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SUITABILITY OF G*LIRICIDIA SEPIUM* (JACQ.) STEUD HEARTWOOD EXTRACT AS FUNGICIDE AGAINST *ANINGERIA ROBUSTA* A. CHEV. WOOD DECAY

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Abstract:

Around the globe, the need for new preservatives to increase the life span of non-durable wood species arose because of increase in cost of the proprietary preservative, non availability when needed and even threat they pose both to workers health and to the environment. Test samples of 15x25x50mm obtained from 5 stands of Aningeria robusta, sampled at the base, middle and top and partitioned into innerwood, middlewood and outerwood along the radial plane were treated with N-hexane extracted heartwood of Gliricidia sepium (GHE) and diluted with kerosene into 0%, 25%, 50%, 75% and 100% before exposure to Lentinus sajor-caju and Trichoderma viride for 16 weeks; untreated test samples were the control. Level of degradation was measured using a 5 - Point Mycelia Growth Scale. A 2x3x3x6 factorial experimental design was adopted; data obtained analysed using descriptive statistics and analysis of variance. Preservative absorption ranged from 0.09 to 12.55Kg/m^3; it increased with increase in concentration level up to 50% when it decreased. The phytochemical analysis showed the presence of alkaloid, tannin, saponin, glycoside and cadenolide. Visual rating showed that the untreated and kerosene treated blocks were almost covered with mycelia growth while 50% had sparse growth. Weight loss decreased with increase in concentration levels from the untreated to 50% GHE, this was the preservative threshold (11.63Kg/m^3) where the GHE performed best; above that level weight loss increased and resistance decreased for both fungi strains. The extract could not prevent complete fungal growth but minimized it greatly.

Key words: *Lentinus sajor-caju; phytochemical analysis; preservative threshold; Trichoderma viride; weight loss.*

INTRODUCTION

The importance of wood in the world economy cannot be over emphasized. It is a unique material in which the chemical composition, anatomical features, physical and mechanical properties and natural durability are interrelated (Winandy 1994; Simpson and TenWolde 1999; Chowdhury et al. 2007). Utilisation of wood is limited due to its susceptibility to biodegradation. The inherent ability of wood species to resist biological deterioration is called natural durability or decay resistance (Sudararaj et al. 2015). Natural durability varies between wood species and is explained mainly by the composition and amount of wood extractives (Alli 2011). The principal biological agents of wood degradation are bacteria, fungi, insects (e.g. termites and beetles) and marine borers. Its excellent potential as a structural material can be limited by the attack of these biodeteriorating agents. Ogunsanwo et al. (2002) noted that large quantities of timbers are destroyed annually by these agents of biodetereoration. Decay and discoloration caused by fungi are major sources of loss in both timber production and wood use, with losses of 15 to 25% marketable wood volume

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in standing timber and of 10 to 15% in wood products during storage and conversion (Zabel and Morrell 1992).

One of the methods usually adopted to guide against wood biodegradation is wood preservation. Less naturally durable wood species require treatment or impregnation with preservatives to increase their resistance to wood destroying organisms. Studies have shown that wood preservation increases the service life of wood by factor of five to fifteen depending on the wood species use and efficacy of treatment (Forest Products Laboratory 2000; Stephens et al. 2001). In recent years, much attention has been centred on finding new alternative locally sourced preservative for the protection of wood-based materials. The need for the new preservatives arose because of increase in cost of the proprietary preservative, non availability when needed and even threat they pose both to workers health and to the environment (Adetogun et al. 2009; Venmalar 2017).

Gliricidia sepium (Jacquin) Steudel is a leguminosae, a versatile fast-growing tree that grows well on many soil types (Simons 1996; Craig and John 2006). Bultman and Southwell (1976); Quintanar et al. 1997 noted that *G. sepium* is highly resistant to insects termites and fungus while Craig and John (2006) reported the presence of tannins, isoflavins, medicarpin and afrormosin as active ingredients in it.

Aningeria robusta (A. Chev.) is a Sapotaceae, a hardwood. The species is well embraced and utilised in Nigerian timber markets and especially recommended for high quality sliced veneer, light carpentry, interior joinery and high class furniture (Chudnoff 1980; PROTA 2010). However, the wood is not durable and liable to attacks by fungi, termites and dry-wood borers (Chudnoff 1980; PROTA 2010).

OBJECTIVE

The objective of this study was to investigate the suitability of heartwood extract of *Gliricidia sepium* as a fungicide against *Aningeria robusta* wood decay with a view of using it as a substitute for the hazardous synthetic preservatives.

MATERIALS AND METHODS

Wood Sample Preparation

Five trees of *Aningeria robusta* were selected based on absence of reaction tendencies; with clear and straight grain and absence of dead knots. They were harvested from a virgin forest within Cocoa Research Institute of Nigeria, Oyo State, South-West, Nigeria. Bolts of 50cm long were collected at the base, middle and top of the merchantable height and partitioned into three equal zones, namely, inner wood, middle wood and outer wood along the radial plane as in Ajala and Ogunsanwo (2011). Test samples of dimension 15 x 25 x 50 mm in accordance with Arora (2006) and Sarker et al. (2006) were used. The test blocks were coded and prepared for test by drying and sterilizing in the oven for 18 hours at 103±2^{o}C until constant weight. Percentage moisture content was calculated using the formula:

$$\%Moisture\ Content\ (MC)\ \frac{(W1-W2\times100)}{W2} \quad (1)$$

where: W1 = weight of wood samples before oven drying
W2= weight of wood samples after oven drying

Extraction of Gliricidia Heartwood Extract (GHE)

Heartwood of a 30 year old *Gliricidia sepium* was milled into fine particles with hammer mill; 500 g of milled heartwood was transcended into the Soxhlet extractor mounted on a steam bath. The processed used was in accordance with the T2 0403-76 Standard (Kazemi et al. 2006). The extract was diluted with kerosene to have 0% (kerosene alone), 25%, 50%, 75% and 100%. Dipping impregnation method (FAO 1986; Adetogun 1998) was adopted and the test blocks were conditioned and completely immersed in cold bath of the fungicide's various concentrations for 48hours in accordance with (BCMAFF 1993) so as to obtain maximum absorption. After treatment the blocks were drained and reweighed to determine absorption level using the BSI (1961) formula:

$$Absorption\ = \frac{\text{Total absorption x concentration x10in Kg/m3}}{\text{Vol. of Wood x Number of Pieces}} \quad (2)$$

Growth medium preparation

Ready made potato dextrose agar (PDA) was used. Thirty-nine (39) g of PDA was dissolved in 1litre of distilled water, homogenized and sterilized in the autoclave at 1.05kg/cm^2 for 15 minutes. After sterilization, the medium was allowed to cool and maintained at 45^{o}C and later dispensed into petri dishes. The PDA was

incorporated with streptomycin to avoid bacteria contamination. Pure isolates of *Lentinus sajor-caju* and *Trichoderma viride* used for the study were obtained from the pathology section of the Forestry Research Institute of Nigeria, Ibadan. The isolates were then subcultured into fully solidified PDA with a 4mm corkborer. The plates were incubated at room temperature. After full ramification, the test blocks were introduced into the two fungi species.

Inoculation of Woodblocks and incubation of fungi

The test blocks were introduced to the fungi strains in a petri dish for sixteen weeks (AWPA, 1997). Durability test against the fungi was carried out using the test blocks in accordance with Zabel and Morrell (1992); EN 113 (1996). The mycelia growth on the surfaces of the blocks were cleaned with dry cotton wool and weighed after sixteen weeks of exposure to the fungi strains. The blocks were dried in the oven for a period of eighteen hours at 103±2^{o}C until a constant weight was obtained and weight loss was determined using the formula:

$$WL = \frac{(DW1 - DW2 \times 100)}{DW1} \quad (3)$$

where:

WL - Weight loss
DW1 - Weight of test blocks before incubation
DW2 - Weight of test blocks after incubation

In examining the test blocks after the incubation period, colour changes and degree of softening were used as parameters of decay assessment (Greaves et al. 1988). Based on this visual method, ratings were allotted to indicate the protective ability of each treatment level on the wood. Six ratings exist; these are 0, 1, 2, 3, 4, and 5. Rating 5 denotes badly decayed wood (Very good fungal growth all over the blocks). Rating 4 indicates fairly decayed wood (Good fungal growth), that is, a situation of marked colour changes and easily recognizable softening. Rating 3 denotes moderate fungal growth. Rating 2 denotes slightly decayed sound wood (poor fungal growth), indicating depletion of the colour of the wood. Rating 1 denotes very rare decayed wood (very poor fungal growth). Rating 0 denotes sound wood, a situation whereby there is a complete suppression of the test fungi by the GHE. Data were analyzed using Analysis of Variance (ANOVA) and Least Significance Difference (LSD) test for mean separation (Gomez and Gomez 1984).

Phytochemical Analysis

Phytochemical screening of GHE was done in accordance with the method of Brain and Turner (1975) to investigate the presence of secondary metabolites such as saponins, tannins, anthraquinone, flavonoids, cardiac glycosides and alkaloids.

Statistics of Experimental Data

A 2x3x3x6 factorial experimental design was adopted; data obtained analysed using descriptive statistics and analysis of variance.

RESULTS AND DISCUSSION

Moisture content

The mean moisture content of test blocks subjected to *L. sajor-caju* is presented in Table 1; it ranged from 43.68% to 77.54% while that of *T. viride* ranged from 48.12% to 63.26% (Table 2). High moisture content was observed at the top between 25% and 75% concentration level (Table 1). Moisture content had significant effect on fungi, preservative concentration level as well as on interaction between fungi used and preservative concentration level at 5% probability level (Table 3). This revealed that the test blocks were exposed to moisture content above fibre saturation point (average 30%) which is required for fungal growth and decay as reported by Kollman and Cote(1968); Highly (1999); Rolls (2003); Raberg et al. (2005); Flæte et al. (2006). The high moisture content observed at the top due to the tender nature of sapwood, therefore, it will be able to retain more water than the lower older part.

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Table 1

Percentage Moisture Content of A. robusta After Inoculation with L. sajor- caju

CL	WZ	Base (%)	Middle (%)	Top (%)	Mean (%)
	Innerwood	44.25	51.61	41.13	45.66±5.38
	Middle	44.19	37.55	50.12	43.95±6.29
Control	Outerwood	42.61	44.21	51.34	46.05±4.64
	Mean	43.68±0.93	44.46±7.03	47.53±5.58	45.22±3.19
	Innerwood	50.61	49.51	47.28	49.13± 1.70
	Middle	52.86	45.92	53. 18	50.65 ± 4.10
0%	Outerwood	36.31	50.25	59.85	48.80± 11.84
	Mean	46.59 ± 8.98	48.56 ±2.31	53.44± 6.29	49.53± 3.35
	Innerwood	66.42	61 .20	68.58	65.40±3.79
	Middle	66.89	70.03	69.71	68.88±1.73
25%	Outerwood	60.45	66.84	65.67	64.32±3.40
	Mean	64.59±3.59	66.02±4.47	67.99±2.08	66.20±1.21
	Innerwood	62.69	64.82	65.76	64.42±1.57
	Middle	67.68	63.90	69.81	67.13±2.99
50%	Outerwood	71.57	60.22	70.41	67.40±6.24
	Mean	67.31±4.45	62.98±2.43	68.66±2.53	66.32±1.14
	Innerwood	71.84	76.34	82.17	76.78±5.18
	Middle	74.88	79.25	78.11	77.41±2.27
75%	Outerwood	77.08	77.02	70.25	74.78±3.93
	Mean	74.60±2.63	77.54±1.52	76.84±2.06	76.33±2.37
	Innerwood	65.27	53.96	65.04	61.42±6.47
	Middle	56. 14	52.84	57.84	55.61±2.54
100%	Outerwood	58.01	55.74	60.15	57.97±2.21
	Mean	59.81±4.82	54.18±1.46	61.01±3.68	58.33±1.71

Legend: CL=Concentration Level of extract, WZ= Wood area zone

Table 2

Percentage Moisture Content of A. robusta After Inoculation with T. viride

CL	WZ	Base (%)	Middle (%)	Top (%)	Mean (%)
	Innerwood	47.89	49.46	45.83	47.73±1.82
	Middle	42.70	48. 15	56.92	49.26±7. 17
Control	Outerwood	54.82	46.76	53.34	51.64±4.29
	Mean	48.47±6.08	48. 12±1.35	47.53±5.58	49.54±2.68
	Innerwood	52.35	56.24	54.32	54.30± 2.36
	Middle	58.69	63. 18	62.45	61.44± 2.20
0%	Outerwood	59.90	69.30	60.49	63.23± 0.95
	Mean	56.98± 20.40	62.91± 22.10	59.09± 19.61	59.66± 0.75
	Innerwood	57.07	52 .23	59.80	56.37±2.09
	Middle	50.45	43. 11	51.73	48.43±2.07
25%	Outerwood	53.48	59.51	57.28	56.76±1.09
	Mean	53.67±18.39	51.62±19.37	56.27±18.32	53.85±0.73
	Innerwood	63.08	59.37	54.77	59.07±1.82
	Middle	54.58	55.33	58.41	56.11±1.82
50%	Outerwood	58.53	58.23	59.93	58.90±0.99
	Mean	58.73±18.39	57.64±18.64	57.70±17.99	58.02±0.68
	Innerwood	66.57	54.72	54.89	58.73±1.64
	Middle	63. 12	58.04	60.40	60.52±1.64
75%	Outerwood	60. 10	60.97	58.29	59.79±0.93
	Mean	63.26±18.91	57.91±18.32	57.86±17.85	59.68±0.65
	Innerwood	53.67	59. 14	54.68	55.83±1.52
	Middle	54. 99	53.31	56.21	54.84±1.52
100%	Outerwood	54.17	60.09	57.58	57.28±0.88
	Mean	54.28±0.67	57.51±3.67	56.16±1.45	55.98±0.63

Legend: CL=Concentration Level of extract, WZ=Wood Area Zone

Table 3

Analysis of Variance for Moisture Content A. robusta After Inoculation

Source of Variance	Degree of Freedom	Sum of Square	Mean Square	F-Cal	F-Tab
Fungi	1	2486.61	2486.61	25.47*	3.86
Concentration level (CL	5	22080.96	4416.91	45.24*	2.23
Sampling height (SH)	2	574.33	287. 17	2.94ns	3.02
Radial position (RP)	2	289.75	144.87	1.48ns	3.02
Fungi * CL	5	11352..94	2270.59	23.56*	2.23
Fungi * SH	2	275.03	137.52	1.41ns	3.02
CL * SH	10	382.08	38.21	0.39ns	1.85
Fungi * CL * SH	10	978.45	97.85	1.00ns	1.85
Fungi * RP	2	190.06	95.03	0.97ns	3.02
CL * RP	10	780.41	78.04	0.80ns	1.85
Fungi * CL * RP	10	1007.42	100.74	1.03ns	1.85
SH * RP	4	446.69	111.67	1. 14ns	2.39
Fungi * SH * RP	4	139.49	34.87	0.36ns	2.39
CL * SH * RP	20	1740.74	87.04	0.89ns	1.60
Fungi * CL * SH *RP	20	1498.40	74.92	0.77ns	1.60
Error	432	42172.38			
Total	539	86395.73			

*Legend: *Significant at α = 0.05. ns = not significant*

Absorption of Preservative

The Mean absorption values of test fungicide and diluents by the wood of *Aningeria robusta* are shown in Table 4. For kerosene treated wood blocks, absorption was least at the innerwood (7.59±0.28) and highest at the middle (7.98±1.20). Along the axial direction, absorption was highest at the top (8.20±1.02) and least at the middle (7.38±0.13). For blocks treated with 25% HEG, absorption was highest at the outerwood (10.82±1.27) and least at the middlewood (10.23±1.14). Axially, the least absorption was found at the base (9.50±0.13) and the highest at the middle (11.08±0.46). Along the sampling height, wood obtained from the top had the highest absorption (12.08±1.03) while those obtained from the middle had the least (11.26±0.40). Concentration levels have effect on the absorption of preservative at 5% probability level (Table 5). Since heartwood extractive will naturally retard preservative absorption, the relative position of innerwood when compared with that of middlewood to the outerwood may have been responsible for the observed trend. The trend along the axial direction is contrary to the normal absorption trend of increasing with increasing tree height because less heartwood is formed at the top than the base. Absorption of blocks treated with 25% GHE was highest at the outerwood because heartwood formation is least at the back; the preservative will then be able to penetrate more. Axial trend was due to the fact that the base is the oldest part of the tree with more phenolic compounds that prevented absorption. At 50% GHE, equal volume of the diluent and the preservative was mixed, therefore making it easier to move within the wood cell than other concentration level. Absorption increased with increasing concentration level up to 50% before decreasing. The trend of absorption could be adduced to the viscousity of the GHE preservative. Viscousity was highest at 100% concentration while corresponding absorption was least at this level. This is because there was no diluent to convey the preservative through the wood cell. However, this contradicts the findings of Olajuyigbe (2007) that when *Gmelina arborea* and *Triplochiton scleroxylon* woods were treated with heartwood extract of *Tectona grandis*, the absorption values ranged from 20.27 – 24.26Kg/m^3 and 54.86 - 67.30Kg/m^3 respectively. The characteristics of the wood species involved in the studies, variation in the porousity of wood species may have been responsible for the variation in absorption results (Kazemi et al. 2006; Viitanen et al. 2006). The type of solvent coupled with the viscousity of the chemicals used must also have influenced the penetration of the preservatives (Kazemi et al. 2006).

Table 4

Mean Values of Absorption of Preservative by Aningeria robusta Wood

CL	WZ	Base (Kg/m^3)	Middle(Kg/m^3)	Top (Kg/m^3)	Mean (Kg/m^3)
	Innerwood	7.71	7.27	7.79	7.59± 0.28
	Middle	7.22	7.35	9.36	7.98± 1.20
0%	Outerwood	8.40	7.52	7.46	7.79± 0.53
	Mean	7.78± 0.60	7.38± 0.13	8.20± 1.02	7.79± 0.44
	Innerwood	9.66	10.61	10.76	10.34±0.59
	Middle	9.41	11.53	9.74	10.23±1.14
25%	Outerwood	9.44	11.09	11.93	10.82±1.27
	Mean	9.50±0.13	11.08±0.46	10.81±1. 10	10.46±0.49
	Innerwood	10.43	11.64	11.36	11.14±0.63
	Middle	13.10	11.28	13.26	12.55±1.09
50%	Outerwood	11.13	10.85	11.63	11.20±0.39
	Mean	11.55±1.38	11.26±0.40	12.08±1.03	11.63±0.50
	Innerwood	2.65	3.24	2.70	2.86±0.32
	Middle	3.13	2.75	2.98	2.95±0.19
75%	Outerwood	2.83	2.88	3.02	2.91±0.09
	Mean	2.87±0.24	2.96±0.25	2.90±0.17	2.91±0.04
	Innerwood	0.09	0.12	0.07	0.09±0.03
	Middle	0.11	0.09	0.10	0.10±0.01
100%	Outerwood	0.11	0.09	0.11	0.10±0.01
	Mean	0.10±0.01	0.10±0.02	0.09±0.02	0.10±0.005

Legend: CL=Concentration Level of extract, WZ=Wood Zone Area

Table 5

Results of the Analysis of Variance for preservative absorption of Aningeria robusta wood

Source of Variance	Degree of Freedom	Sum of Squaree	Mean Square	F-Cal	F-Tab
Concentration level (CL	4	4288.60	1072.15	460.44*	2.23
Sampling height (SH)	2	9.35	4.68	2.01	3.04
Radial position (RP)	2	5.90	2.95	1.27ns	6.04
CL * SH	8	34.65	4.33	1.86	1.98
CL * RP	8	30.14	3.77	1.62	1.98
SH * RP	4	4.93	1.23	0.53	2.41
CL * SH * RP	16	41.51	2.60	1. 1	1.69
Error	180	419. 14	2.33		
Total	224	4834.21			

* Significant @ 5%

Visual rating of fungal growth

Visual rating of fungal infestation of the test blocks as proposed by Greaves et al. (1988) was adopted (Table 6) and it revealed that the untreated (control) and the kerosene treated (0%) woodblocks were almost covered with the fungi mycelia. Woodblocks treated with 25% GHE had sparse growth of *L. sajor-caju but* much fewer with those subjected to *T. viride.* Woodblocks treated with 50% had the least growth of the fungi despite the fact that their moisture content was above 30% just like others, which is the minimum required for fungal growth whereas those treated with 75% and 100% GHE had poor fungal growth but lesser than those of 50%.

Table 6

Visual rating of mycelia growth on the wood blocks of A. robusta after sixteen weeks

Fungus	Rating of mycelia growth/ GHE concentration					
	Control	0%	25%	50%	75%	100%
Lentinus sajor-caju	4	4	2	1	2	2
Trichoderma viride	4	4	1	1	2	2

Weight Loss

The comparison of weight loss in the two fungi is presented in tables 7 and 8 while table 9 shows the results of the analysis of variance. Greater weight loss was recorded with wood blocks exposed to *Trichoderma viride* (brown) than those exposed to *Lentinus sajor-caju* (white)**.** The mean percentage weight loss of *L. sajor-caju* inoculated sampes ranged from 7.93 to 75.55 (Table 7), it decreased with increase in concentration level up to 50% when it increased up to 100%. Weight loss of untreated wood blocks (control) subjected to *L. sajor-caju* was between 51.50% and 90.64% while that of 0% GHE (kerosene alone) level of treatment was between 60.00% and 79.00%. The weight loss of test blocks subjected to GHE at 25% was between 8.30% and 12.58%. The preservative threshold was 50% GHE, where mean percentage weight loss started increasing up to 100% GHE. The greatest attack was found in the untreated woodblocks followed closely by the kerosene treated blocks, hence, resistance was increasing with increase in concentration level up to 50%. The percentage mean weight loss of *T. Viride* inoculated wood blocks ranged from 8.40% to 82.01%, it decreased with increase in concentration level from the untreated (82.01%) to 50%GHE (8.40%) when it increased slightly up to 100% (19.10%).

Weight loss of wood blocks subjected to *T. viride at* zero level of treatment (kerosene only) was between 65.70% and 88.40% while that of test blocks subjected to25% GHE was between 9.01% and 10.48%. At 50% GHE, weight loss was between 7.13% and 9.64%. Resistance of woodblocks to fungi attack reached the peak at 50%GHE level when it started to decrease up to 100% (Table 8).

Statistical analysis revealed that at 5% level there are variations in fungi used, sampling height, concentration levels, interaction between fungi and sampling height as well as interaction between fungi and concentration level (Table 9). However, post mortem analysis revealed that all the levels of concentration as well as the fungi too were significantly different from one another. Greater weight loss was recorded for test blocks exposed to *Trichoderma viride* (brown rot fungus) than those exposed to *Lentinus sajor-caju* (white rot fungus). A school of thought disagreed with this, but, it is in conformity with the report of Adetogun et al. (2006) on *Afzelia africana* and *Nesogordonia papaverifera,* Kamdem (1994) on aspen blocks treated with heartwood extracts, Zabel and Morrell (1993); Green and Highley (1997) that brown rot fungi cause more weight loss than white rot fungi. The weight loss, despite preservative treatment confirms the suggestions of Kazemi et al. (2006); Humar et al. (2006) that treatment may only reduce and not stop degradation unless applied at toxic levels. Resistance was increasing with increase in concentration level of GHE. This agreed with the report of Adetogun (1998) that resistance was increasing with increase in concentration level of Obeche subjected to a white rot fungus after treated with cashewnut shell liquid. Olajuyigbe (2007) also reported 16% weight loss for Obeche exposed to white rot fungus after treated with a chemical preservative and 28% for untreated samples. At both 75% and 100% GHE, absorption of preservative had decreased to 2.91kg/m^3 and 0.10kg/m^3 respectively because of the viscosity of the preservative. Olajuyigbe (2007) reported weight loss ranging from 6% to 9% and 9% to 20% respectively for Obeche exposed to brown rot fungus both for chemical treated and untreated wood samples. The disparity in this result and previous studies could have been attributed to variation in durability of heartwood in the radial direction; outer heartwood is said to have more decay resistance than those close to the pith (Panshin and deZeeuw 1980). Moreover, heartwood of older trees are decay resistant than younger trees of a comparable size. Scheffer and Cowling (1966); Rao and Nazma (1976); Sundararaj et al. (2015) also said that size and age of trees play a significant role in decay resistance. The variability could also have been caused by differences in composition of extractives even though they are obtained from the same species (Zabel 1948). Various treatments with which the woods were subjected when the studies were being carried out at different locations could also be another source of variation in the results obtained. Also, Scheffer and Eslyn (1961) in a study conducted on the heartwood of three hardwood and six coniferous species found out that when the wood was heated in a dry atmosphere at temperatures varying from 82-176^{o}C the resistance of all the woods tested was reduced by only comparatively small amounts. The rate and type of extractives are variegated by the site, age, and genetic factors of trees (Kazemi et al. 2006).

Table 7

Mean Percentage Weight Loss Values of Aningeria robusta Along and Across the Bole After Inoculation with Lentinus sajor-caju

CL	WZ	Base (%)	Middle (%)	Top (%)	Mean (%)
Control	Innerwood	49.62	82.90	87.84	73.45±20.79
	Middle	51.50	83.80	88.55	74.62±20.16
	Outerwood	53.39	86.81	95.53	78.58 22.16
	Mean	51.50±1.88	84.50±2.05	90.64±2.58	75.55±1.32
0%	Innerwood	50.39	59.77	70.45	60.20± 10.03
	Middle	64.27	69.65	77.20	70.37± 6.49
	Outerwood	65.35	80.60	89.37	78.44± 12.15
	Mean	60.00± 8.34	70.00± 10.42	79.00± 9.59	69.67± 1.05
25%	Innerwood	14.76	10.86	9.02	11.49±2.95
	Middle	13.66	8.49	6.84	9.65±3.56
	Outerwood	9.33	9.20	9.05	9.19±0.14
	Mean	12.58±2.87	9.46±1.12	8.30±1.27	10.11±0.97
50%	Innerwood	13.34	9.75	7.73	10.27±2.84
	Middle	7.21	4.65	6.39	6.08±1.31
	Outerwood	10.06	4.04	8.23	7.44±3.08
	Mean	10.20±3.07	6.15±3.13	7.45±0.95	7.93±1.24
75%	Innerwood	15.54	19.43	17.05	17.34±1.96
	Middle	16.87	9.92	13.94	13.58±3.49
	Outerwood	22.37	19.09	12.88	18.11±4.82
	Mean	18.26±3.62	16.15±5.39	14.62±2.16	16.34±1.62
100%	Innerwood	19.50	24.50	18.04	20.68±3.38
	Middle	19.37	19.00	16.02	18.13±1.84
	Outerwood	19.91	17.01	14.46	17.13±2.72
	Mean	19.59±0.28	20.17±3.88	16.17±1.79	18.65±1.81

Legend: CL=Concentration Level, WT=Wood Type

Table 8

Mean Percentage Weight Loss Values of Aningeria robusta Along Across the Bole After Inoculation with Trichoderma viride

CL	WZ	Base (%)	Middle (%)	Top (%)	Mean (%)
Control	Innerwood	64.25	76.28	87.15	75.89±11.45
	Middle	68.40	87.14	89.62	81.72±11.60
	Outerwood	77.40	91.65	96.25	88.43± 9.82
	Mean	70.01±6.72	85.02±7.90	91.00±4.71	82.01±6.28
0%	Innerwood	54.65	67.14	80.00	67.26±12.68
	Middle	68.71	77.09	89.00	78.27± 10.19
	Outerwood	73.74	85.11	96.21	85.02± 11.24
	Mean	65.70± 9.89	76.45±9.00	88.40±8.12	76.85±8.96
25%	Innerwood	10.18	8.52	12.76	10.48±2.14
	Middle	9.30	8.67	9.07	9.01±0.32
	Outerwood	8.49	10.03	8.64	9.05±0.84
	Mean	9.32±0.84	9.07±0.83	10.16±2.26	9.51±0.83
50%	Innerwood	6.11	7.07	8.2	7.13±1.04
	Middle	8.12	8.05	9.13	8.43±0.60
	Outerwood	7.7	10.09	11. 12	9.64±1.75
	Mean	7.31±1.06	8.40±1.54	9.48±1.49	8.40±1.26
75%	Innerwood	16.14	10.92	17.47	14.84±3.46
	Middle	14.46	14.81	15.4	14.89±0.48
	Outerwood	18.48	15.60	15.38	16.49±1.73
	Mean	14.08±2.02	17.76±2.51	19.16±1.20	17.00±1.66
100%	Innerwood	14.17	17.04	19.25	16.82±2.55
	Middle	18.21	18.01	23.12	19.78±2.89
	Outerwood	19.01	19.01	24.08	20.70±2.93
	Mean	17.13±2.59	18.02±0.99	22.15±2.56	19.10±2.02

Legend: CL=Concentration Level of extract, WT=Wood Zone Area

Table 9

Results of the Analysis of Variance for Weight Loss of Aningeria robusta after inoculation

Source of Variance	Degree of Freedom	Sum of Square	Mean Square	F-Cal	F-Tab
Fungi	1	1134.25	1134.25	223.80*	3.86
Concentration level (CL	5	21737.95	4347.59	857.83*	2.23
Sampling height (SH)	2	75.74	37.87	7.47*	3.02
Radial position (RP)	2	29.24	14.62	2.88ns	3.02
Fungi * CL	5	4571.47	914.29	180.40*	2.23
Fungi * SH	2	44.99	22.50	4.44*	3.02
CL * SH	10	657.41	65.74	12.97*	1.85
Fungi * CL * SH	10	217.45	21.75	4.29	1.85
Fungi * RP	2	99.86	49.93	9.85	3.02
CL * RP	10	203.27	20.33	4.01	1.85
Fungi * CL * RP	10	89.28	8.93	1.76ns	1.85
SH * RP	4	2.76	0.69	0.14ns	2.39
Fungi * SH * RP	4	34.81	8.70	1.72	2.39
CL * SH * RP	20	80.40	4.42	0.87ns	1.60
Fungi * CL * SH *RP	20	53.27	2.66	0.53	1.60
Error	432	2189.44	5.07		
Total	539	31299.60			

* Significant @ 5%

Phytochemical Analysis

The phytochemical analysis of heartwood extract of *Gliricidia sepium* was determined quantitatively and the results are presented in table 10. The presence of these secondary metabolites (Alkaloid, Tannin, Saponin, Glycoside and Cadenolide) is an indication that the plant species is durable for timber because these bioactive components are anti- microbial agents. This conforms to the assertion of Quiroga et al. (2001); Carpinella et al. (2003); Kawamura et al. (2004), Kawamura and Ohara (2005); Kusuma et al. (2005); Yen et al. (2008) that many secondary metabolites of timber have antifungal properties and can be used as natural biodegradable fungicides to replace the traditional toxic wood preservatives, which create environmental hazards. These agreed with the reports of Craig and John (2006); C.U. (2008) that wood extract of *Gliricidia sepium* have insecticidal and antimicrobial and that it is very resistant to insects, termites and fungus (Standley 1961; Bultman and Southwell 1976; Duke 1983; Stewart 1996; Quintanar et al.1997; Scheffer and Morrell 1998; Craig and John 2006; C.U 2008) due to the presence of tannins, isoflavins, medicarpin and afrormosin. Conversely to the findings of these results, isoflavins, medicarpin and afrormosin were not found in the extract. These variations could be related to environmental conditions in which plants were cultivated which could evoke secondary metabolites production as an adaptive strategy (Zhao et al. 2005). Also, genetic polymorphism in plant taxa could result in differences in chemical composition in secondary metabolites (Izco 1997). Quantitatively, tannins had the highest value in the heartwood extract and this has been reported to protect trees from being infected by fungi and bacteria (USDA 2019). The presence of tannins could have contributed to the reduced weight loss of the wood samples treated with the heartwood extract after fungal attack.

Saponins and Glycosides found in the heartwood extract are also higher in concentration but next to tannins. Saponins constitute a major family of secondary metabolites that occur in a wide range of plant species and are reported to be involved in plant disease resistance because of their well–known antimicrobial activity (Osbourn 1996; Elisa et al. 2007). Glycosides are prominent secondary metabolite in major plant species and are reported to possess antimicrobial property (Neethu and Neethu 2016). The impact of these metabolites must have reflected in the reduction of weight of the test samples after fungal attack.

Alkaloids contain nitrogen, which is usually derived from an amino acid. Alkaloids have been reported to have antimicrobial properties which are effective against fungal growth (Carson and Hammer 2010; Neethu and Neethu 2016). Phytochemical analysis of *Gliricidia sepium* heartwood extract revealed the traces of alkaloids which could have contributed to the reduction in weight of the test samples after exposure to the fungal strains.

Table 10

Phytochemical Analysis of Gliricidia sepium heartwood extract

Component	Degree
Alkaloid	(25%)
Tannin	**(75%)**
Saponin	**(50%)**
Anthraquinone	**(0%)**
Glycoside	**(50%)**
Cadenolide	(25%)

CONCLUSION

This study focused on the suitability of *Gliricidia sepium* heartwood extract in preserving wood against decay fungi. It revealed that 50% GHE was the preservative threshold where the GHE performed best; above that level weight loss increased and resistance decreased for the two fungi strains used. The phytochemical screening revealed the presence of alkaloid, tannin, saponin, glycoside and cadenolide which are reported to have antimicrobial properties and may have been responsible for the little preservation conferred on the test blocks. *T. viride,* a brown-rot fungus was more virulent than *L. sajor-caju* in degrading the test blocks. Statiscal analysis revealed that the fungi, the preservative used and the sampling height had significant effect on the weight loss at 5% probability level. It is recommended that further studies be carried using other organic biocides.

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