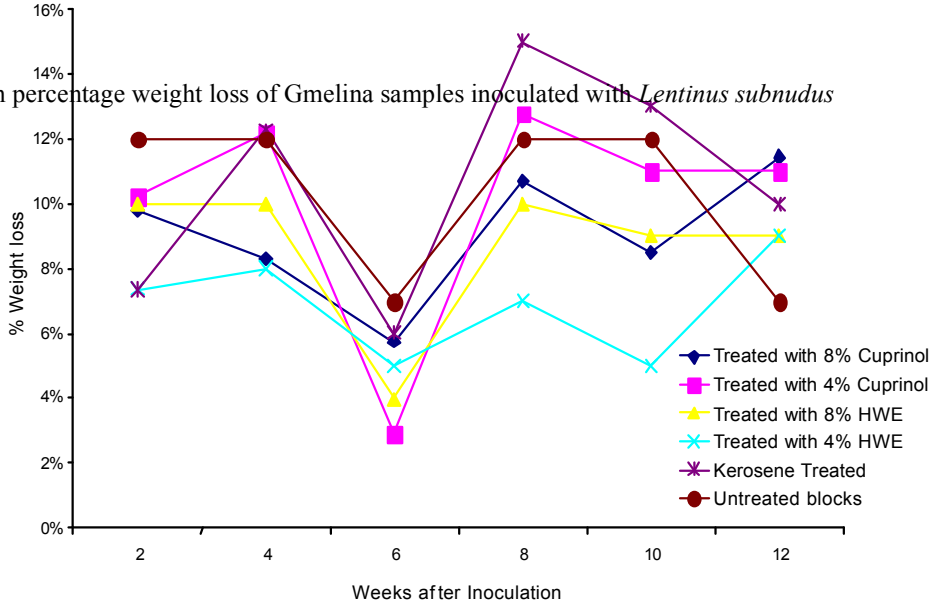


Fig. 2: Mean percentage weight loss of Gmelina samples inoculated with *Lentinus subnudus*

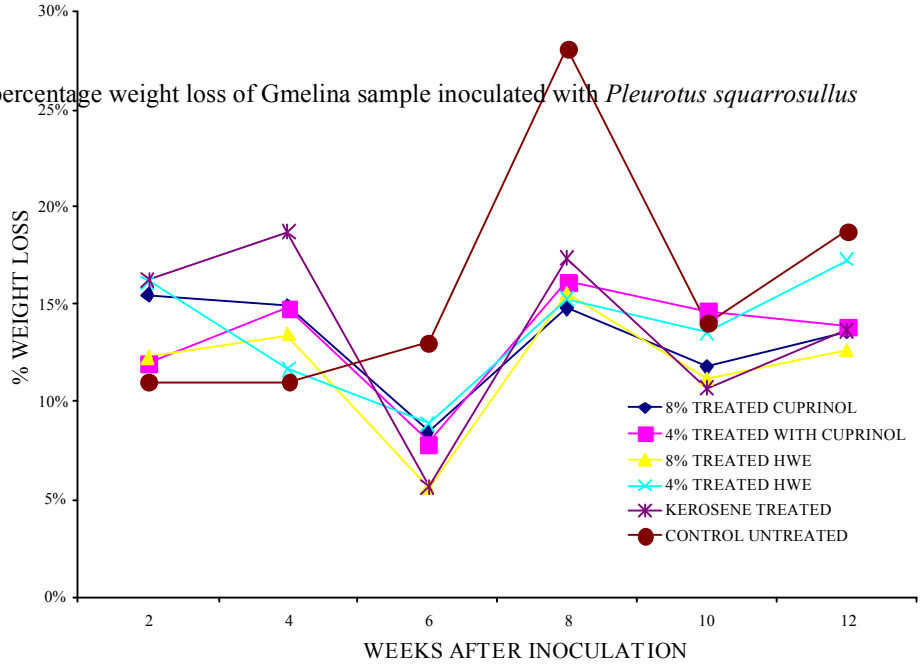


Fig. 3: Mean percentage weight loss of Gmelina sample inoculated with *Pleurotus squarrosullus*

Fig. 4: Mean percentage weight loss of Obeche inoculated with *Pleurotus squarrosullus*

For gmelina test blocks innoculated with *Pleurotus squarrosullus*, the lowest weight loss were recorded in samples treated with 4% HWE (5-9%), while kerosene-treated blocks had the highest weight loss (15%) after 8 weeks, Fig.3. Results further shows that 4% cuprinol-treated block showed the lowest weight loss at the 6th week of inoculation, this is followed again by 4% HWE-treated blocks.

Effects of Wood Species and Type of Inoculums on Weight Loss: There were significant differences in the effects of wood types ($p< 0.05$) and the periods of inoculation ($p< 0.05$) on the percentage weight loss of the treatment combinations, Table 1. This is because *Gmelina arborea* is a semi-durable species while *Triplochiton scleroxylon* is a non-durable species, it is expected therefore to find variations in their responses to attack by agents of biodeterioration. The follow up tests Table 2 revealed that the wood samples were significantly different from each other in their weight loss, while, the weight loss after 6 weeks (0.46) was significantly different from other time periods.

It was further shown that the lowest weight loss was recorded after 6 weeks (0.46) and the highest was recorded after 4 weeks (0.79) of inoculation. The main effect plot revealed that there is no difference in the type of fungi used. Ogunsanwo *et al.* [8] had observed the significant difference in weight loss as a result of differences in fungus type, the result obtained from the present study may be connected to the fact that the fungus in the present study are both white rot decaying organisms and are therefore likely to have similar degradation effects. The main effect plots of the factors revealed that the wood type had a very significant influence on weight loss, as obeche had an overall loss of 0.73, while Gmelina had a weight loss of 0.53, which was significantly lower. Also, the 4% HWE treated samples had the highest main effect on reduction of weight loss.

ANOVA revealed that there were significant differences in the wood type effects and the interaction between the wood types and chemicals used ($p < 0.05$). Other treatments showed no significant differences in the measured variables.

CONCLUSION

This study has revealed the efficacy of heartwood extractive of *Tectona grandis* as an alternative to Cuprinol clear (synthetic chemical) for the preservation of *Gmelina arborea* (Gmelina) and *Triplochiton scleroxylon* (Obeche) against white rot degradation. It was discovered that, the heartwood extractive (HWE) and Cuprinol-clear had similar inhibitory influence on the activities of white rots through weight loss reduction.

HWE has the best inhibitory effect on Gmelina and Obeche test blocks. While HWE at 4% concentration produced the most effective result in terms of weight loss reduction in test blocks, effect of extract on biodegradability of test blocks was higher in *Gmelina arborea* than for *Triplochiton scleroxylon*.

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