Journal of Research in Forestry, Wildlife & Environment Vol. 14(1) March, 2022
E-mail: *jrfwe2019@gmail.com; jfewr@yahoo.com*
http://www.ajol.info/index.php/jrfwe

jfewr ©2022 - jfewr Publications
ISBN: 2141 – 1778
Olaniran et al., 2022

ASSESSMENT OF THE FUNGICIDAL EFFECT OF *Tithonia diversifolia* LEAF EXTRACT ON RUBBERWOOD (*Hevea brasiliensis* Müll. Arg.)

[1]Olaniran, S.O., [1]Adara, O.H., [2]Hassan, G.F and [1]Olufemi, B.

[1]Department of Forestry and Wood Technology, Federal University of Technology Akure, Ondo State
[2]Plant Pathology Unit, Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Ondo State

***Corresponding Author**: *soolaniran@futa.edu.ng*; +234 806 620 8008

ABSTRACT

This study was carried out to assess the fungicidal effect of Tithonia diversifolia (sunflower leaves) extract as a preservative material against fungal attack on rubberwood. The wood samples were cut into sample size of 20mm×20mm×9mm then oven dried at 103˚C until constant weight was attained. The wood samples were treated with extracts of sunflower leaves prepared through three methods (methanol, hot water and cold water) and absorption and retention of extracts by wood samples were calculated. After treatment, the wood samples were exposed to three fungal species including Phanerochaete chrysosporum (white rot), Serpula lacrymans (brown rot) and Gilbertella persicaria (soft rot) for a period of 8 weeks followed by the assessment of weight loss. The result obtained revealed that hot water extract had the highest (40.84%) of preservative absorption by the rubber wood, while cold water extract indicated the least quantity (35.23%). The wood treated with methanol extract had the highest retention value of 3.52% while the lowest value was reported for the wood treated with cold water extract with 2.12%. Results obtained revealed that there was no significant difference (p>0.05) in the percentage weight loss of the treated and untreated wood samples. Untreated rubber wood samples had the highest percentage weight loss of 15.51% followed by cold water extract treated wood samples (14.17%) and samples treated with hot water extract (10.73%), while the least was recorded for wood samples treated with methanol extract (10.19%). These results implied that rubber wood samples treated with methanol extract exhibited only a minimal resistance to fungi, while the untreated wood samples exhibited the highest percentage weight loss. Therefore, it is clear that Tithonia diversifolia leaf extract did not give considerable protection to rubber wood against fungi attack due to the low content of tannin and flavonoid present in the leaves which could inhibit fungal growth, and there is a need for further research to find suitable bio preservatives for rubber wood.

Key words: *Tithonia diversifolia*, *Hevea brasiliensis*, Cold water extract, Hot water extract, Methanol extract, Fungi, Weight loss.

Correct Citation of this Publication

Olaniran, S.O., [1]Adara, O.H., [2]Hassan, G.F and [1]Olufemi, B. (2022). Assessment of the fungicidal effect of *Tithonia diversifolia* leaf extract on rubberwood (*Hevea brasiliensis* Müll. Arg.). *Journal of Research in Forestry, Wildlife & Environment*, 14(1): 128 - 134

INTRODUCTION

Rubber trees are an economical species, which is widely used by individuals, groups, companies in the production of many wood products. The primary and major products of rubber tree is the bark, which contains the latex –producing tissue. The latex is used for the manufacture of adhesive, balls, industrial glove among others

(Abolagba *et al.*, 2003). Apart from latex, the rubber tree produces seeds and wood which are also of economic value. Moreover, when felled for replanting, the rubber tree is sawn to give rubber wood (i.e., timber). However, rubber wood logs or sawn timber are very susceptible to fungi and insects such that when it is not treated, bio-deteriorating organisms will lower the values that makes its suitable for wood products. It is therefore necessary for rubber wood to be treated with preservatives for protection against the attacks of bio deteriorating organisms (Mohd, *et al.*, 1999). Much study concerns have been raised on the use of synthetic chemicals in wood preservation due to their unavailability, cost, toxicity and negative environmental impacts (Tiilikkala *et al*., 2010). Based on the detrimental effects of these chemicals available in the market, it is imperative to search for user and environmentally friendly alternative. Green plants have been discovered to be reservoirs possessing inexhaustible harmful fungicides/pesticides that are innocuous to man when compared to their synthetic counterparts (Venmalar and Nagaveni 2005; Falemara, *et al*., 2018). Various research works had been done by researchers to investigate the biological or pharmacological activities associated with *T. diversifolia*. The *T. diversifolia* is the hub of medicinal values which is used as food and medicine worldwide but research has not been done on the importance of using this leaf as a preservative or for the treatment of wood. This therefore necessitated the study to assess the fungicidal effect of *Tithonia diversifolia* leaf extract on rubber wood.

MATERIALS AND METHODS

Preparation of Materials

Rubberwood samples for this study were obtained from a local sawmill within Akure metropolis, Ondo State. Thereafter, the wood samples were sawn into 20 × 20 ×9 mm^3 required for test as specified in ASTM D-2017 (2008). A total of 60 samples were prepared for the experiment. The wood samples were oven dried at 103°C until a constant weight was achieved. The weight was measured and recorded as W_o. Cultured white rot fungi (*Phanerochaete chrysosporum),* brown rot fungi *(Serpula lacrymans)* and soft rot fungi (*Gilbertella Persicaria)* were collected and identified at Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State. The *Tithonia diversifolia* (sunflower) leaves were collected, sundried and grinded into powder.

Extraction sunflower leaves

The extraction of the sunflower leaves was done by using the soxhlet extractor procedure of Abdullahi *et.al.,* (2020). Dried, grinded and finely powdered leaves weighing 120 g was placed inside a thimble or a strong filter paper. Methanol was used as the extraction solvent and it was poured into the bottom flask followed by the thimble into the extraction chamber. The solvent was heated from the bottom flask, it evaporated and passed through the condenser and flowed down the extraction chamber and extracted the leaf by coming in contact. Consequently, when the level of the solvent in the extraction chamber reaches the top of the siphon, the solvent and extracted leaves flowed back to the flask. The process was repeated until desirable amount of extract is acquired, it was then stored in a container and labelled for use. To perform the cold-water extraction, 120 g of the powdered leaf of *Tithonia diversifolia* was mixed with 2 liters of distilled water inside a container for 24 hours. This was also done for the hot water extraction, i.e., 120g of the powdered leaf of *Tithonia diversifolia* was mixed with 2 liters of hot water inside another container for a short period of time. It was then sieved and further filtered using filter paper. The extract was transferred into a container and labelled for use.

Treatment of Rubberwood with *Tithonia diversifolia* extracts

Fifteen (15) samples each were treated in the prepared extracts from methanol, hot water and cold water, while another 15 samples were left untreated (control samples). The treatment of samples in cold-soaking in the extracts for 24 hours. After the treatment, the samples were drained of excess extracts, while their absorption and retention were calculated as follows:

$$Absorption\ \% = \frac{W_1 - W_0}{W_1} \times 100 \quad \ldots\ldots\ldots (1)$$

$$Retention\ (kg/m^3) = \left(\frac{G \times C}{V}\right) \times 10 Kg/m^3 \quad \ldots (2)$$

Where:

W_0 = Oven dried weight, W_1 = Treated weight, G =(W_1-W_0), C = grams of preservative in 100g of treating solution, V = volume of sample in m^3.

Phytochemical Analysis of *Tithonia diversifolia* extracts

The chemical profiles of the extracts were determined by gas chromatography-mass spectroscopy (GC-MS) at the Bio-chemistry Department, Federal University of Technology, Akure. The procedure forthe analysis included the following details. Column temperature was set at 80°C, injectiontemperature at 250°C, pressure at 108.0 kPa, total flow was at 6.2 ml/min and linear velocity at 46.3 cm/s. The start time was 3.00 min and end time was 28.00 min. The compounds were identified using molecular weight and formula of the compounds and the retention time. Compound identification was obtained by comparing these values and the spectra data with thoseof authentic compounds from the library data of the corresponding compounds using automated Shimadzu software.

Laboratory testing of resistance to decay fungi

Decay resistance was assessed according to American Society for Testing and Materials (ASTM) D 4445-91 (1998). The fungus was sub-cultured into plates and later inside culture bottles where the wood (treated and untreated) samples were inoculated. Visual ratings were carried out for a period of Eight weeks on a scale of 0-5 (0-100%). At the end of the eight weeks, percentage weight loss of the wood test samples was calculated with the following equation:

$$\%Weight\ loss = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots\ldots\ldots\ldots (3)$$

Where:

W_1 = treated weight, W_2 =Final weight after fungi exposure

Experimental Design and Statistical Analysis

The experimental design for this study was Randomized Complete Block Design involving three fungi (white rot, brown rot and soft rot), three methods of extraction and one treatment technique, control(untreated) replicated five times. Data was subjected to analysis of variance (ANOVA) for significant differences and where significant differences existed, Duncan Multiple Range Test (DMRTs) was used.

RESULTS

Percentage Preservative Absorption and Retention

The result of the effects of the preservatives treatment on preservative absorption (%) as presented in Table 1 revealed that that wood treated with hot water extract has the highest absorption value of 40.84%, higher than the wood treated with methanol extract with 38.04% and while the least percentage absorption was recorded for cold water extract with 35.23%. The statistical analysis revealed that there were significant differences in the absorption of preservatives between the three selected preservative extraction methods. The result of the retention of the preservatives presented in Table 1 revealed that the treated wood with methanol extract has the highest retention value of 3.52% higher than the wood treated wood with hot water extract with 2.23%, while the lowest value was reported for the wood treated with cold water extract with 2.12%. The statistical analysis revealed that there were significant differences in the retention of preservatives between the three selected preservative extraction methods.

Table 1: Mean percentage preservative absorption and Retention of by *Hevea brasiliensis* wood samples

Preservative	Absorption (%)	Retention (kg/m^3)
Methanol extract	38.04^b	3.52^a
Hot water extract	40.84^a	2.23^b
Cold water extract	35.23^c	2.12^c

Means with different alphabets vertically are significantly different ($p \leq 0.05$)

Phytochemical constituent of Tithonia diversifolia extract

The result in Table 2 shows the Qualitative and quantitative of analyzed phytochemicals in *Tithonia diversifolia* leaves. From the Table Nine (9) phytochemicals were observed in *Tithonia diversifolia* which are; tannin, saponin, alkaloids, steroids, terpenoides, flavonoids, oxalate, phytin and cardiac glycosides. There were variations in the quantity and abundance of compounds present in the extract obtained from *Tithonia diversifolia* leaves depending on the solvent used for extraction. In the Table it can be observed that higher quantities of compounds were present in the methanol extracts compared to those extracted with hot water.

Table 2: Qualitative and quantitative analysis of analyzed phytochemicals in *Tithonia diversifolia* leaves

Phytochemicals analyzed	Methanol extract		Hot water extract	
	Qualitative analysis	Quantitative analysis (mg/g)	Qualitative analysis	Quantitative analysis (mg/g)
Tannin	++	6.03	++	4.02
Saponin	++++	18.90	+++	12.80
Alkaloides	++++	11.60	++	7.50
Steroids	+	6.50	+	3.28
Terpenoides	++	12.65	++	6.70
Flavonoids	++	3.20	+	1.28
Cardiac-glycocides	+	3.28	+	1.35
Oxalate	+	1.98	+	0.65
Phytin	++++	22.24	++++	18.80

Key: *+ Present, ++ moderately present, ++++ abundantly present*

Resistance of rubberwood treated with Tithonia diversifolia extract to wood-decay fungi

The result of visual assessment of the *Hevea brasiliensis* wood treated with methanol extract, hot water and cold-water extract are presented in Figures 1 to 4. Results showed that for samples treated with hot water extracts, the spread of decay fungi increased with the exposure period and was over 80% by the end of the 8th week (Figure 1). The growth of decay fungi on samples treated with cold water extracts attained 100% after three weeks of exposure (Figure 2), while samples treated with methanol extracts showed a slightly different trend. For samples treated with methanol extracts, the spread of white rot and soft rot fungi was 100% by the eighth week, but the treated samples were slightly resistant to brown-rot fungus as the proportion of brown-rot fungus that covered the surface was 72% (Figure 3). Comparing the above results to the untreated samples (Figure 4), most of the samples have attained a visual rating of over 70% from the first week of exposure to the three species of fungi.

From the result Table 2 shows the percentage weight loss of treated and untreated *Hevea brasiliensis* wood exposed to different types of fungi. It was observed that wood samples exposed to *Gilbertella persicaria* has the highest mean percentage weight loss of 13.52% higher than that of the wood samples exposed to *Phanerochaete chrysosporum* with value of 13.32%, while the least value was recorded for wood samples exposed to *Serpula lacrymans* with 12.46%. For *Gilbertella persicaria*, the untreated samples show the highest percentage weight loss of 14.71% followed by wood samples treated with cold water extract with 13.60% higher than that treated with hot water extract with 13.25%, while the least was recorded for wood samples treated with methanol extract with 12.53%. For brown rot it was recorded that the untreated wood samples show the highest percentage weight loss of 15.15% followed by wood samples treated with cold water extract with 13.77% higher than that treated with hot water extract with 10.73%, while the least was recorded for the wood samples treated with methanol extract with 10.19%. For white rot fungi, the untreated wood samples show the

highest percentage weight loss of 15.51% followed by wood samples treated with cold water extract with 14.17% higher than that treated with hot water extract with 13.09%, while the least was recorded for the wood samples treated with methanol extract with 10.52%. As observed from Table 3, the analysis of variance showed that the effect of the three types fungi is not significantly different in percentage weight loss. Also, there is no significant difference in the treated and untreated wood samples. These results revealed that the non-significant difference in the effect of *Tithonia diversifolia* extracted with methanol, hot and cold water may be that the active ingredients are not effective in resisting the attack of fungi on rubber wood.

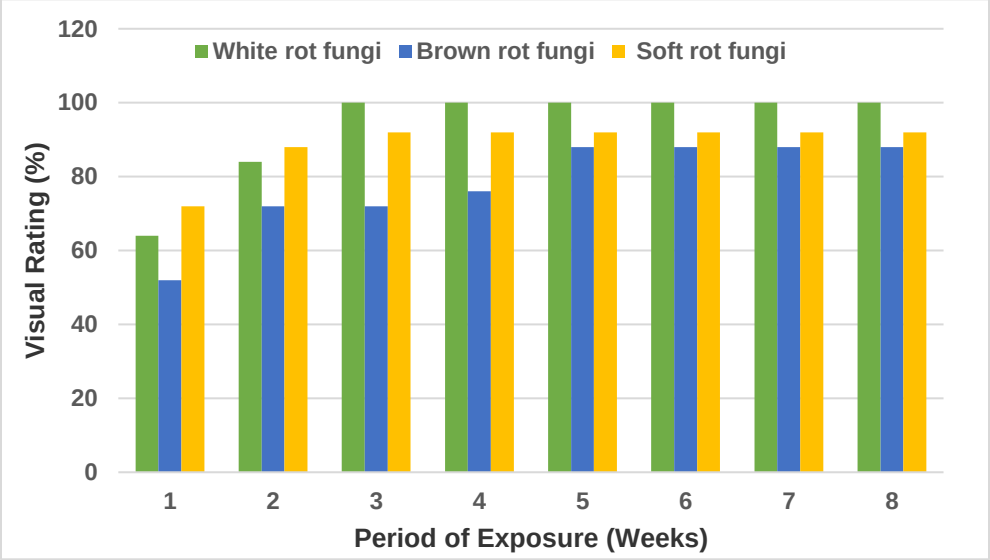

Figure 1: Visual ratings of Fungi species growth on rubberwood samples treated with hot water extract for a period of eight weeks

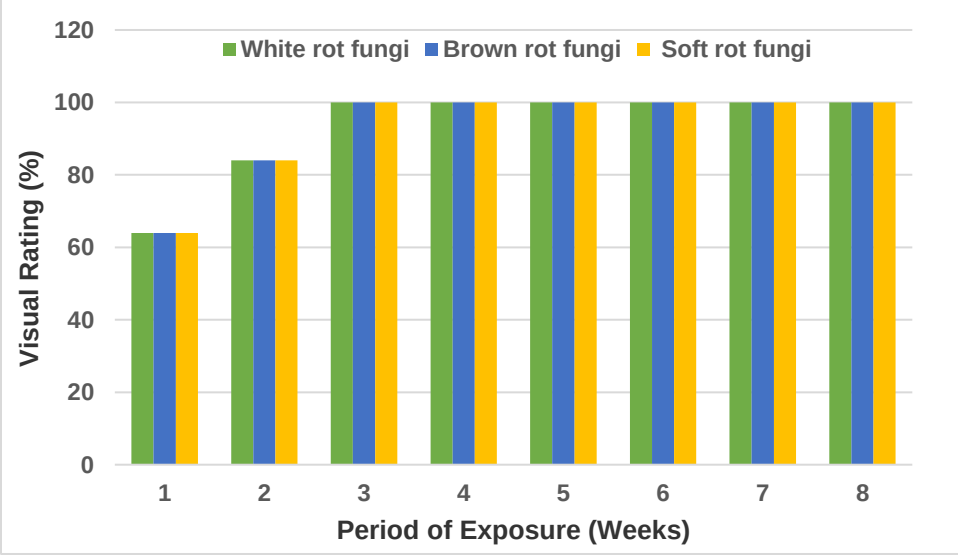

Figure 2: Visual ratings of Fungi species growth on cold-water extract treated rubberwood for a period of eight weeks

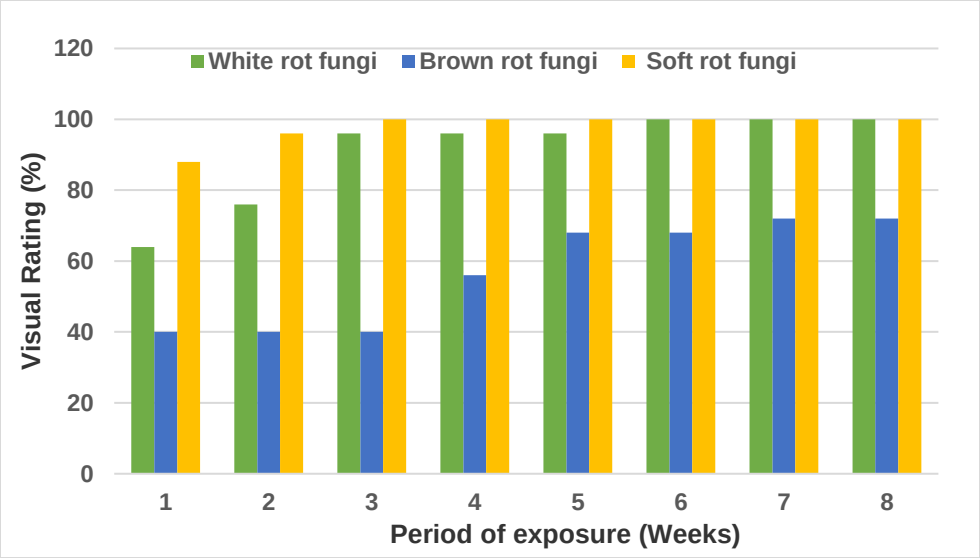

Figure 3: Visual ratings of Fungi species growth on rubberwood samples treated with methanol extract for a period of eight weeks

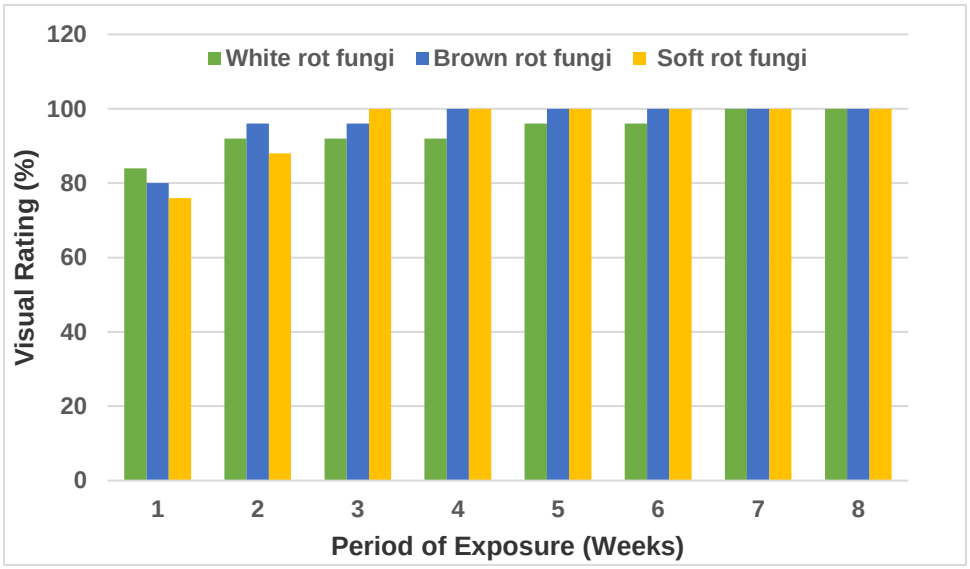

Figure 4: Visual ratings of Fungi species growth on untreated rubberwood wood samples for a period of eight weeks

Table 3: Percentage mean weight loss of *Hevea brasiliensis.*

Fungi species	Treatment	Weight Loss (%)	Average
Phanerochaete chrysosporum	Methanol	10.52±2.59	13.32±3.92[a]
	Hot water	13.09±2.59	
	Cold water	14.17±2.58	
	Control	**15.51±5.84**	
Serpula lacrymans	Methanol	10.19±5.49	12.46±4.11[a]
	Hot water	10.73±2.69	
	Cold water	13.77±3.05	
	Control	**15.15±3.33**	
Gilbertella persicaria	Methanol	12.53±2.01	13.52±2.63[a]
	Hot water	13.25±2.72	
	Cold water	13.60±3.42	
	Control	**14.71±2.56**	

Means with the same alphabets are not significantly different (p>0.05)

DISCUSSION

As shown in the results, wood samples treated with hot water has the highest absorption rate this result agrees with ASTM (1974) stating that absorptions rate are higher for water soluble preservatives than oil types preservatives. This is because water does not only fill the cell cavity but it is also absorbed in the cell wall (Olaniran and Olufemi 2015). The retention of preservatives of *Hevea brasiliensis* wood samples are differs among the methods of extractions as shown through the results obtained. The wood samples treated with methanol extract had the highest percentage retention values of 3.52% compared to other extraction methods, indicating that the quantity of active ingredients retained in the rubberwood are more in the methanol extracts compared to other methods. Although wood exhibit variation in absorption and retention of preservatives when treated as previously stated by Schultz and Nicholas (2007), retention of active ingredients may also be dependent on the nature of solvent of used for extraction of plant parts as revealed in this study.

The efficacy of the extracts depends solely on the potency of the active ingredients that inhibit the growth of fungi as previously asserted by Okigbo and Ogbonnaya (2006). For any plant extract to inhibit fungal growth, active ingredients such as Tannins and Flavonoids must be present (Oliveira et al. 2010). However, it is revealed in this study that, though Tannin and Flavonoid are present in the extracts of *Tithonia diversifolia*, they are not abundant as shown in the phytochemical analysis. Okigbo and Ogbonnaya (2006) opined that the differences in the efficacy of the extracts could be attributed to the differences in their active ingredients. Oliveira, *et.al.*, (2010) reported that Tannins and Flavonoids inhibit fungal growth, but based on the results obtained in the quantitative analysis, tannin and flavonoids in hot water extract and methanol extract of the leaves are low compared to other compounds, this result could have led to the weight loss exhibited by the treated wood samples which are not significantly different from the untreated wood samples.

CONCLUSION

From the results of the study, methanol extract treated wood samples exhibited a considerable resistance to attack by fungal attack after 8 weeks of exposure. The result showed that *Hevea brasiliensis* both the treated and untreated wood samples have no significant difference in the weight loss. *Tithonia diversifolia* leaf extract did not completely protect the wood against the infestation of the three fungi, and this may be as a result of the presence low content of Tannin and Flavonoids which can inhibit fungal growth.

Therefore, *Tithonia diversifolia* leaf extract may not be considered as an alternative for synthetic chemicals for preserving Rubber wood because it does not in any way meet the standards for effective preservative. *Hevea brasiliensis* wood is less durable and it requires an effective preservative to prolong its service life. Therefore, it is recommended that more studies be carried out on other plant extracts in a bid of finding an efficacious preservative that is friendly to its user and the environment and researchers and agencies be intentional with making the environment conducive for living.

REFERENCES

Abdullahi, R.A. and Haque, M. (2020). Preparation of Medicinal plants: Basic Extraction and Fractionation procedures for Experimental Purposes. *Journal of Pharmacology. and Bio-Allied Science*,12(1):1-10.

Abolagba, E.O., Aigbokaen, E.O. and Omokhafe, K.O. (2003). Farm Gate Marketing of Natural Rubber in the South East Rubber Growing zone of Nigeria. *Nigerian Journal of Agriculture and Rural Development*, 6 (2003): 40-48.

ASTM (1974): Standard methods of laboratory evaluation of wood and other cellulosic materials for resistance to termites. D-3345-74. Annual Book of ASTM Standards pp. 927-929.

ASTM D 4445-91. (1998). Standard test method for fungicides for controlling sap stain and mold on unseasoned lumber (laboratory method). Annual Book of ASTM Standard: Pp 497-500.

American Society for Testing Materials (ASTM) D-2017. (2008). Standard Method of Accelerated Laboratory Test of Natural Decay Resistance of Wood. Annual Book of ASTM Standards, ASTM International, West Conshohocken, USA.

Falemara, B.C., Olatunbosun, O.N. and Lawal, H.U. (2018). Assessment of the Resistance of *Bambusa Vulgaris* Schrad treated with Cashew Nut Shell Liquid against fungi and termite deterioration. *Pro Ligno* 14(2):28-37.

Mohd. Dahlan Jantan, Hong, L.T., Azlan Mohamad and Wong, A.H.H. (1999). Preservation of rubberwood. *http://www.researchgate.net/publication/291728100*.

Okigbo, R.N., and Ogbonnaya, U.O. (2006). Antifungal Effects of Two Tropical Plant Extracts (*Ociumu gratissiumum* and *Afromomum melegueta*) on post-harvest yam (*Dioscorea spp*.) rot. *African Journal of Biotechnology*. 5: 5727-731.

Oliveira, L.S., Santana, A., Claudia, A.M., Miranda, R.M., Galvao de Lima, V.A., Da Silva, S.I., Nascimento, M.S., and Bieber, L. (2010). Natural resistance of five woods to *Phanerochaete Chrysosporium* degradation. *International Biodeterioration and Biodegradation*, 64: 711-715.

Olaniran, S.O., and Olufemi, B. (2015). Flexural Strength of Incised Rubber-wood (*Hevea brasiliensis Müll. Arg*.) Treated with Creosote oil and Liquefied Biomass. *Applied Tropical Agriculture,* 20(1): 57-61.

Schultz, T.P. Nicholas, D.D. (2007). Totally organic wood preservative for exterior residential applications. In: Barnes HM (ed) Wood protection 2006, Forest Products Society, Madison, pp 28: 9294.

Tiilikkala K, Fagernas L, and Tiilikkala, J. (2010). History and use of wood pyrolysis liquid as biocide and plant protection product. *Open Agriculture Journal*, 4:111-118.

Venmalar, D., and Nagaveni, H.C. (2005). Evaluation of copperised cashew nut shell liquid and neem oil as wood preservatives, In: Proceedings of 36th Annual Meeting of International Research Group on Wood Protection, Bangalore, India, IRG/WP 05-30368.