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FUNGAL RESISTANCE OF OBECHE (*Triplochiton Scleroxylon* K. SCHUM*)* WOOD TREATED WITH NEEM (*Azadirachta indica,* A. JUSS) SEED OIL EXTRACT

Adebawo, F. G.

Federal College of Forestry, P. M. B. 5087, Jericho Hill, Ibadan, Nigeria
Correspondents email: *adebawofunke@yahoo.com*; 08062077780

ABSTRACT

This study aimed to providing eco-friendly wood preservatives from neem (Azadirachta indica) seed oil extract against wood decay fungi. Seed oil was extracted from the neem seed using soxhlet apparatus with N-hexane as the solvent. After extraction, the phytochemical screening of the oil was carried out. Sixty wood blocks of Triplochiton scleroxylon were treated with five different concentration levels (0 mL, 25 mL, 50 mL, 75 mL and 100 mL) of the seed oil extract and the untreated wood samples served as the control. The wood blocks were exposed to Sclerotium rolfsii (brown rot fungus) and Pleurotus ostreatus (white rot fungus) for 16 weeks and their weight loss determined. The phytochemical screening of the oil revealed that alkaloids (2141.7 mg/100g), tannins (975.0 mg/100g), flavonoids (1418.3 mg/100g), terpenoids (826.7 mg/100g), saponins (43.3mg/100g) and steroids (218.3mg/100g) were present. There were significant differences in the weight loss at different concentration levels at p< 0.05. The control has the highest weight loss of 36.48% ±2.03 and 47.89% ±2.34 for Pleurotus ostreatus and Sclerotium rolfsii respectively while the lowest weight loss for white and brown rot was 8.23% ± 1.07 and 11.84% ± 1.24 for Pleurotus ostreatus and Sclerotium rolfsii respectively. From this study, it is established that Azadirachta indica seed oil extract is a potential bio-preservative against wood decay fungi.

Keywords: *Azadirachta indica*, fungi, bio-deterioration, phytochemical screening

INTRODUCTION

Wood is one of the most valuable natural resources that is readily available. It is easily worked with tools and machines and it has a very high strength to weight ratio. The fact that wood can be used for both in-door and out-door services and their exposure to different weather conditions shows that wood can be used for many years if properly preserved. Some timbers have excellent resistance to various agents of deterioration and are therefore highly valued for this property - usually summed up as durability. Many others have only moderate resistance or hardly any resistance and it is in these cases, which are very numerous, that wood preservation becomes a necessity (FAO, 1986). Hence, due to the nature and character of wood, durable wood species are being selected and exploited for structure and construction purposes (Oluwafemi and Adegbenga, 2007). However, there are other wood species of low durability that can be used for construction purposes if properly treated with wood preservatives. Wood preservatives such as creosote and inorganic preservative chromate copper arsenate (CCA) are highly effective in protecting wood. However, due to their harmfulness, there has been a restriction in their use. Hence, developing biocides that are environmental friendly has become a viable option.

Recently, great interest has been focused on some wood preservatives that are relatively cost – effective and eco- friendly. Ability of wood and natural plant extractives to protect wood against wood degrading fungi and insects has been one possible approach for developing new wood preservatives (Kartal *et al*., 2004). Several scientist have reported that plant extracts are good source of fungicides (Kabir *et al*., 2007). Most reported works on the use of ecofriendly wood preservatives is on

extractives from heartwood, leaf, bark, root and oil from herbaceous plants (Onuorah, 2000; Saxena and Dev, 2002; Swathi *et al*., 2004 and Adetogun *et al*., 2007). Moreover, there are also reports on the use of neem oil to preserve different wood species evergreen tree (Venmalar and Nagaeveni, 2005). Despite the extensive studies on the use of preservative of plant origin to protect wood, there is little or no information on the use of neem seed oil to preserve *Triplochiton scleroxylon* and *T. scleroxylon* is a tropical species, a timber of commerce that is used in many wooden applications.

Neem tree (*Azadirachta indica*) is an evergreen tree; it belongs to Meliaceace family, and grows rapidly in the tropic and semi-tropic climate (Puri, 1999). It is properly known as village pharmacy as all parts of this plant are used for several types of diseases since centuries. Extracts of leaves and seeds exhibit the property of antibacterial, antifungal, antiviral (Tewari, 1992). Neem wood is known to be durable against wood rotters (Rao, 1990). Neem seed oil with the main constituent of Azadirachtin has been evaluated to find out the effectiveness as wood preservatives. In this vein, this study was undergone to assess the antifungal activity of neem seed oil against *Pleurotus ostreatus* and *Sclerotium rolfsii* fungi on *T. scleroxylon* wood.

MATERIAL AND METHODS

Preparation of Wood Samples: A total of 60 wood blocks of dimension 2x 2 x 6 cm were obtained from 22 years old *Triplochiton scleroxylon* wood species according to the ANSI/ASTM D 1413 – 76/10 – 11 standard procedure. The initial weight of the samples (W_1) were taken using an electronic weighing balance and then oven dried for 24 hours at 103±2 ^{o}C to constant weight. After oven drying, the weight of the samples (W_2) was taken and percentage moisture content was estimated using the equation (1).

$$\% \text{ M. C.} = \frac{W_1 - W_2}{W_2} \text{ X } 100 \quad ---1$$

Where:

M.C. = Moisture content

W_1 = weight of wood samples before oven drying

W_2 = weight of wood samples after oven drying

Preparation of *Azadiracta indica* seed oil extract: Matured neem fruits were collected by picking from neem stands within the Polytechnic of Ibadan, Ibadan, Oyo state, Nigeria. The seed coats were removed and sun dried for seven days to reduce the moisture and ground with an electric blender. 100 g of neem powder was placed into the thimble and 500 mL of N-hexane was poured in a round bottom flask and placed in the soxhlet chamber to extract the oil from the neem seed. The extract obtained was stored in a sterilized bottle.

Phytochemical Analysis of Neem Seed Oil Extract: Phytochemical screening of Neem seed oil was done following the standard procedure by the method of Brain and Turner (1975). The seed oil extract was subjected to phytochemical screening for the presence of alkaloids, tannins, flavanoids, fats, saponins, phenolic compounds, steroids and terpenoids.

Preparation of Test Fungicide: The volume-to-volume method according to Ajala *et al*., 2014 was used for the preparation of different concentration levels of the oil extract using kerosene as the diluent. This implies that, 1mL of neem oil in 99 mL of Kerosene (diluent) is equivalent to 1% dilution. Hence, 0 mL neem oil in 100 mL kerosene, 25 mL neem oil in 75 mL kerosene, 50 mL neem oil in 50 mL kerosene, 75 mL neem oil in 25 mL kerosene and 100 mL neem oil in 0 mL kerosene is represented as 0 mL, 25 mL, 50 mL, 75 mL and 100 mL respectively while the untreated sample is represented as control. For each concentration, ten replicates were used for each oil treatment which was thereafter separated into five replicates each for *Pleurotus ostreatus* and *Sclerotium rolfsii* fungi.

Treatment of Test Blocks: Dipping impregnation method (FAO, 1986) was used for treatment of the wood test blocks with the preservatives. They were completely immersed in the fungicides for 24 hours. The wood blocks were conditioned and treated with various concentrations of neem oil (0 mL, 25 mL, 50 mL, 75 mL and 100 mL) so as to obtain maximum absorpti. They were removed and air dried for three days and then weighed (W_3).The absorption rate was calculated using equation (2) according to Adetogun (2009).

$$A = \frac{TA \times Conc. \times 10}{VW \times PN} \text{ - - - - - -2}$$

Where:
A = Absorption, (kg/m^3)
TA= Totaal Absorption
Con. = Concentration
VW = Volume of wood
NP = Number of piece

Preparation of Culture Medium: The pure culture of brown rot fungi (*Sclerotium rolfsii*) was obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State; while the white rot fungi (*Pleurotus ostreatus*) was obtained from the Pathology Department of Forestry Research Institute of Nigeria (FRIN). A nutrient medium of Potato Dextrose Agar (PDA) in distilled water was prepared. PDA of 40 mL was poured into McCartney bottles and sterilized by autoclaving at 0.1 N/mm^2 (120 °C) for a period of 20 minutes. The medium was inoculated with the test fungi within 6 days after preparation of the bottles (Sarker *et al.*, 2006).

Infection of Test Blocks: The blocks were infected by placing them in the bottles in which there were actively growing cultures of the test fungi. The blocks were placed in the bottles containing each of the two test fungi such that they came in contact with the aerial mycelium of the fungus. The control test blocks were wrapped in aluminum foil and sterilized in the oven before introduction to the test fungi.

Duration of test: The bottles were inoculated with the test fungi and then incubated at room temperature (27 ± 2 ^{o} C) in the laboratory for 14 weeks. At the end of incubation period, the blocks were removed from the culture bottles, cleaned of the adhering mycelium and oven dried at 103 ^{o}C) to constant weight (Sarker *et al.*, 2006).

Weight Loss Determination of treated wood samples: At the end of incubation period, test blocks were carefully removed, oven dried and reweighed to determine weight loss. Percentage weight loss of each sample due to fungi attack was calculated using equation (3)

$$\% \text{ WL} = \frac{T_3 - T_4}{T_3} \times 100 - - - -3$$

Where:
WL = Weight Loss
T_3 = weight of test block after treatment.
T_4 = weight of test block after exposure to fungi attack.

Statistical Analysis: All the data obtained were subjected to an analysis of variance and means were separated with the aid of Duncan Multiple Range. Statement of significance are based on P≤0.05

RESULTS

Phytochemical Constituents of Neem Seed Oil

The results of quantitative phytochemical analysis of *Azadirachta indica* seed oil extract are presented in Table 1. Phytochemical screening of the *T. peruviana* oil revealed that the main constituents found at highest concentrations were alkaloids (2141.7 mg/100 g) followed by flavonoids (1418.3 mg/100g). Tannins, terpenoids, steroids and saponins are also present but saponins gave the lowest constituents of 43 mg/100 g.

Absorption Rate of Neem seed oil by wood blocks

The absorption rate ranged between 0.69 – 73.07 kg/m3 with 75 mL concentration level having the highest absorption of 73.07 (kg/m^3) as presented in Table 2.

Table 1: Phytochemical constituents of Neem seed oil (NSO)

Parameters	Value (mg/100 g)
Saponins	43.3
Flavonoids	**1418.3**
Tannins	975.0
Alkaloids	**2141.7**
Steroids	218.3
Terpenoids	826.7

Table 2: Mean values of percentage absorption of Neem seed oil by *T. scleroxylon* wood

Concentration level (mL)	Mean (kg/m^3)
0	0.69[a]
25	23.63[b]
50	38.15[c]
75	73.07[e]
100	58.75[d]

Mean with the same alphabet are not significantly different from each other at α = 0.05

There were significant differences among the treatments for the absorption rate of wood samples treated with Neem seed oil extract at different concentration levels. The absorption rate increases as the concentration level increases except for the 75 mL and 100 mL. The least absorption was recorded in the test blocks treated with kerosene i.e. 0 ml concentration level.

Determination of decay resistance by weight loss:
Resistance of *T. scleroxylon* wood treated with Neem seed oil at varying concentrations was assessed by exposure to fungi for 16 weeks. The decay resistance of treated and untreated wood samples was determined by weight loss as presented in Figure 1. The weight loss ranged from 8.23% - 36.48% and 10.24% - 47.89% for *pleurotus ostreatus* and *Scleorotium rolfsii* respectively. The highest weight loss (47.89%) is observed in control for wood blocks exposed to *Sclerotium rolfsii*, brown fungus while its counterpart, *Pleurotus ostreatus* produced a weight loss of 36.48%. However, there is no significant difference in the weight loss of the wood blocks at 100 mL concentration level for both fungi.

Table 3: Mean values of percentage weight loss of *Triplochiton scleroxylon* after 14 weeks exposure to fungi

Treatment (mL)	Fungi	
	Pleurotus ostreatus **Mean ± Sdv**	***Sclerotium rolfsii*** **Mean ± Sdv**
Control	36.48±2.03[a]	47.89±2.34[a]
0	32.13±1.33[b]	44.56±1.58[b]
25	31.4±1.25[b]	36.63±1.25[c]
50	21.46±0.68[c]	29.67±1.17[d]
75	8.23±1.07[d]	11.84±1.24[e]
100	10.24±1.20[e]	12.53±0.98[e]

Each value is an average of 5 replicates. Mean with the same alphabet in each column are not significantly different from each other at α = 0.05

DISCUSSION

Phytochemical Constituents of Neem Seed Oil
Phytochemicals generally exert their antimicrobial activities through different mechanisms and are known to be biologically active because they protect the plants against infections (Scalbert, 1991). From the present study, it was observed that Saponins, Flavonoid, Tannins, Alkaloids, Steroid and Terpenoids were present in the extract. This agrees with the findings of Dooh *et al.* (2014), who

reported the same results for the qualitative analysis of methanol extract of *Thevetia peruviana*. Quantitatively, alkaloids had the highest value in the seed oil extract and this has been reported that it acts as deterrent against microbial and insect attacks (Smith 1996; Macel 2011). Alkaloids are organic heterocyclic nitrogen compounds that are basic-forming water-soluble salts. They 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. The higher concentrations of alkaloids (2141.7 mg/100g) could have contributed to the reduced weight loss of the wood samples treated with the oil after the fungal attack.

Flavonoids are phenolic structures found abundantly in photosynthesizing cells. Esmaeili *et al.* (2013) reported that they are secondary metabolites that provide UV protection and color to almost all terrestrial plants and fruits. They are usually found in many common edible plant parts such as fruits, vegetables, nuts and seeds. Flavonoid compounds have a structural feature of the 2-phenyl-benzopyrane or flavine nucleus, which consists of two benzene rings linked through a heterocyclic pyrane ring (Savoia 2012; Carson and Hammer, 2010). Flavonoids are important plant components due to their active hydroxyl groups and anti-oxidative properties (Güder *et al*., 2014). They are known to have antioxidant, anti-inflammatory and antitumor activity. Flavonoid compounds have been reported to inhibit wood decay fungi (Onuorah 2000; Yen *et al*., 2008; Tumen *et al*., 2013; Li *et al.*, 2014).

Saponins are a major family of secondary metabolites that occur in a wide range of plant species (Osbourn 1996). These compounds are present in plants and are reported to be involved in plant disease resistance because of their well-known antimicrobial activity (Papadopoulou *et.al*, 1999; Bouarab *et.al*, 2002; Wittstock and Gershenzon, 2002, Elisa *et.al*, 2007). Saponins has been characterized and reported to act as deterrent for biological activity against insects (Macel, 2011).

Flavonoids, steroids and terpenoid have antioxidants and antimicrobial properties. Tannins are formed via the Shikimic acid pathway and have significant anti-feedant properties attributed both in angiosperms and gymnosperms (Barbehenn and Constabel, 2011). They are found in bud and foliage tissues, seeds, bark, roots, heartwood and sapwood. The highest levels are in heartwood and barks. Terpenoids are also used as flavoring agents, insect repellents, fungicides and for medicinal purposes (Johnson and Morgan, 1997).

Absorption Rate of Neem seed oil by wood blocks

The absorption rate indicated that at different concentration levels, wood blocks absorbed the oil extract at different rate. This study has been able to show that *T. scleroxylon* absorbs more oil at higher concentration level. The result revealed that the wood samples were easily impregnated without difficulty with Neem seed oil extract due to low viscosity of the diluents which consequently lowered the viscosity of the extract. This result is in consonance with the work of Adetogun (2011).

Determination of decay resistance by weight loss

It is clear from the result that the control groups suffered the highest level of damage for both fungi. However, *Sclerotium rolfsii*, a brown rotter, was more virulent in their attack than *Pleurotus ostreatus*, a white rot fungus as seen in the result (Table 3). The differences in the weight loss as a result of different fungi used is in accordance with the work of Ogunsanwo *et.al.,* 2006 who also observed variation in the weight loss as a result of different fungi used. Analysis of variance (Table 3) further established the fact that level of attack was minimal at higher concentration level of the seed oil extract (75 mL and 100 mL) for both the white rot and brown rot fungi. However, there is no significant difference in the weight loss of the wood blocks exposed to brown rot at 75 mL and 100 mL concentration level. The results also showed that higher concentration of the oil seems to have higher phytochemicals that accorded the wood blocks with resistance against fungal attack. In view of the fact that phytochemicals have been reported to have antifungal properties, this could have contributed to the reduced weight loss seen in the wood blocks after 16 weeks as compared to their untreated counterparts. The effect of the seed oil extract was clearly seen in the wood blocks which were

significantly different from each other in their weight loss at different concentration levels.

CONCLUSION

This study has shown the potential of Neem seed oil extract as a biopreservative against decay fungi on *Triplochiton scleroxylon* wood. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, steroids and saponins in the oil. The results showed that the rate of attack of the treated wood blocks by *Sclerotium rolfsii* was more virulent than the *Pleurotus ostreatus*, however, the untreated wood blocks had the highest weight loss. The treatment of the wood with the oil provided resistance against *Pleurotus ostreatus* and *Sclerotium rolfsii* at 75 mL and 100 mL concentration levels. It is therefore concluded that neem seed oil is effective as biopreservative against decay fungi on *Triplochiton scleroxylon* wood.

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