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Treatment of *Delonix regia wood with* castor oil (*Ricinus communis*) protects it against white and brown rot fungi

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Abstract

This study was aimed at assessing the resistance of *Delonix regia* wood treated with an eco-friendly bio preservative exploring castor (*Ricinus communis*) seed oil identified as a promising candidate for wood antifungal treatment, attributed to its documented antimicrobial properties. Sixty wood block samples of conditioned *Delonix regia* were treated with five different concentration levels (0 %, 25 %, 50 %, 75 % and 100 %) of formulated fungicides prepared from Sohxlet extracted oil of castor seeds and the untreated wood samples served as the control. The wood blocks were exposed to *Pleurotus ostreatus* (white rot fungi) and *Sclerotium rolfsii* (Brown rot fungi) for 14 weeks and parameters including preservative absorption, oil yield, and weight loss of the wood samples subjected to treatment were assessed. Using Analysis of variance (ANOVA) to analyze the data obtained, the mean oil yield of seeds of *Ricinus communis* extracted was 37.75%. The100% concentration level had the best performance with the highest absorption of 92.68 (kg/m^3) while the least absorption (69.96 kg/m^3) was recorded for the 25% concentration except for the control (0%) which was 118.60kg/m^3. Statistically significant variations in weight loss were observed across different concentration levels, with a significance level of $p < 0.05$ and it ranged from 35.02 -31.42(%) for white rot while it ranged from 42.32 to 31.98(%) for brown rot apart from the control which was recorded to be 38.25% and 45.28% respectively. It can therefore be established that *Delonix regia* wood can be preserved with *Ricinus communis* seed oil extract against white rot and brown rot fungi.

Keywords: *Ricinus communis*; Fungi; White rot fungi; Brown rot fungi; Weight loss

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INTRODUCTION

Wood has traditionally been recognized as a primary construction material, widely utilized for both domestic and industrial purposes on a global scale. In Nigeria, over 80% of constructions involve wood but despite its usefulness, wood has a short service life which can be caused by various bio-deteriorating agents like termites, insects, marine borers, fungi (especially brown rot and white rot which degrades the major constituents of wood by proportion i.e cellulose, hemicelluloses and lignin that breaking down the polymeric structures of wood molecules (Adebawo, 2019). This breakdown causes a type of decay, which shrinks wood and gives a brown coloration. Wood deteriorates by fire attack and at times mechanical failure, thus the need to treat and condition wood to impact resistance to degradation and deterioration by these living organisms, so, generally speaking freshly cut logs or lumber needs to be treated before they are manufactured into products. In addition to structural wood preservation methods, various chemical preservation methods and procedures have been employed to prolong the lifespan of wood, timber, and related products. Three robust wood preservatives, namely chromated arsenicals, creosote, and pentachlorophenol, have previously been employed for wood treatment. However, assessments have been conducted to ascertain that these products can fulfill their intended function without posing undue risks to human health and the environment, after which they are recommended for outdoor uses only. Some newer wood preservatives which are seen to exhibit reduced toxicity levels in comparison to older wood preservatives are now used for indoor purposes e.g., Alkaline copper quaternary (ACQ), Borates, Copper azole and Copper naphthenate (US EPA 2023) Despite their low toxicity, they are still considered dangerous thus the need for the use of more eco-friendly bio-preservatives which are gotten from plant extracts. Numerous researchers have directed their attention to the chemical constituents present in these extracts (Syofuna *et al*., 2012; Rodrigues *et al*., 2012), with a significant emphasis on the Castor plant (Ricinus communis) as a predominant source. Belonging to the spurge family, Euphorbiaceae (Momoh *et al.*, 2012), it is commonly referred to as the 'palm of Christ.' The seed oil of this plant has been documented to exhibit antimicrobial properties (Momoh *et al.,* 2012) and has proven effective in termite control in wood (Ahmed *et al*., 2014), whereas the leaf extract shows molluscicidal activity against Lymnaea acuminate, and the seed extracts exhibit more potent insecticidal activity (Upasani *et al*., 2003; Sharma *et al.,* 2009; Ramos Lopez *et al.,* 2010).

Delonix regia (*Caesalpinidae*) is a beautiful, semi-deciduous ornamental, leguminous plant tree, which is about 12m to 18m high with a slightly flat or curving crown commonly known as flame of forest in Nigeria. Due to the growing prevalence of the use of widely recognized wood species for structural applications in Nigeria, it is important to explore the lesser-known species a huge part of which *D. regia* is. This informs this study and is also aimed at looking out for the durability of this lesser-known wood specie using well-established bio-preservative *R. communis* seed oil.

MATERIALS AND METHODS

Material Source and Preparation

A twenty- year old tree of *D. regia* was felled at a farm in Ijokodo area, Ibadan,Oyo state,Nigeria and was cut into 60 wood blocks of the dimensions of 20mm ×20mm × 60mm according to the standard procedure and the samples were well labeled. The wood samples were oven dried at 100^oC for 24hrs to a constant moisture content and the initial weight of the samples (W_1) were taken using a well calibrated electronic balance and then oven dried for 24 hours at 103±2 ºC till a constant weight was obtained and the weight (W_2) was recorded, the percentage moisture content was estimated using the equation (1).

$$\%\ \mathrm{M.C.} = \frac{W_1 - W_2}{W_2} \times 100 \text{-------------------} (1)$$

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 *R. comminus* seed oil extract

Fresh matured castor (*R. communis*) seeds were picked at a farmland at Iwo Osun State of Nigeria. It was manually cleaned and left to sun-dry for approximately 5 days until the fruit capsules opened to release the enclosed seeds, after which it was weighed (305 g) before oven drying. The seed coats were removed and the castor bean was further dried for 9hrs in a hot air oven to reduce the moisture and obtain a constant weight of 100g and later ground to powdered form with an electric blender after which a Soxhlet chamber in the Department of Wood and Paper Technology laboratory, Federal College of Forestry,Ibadan was used to extract the oil from the castor seed.The experiment was repeated five times with each experiment containing thimble filled with 20 g of powdered castor seed and 100 ml of N-hexane poured in a round bottom flask attached to the Soxhlet chamber while the extracted oil was stored in a sterilized bottle.

Oil yield

The oil yield was determined using the formula below:

$$Oil\ yield\ \% = \frac{Volume\ of\ oil\ extracted}{Weight\ of\ ground\ meal\ used} \times 100$$

Phyto-chemical Analysis of Seed Oil Extract

The seed oil of castor (Ricinus communis) underwent phytochemical screening, following the standard procedure outlined by Brain and Turner (1975). This screening aimed to detect the presence of alkaloids, tannins, flavonoids, fats, saponins, phenolic compounds, steroids, and terpenoids.

Preparation of Test Fungicide (Bio-preservative)

The volume-to-volume method was employed to create various concentration levels of the oil extract, utilizing kerosene as the diluent, following the procedures outlined by Olajuyigbe et al. (2010) and Ajala (2014). Specifically, this method involves the preparation of dilutions where 1 ml of castor oil is combined with 99 ml of kerosene (diluent), resulting in a 1% dilution. Consequently, dilutions of 0%, 25%, 50%, 75%, and 100% were established accordingly while the untreated wood sample serves as control. Due to the availability of the extracted oil and also to ensure adequate soaking of the test wood blocks, the experiment was limited to 5 replicates per concentration, thereafter the wood samples were introduced to the fungi

Treatment of Test Block

The wooden test blocks underwent a full immersion/soaking process in the bio-preservative for a duration of 24 hours, adhering to the guidelines specified by FAO (1986). This treatment involved utilizing varying volumes of castor oil (0 ml, 25 ml, 50 ml, 75 ml, and 100 ml) to ensure optimal absorption. Following the treatment, each test block was extracted from the formulated preservatives, drained, and subjected to re-weighing to assess preservative absorption, employing the method outlined by Olajuyigbe et al. (2010), as described below:

$$\boldsymbol{Absorption} = \frac{\mathbf{10^6} \times \boldsymbol{WPA}}{\mathbf{10^3} \times \boldsymbol{V}}$$

Where: WPA = Weight of preservative absorbed (Kg),

V = Volume of wood sample (m^3).

Preparation of Culture Medium

The pure culture of white and brown rot fungi was sourced from the Pathology Department of the Forestry Research Institute of Nigeria (FRIN). A nutrient medium comprising Potato Dextrose Agar (PDA) in distilled water was formulated, with 40 ml dispensed into McCartney bottles and subsequently sterilized through autoclaving at 0.1 N/mm^2 and a temperature of 120 °C for 20 minutes. Inoculation with the test fungi occurred within 6 days post-preparation of the bottles, following the procedure outlined by Sarker (2006).

Inoculation of Test Blocks

The test blocks, wrapped in aluminum foil, underwent sterilization in the oven and were subsequently inoculated with fungi. They were placed in bottles containing actively growing cultures of the test fungi, ensuring contact with the aerial mycelium of the fungus. The inoculated blocks were then incubated at room temperature (27 ± 2) in the laboratory for a duration of 14 weeks. Following the incubation period, the blocks were extracted from the culture bottles and meticulously cleaned of any adhering mycelium (Sarker, 2006).

Weight Loss Determination of Treated Wood Samples

The test blocks after inoculation were carefully removed and dried in the oven at 103^0C for 18 hours and finally weighed again to determine the weight loss following procedures used in 1993 by Kumar and Dev as given below:

$\boldsymbol{W}$eight loss (%) = $\frac{W1-W2}{W1} \times 100$

Where: W1 = weight of oven dried wood sample before incubation;

W2 = weight 0f oven dried wood sample after incubation

Experimental Design

Experimentally speaking, the design utilized in this study was a completely randomized design (CRD). All collected data underwent an analysis of variance (ANOVA), and the means were differentiated using the Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Oil yield

The average oil yield of Ricinus communis seeds obtained through solvent extraction was 37.75%. This percentage is lower than the 45% oil yield documented by Ogunniyi (2006) for mechanically extracted seed oil from same plant. However, it is comparable with the 33.2% reported by Akpan (2006) for solvent-extracted oil. While Tsaknis (1999) found that the solvent extraction method generally yields more oil than the mechanical extraction method, the lower value reported in this case might be attributed to variations in the plant variety. It's worth noting that castor plants are known to have approximately 36 different varieties. (Ramos, 1984). Hence, castor seeds can be classified as a high oil-yielding plant, holding potential commercial value in the wood industry. This categorization aligns favorably with the oil yields reported by Rossel (1987) for various commercial plant oils, including olive (17%), soya beans (18%), and corn (3.4%). This observation is consistent with the findings of Ikhuoria (2008), who emphasized the economic viability of plant seeds with high oil content when processed for oil extraction.

Table 1. The Oil Yield extracted from the seed of *Ricinus communis*

Ricinus communis seed oil	Oil yield (%)
Replicate 1	38.27
Replicate 2	36.36
Replicate 3	37.27
Replicate 4	38.18
Replicate 5	38. 67
Mean value	37.75

Phytochemical Screening

Phyto-chemicals present in plant samples are connected to the biological activity of such plant and this plays a crucial role in exploring the medicinal properties of plant samples, our observations unveiled the presence of alkaloids, tannins, terpenoids, steroids, saponins, and flavonoids in the seed oil of *Ricinus communis*. This result corroborates with the findings of Dooh (2014) who reported identical findings in the qualitative analysis of the methanol extract of *Thevetia peruviana*. The presence of alkaloids, which are water-soluble salts derived from amino acids and known as organic heterocyclic nitrogen compounds, has been identified in the oil. Alkaloids have been reported to exhibit antimicrobial properties, particularly effective against fungal growth (Carson and Hammer, 2010). It has also been reported by (Smith 1996; Macel 2011) to act as a preventive measure against microbial and insect attacks thus, suggesting the antifungal capability of the oil.

Saponins, compounds found in plants, are known for their antimicrobial activity and are believed to play a role in plant disease resistance (Papadopoulou, 1999; Bouarab, 2002; Wittstock and Gershenzon, 2002, Elisa, 2007) are also present in this seed oil indicating its potential to resist fungal activities. They have been characterized and identified as agents discouraging biological activity, particularly against insects. (Macel, 2011).

Tannins are found in bud and foliage tissues, seeds, bark and roots of woods, they are also present in the oil sample. They possess notable anti-feed properties recognized in both angiosperms and gymnosperms (Barbehenn and Constabel, 2011). Terpenoids present in the castor seed oil sample are also reported by (Johnson and Morgan, 1997) to be used as flavoring agents, insect repellents, fungicides and for medicinal purposes.

Flavonoids which were reported to be phenolic structures, prevalent in photosynthesizing cells and are commonly present in various edible plant parts such as fruits, vegetables, nuts, and seeds was also found in this analysis, have been documented to hinder the growth of wood decay fungi (Onuorah 2000; Yen, 2008; Tumen, 2013). Flavonoids, terpenoids, and steroids exhibit antioxidant and antimicrobial properties.

Absorption of Preservatives by *Delonix regia* wood.

Table 2. Mean Percentage Preservative Absorption of *Delonix regia* wood.

Concentration	Mean (Kg/m^3)
0%	118.6
25%	69.96
50%	76.66
75%	84.3
100%	92.68

The mean absorption rate ranged between 69.96 to 92.68 kg/m^3 with 100% concentration level having the highest absorption of 92.68 kg/m^3 as presented in Table 2 except for the control (0%) while the least was recorded for the 25% concentration. No significant difference ($p > 0.05$) was observed in the effect of concentration. The remarkably high preservative absorption noted in the solvent alone (0%) is likely attributable to the low viscosity of the solvent (kerosene) which allows for easy permeability into the cell walls of the wood sample better than the others mixed with the seed oil. There was a rising trend in absorption noted with increasing concentration of the preservatives increased from 25% to 100%, this may be due to the increasing concentration of the preservatives as more of the oil is been introduced into the cell wall as the concentration increased. With the highest absorption at 100% concentration level, there is an indication that the preservative remained within an acceptable range of effective absorption into the wood.
The result obtained from this study is higher than the one reported by Adebawo (2019) while assessing the resistance of Obeche wood treated with neem seed oil against fungal attack, which could potentially be attributed to the viscosity of the preservatives involved (Owoyemi, 2010) or differences in the anatomical structure of the wood species. This corroborates the findings of Kazemi (2006) who noted that both the type of solvent and the viscosity of the chemicals employed influence preservative penetration in wood. The highest absorption rate obtained in this study at 100% concentration is also in line with what was reported by Okanlawon, *et.,al* 2020(a) while assessing the effectiveness of leaf extract from *Ocimum basilicum* L. as a preservative against termite attack on *Triplochiton scleroxylon* and *Ceiba pentandra*. The absorption rate also shows that wood blocks absorbed the oil extract differently at varying concentrations indicating that the wood samples were easily impregnated with castor seed oil extract due to the low viscosity of the extract influenced by viscosity of the diluents, a result which agrees with the work of Adetogun (2011) while evaluating Cashew nut Shell Liquid Incorporated with Sodium Chloride (Table Salt) as Fungicide against Wood Decay. The mean absorption rate values were observed to be significantly different from each other as it increases with increase in concentration level The result obtained also agrees with the findings of Okanlawon *et al.,* (2020b) who reported that absorption depends on the method of application, viscosity, soaking period and the level of concentration of the preservative.

Determination of Decay Resistance by Weight Loss

Table 3. Mean values of percentage weight loss of *Delonix regia* after exposure to fungi

Treatment (ml)	*P. ostreatus*	*S. rolfsii*
	Mean	
Control	38.25^a	45.28^a
0%	35.02^b	42.32^b
25%	33.68^c	35.67^c
50%	32.48^d	33,82^d
75%	31.92^d	32.52^e
100%	31.42^d	31.98^e

Mean with the same alphabet in each column are not significantly different from each other at $\alpha = 0.05$.

There was a consistent pattern of apparent reduction in weight loss as the concentration of the preservative increased for both brown rot fungi and white rot fungi. The mean values of percentage weight loss of *Delonix regia* after 14 weeks exposure to fungi ranged from 35.02 - 31.42(%) for white rot fungi while it ranged from 42.32 - 31.98(%) for brown rot fungi apart from the control which was recorded to be 38.25(%) and 45.28 (%) respectively. The result clearly shows that the control group was most damaged by the fungi but there was the minimal level of attack at higher concentration levels of the seed oil extract 50ml, 75 ml and 100 ml) for both fungi. The effect of the seed oil extract was significantly different from each other in their weight loss at different concentration levels, however, there is no significant difference at 50ml, 75 ml and 100 ml concentration level for white rot fungi and also at concentration levels 75ml and 100ml for brown rot fungi. This may be as a result of higher concentration of phytochemicals present in larger quantities of the seed oil which made the wood blocks resists the fungal attack since they have been reported to have antifungal properties. The diluting agent (kerosene) exhibited a temporary inhibitory effect on fungal decay, as evidenced by its higher initial weight loss compared to other preservative treatments, which diminished over time due to evaporation. Meanwhile, the most severe attacks observed in the control samples when compared with the 50-100% preservative concentration level which resulted in a reduced weight loss, indicates the efficacy of the preservatives in posing a substantial resistance to fungal attack by the wood samples. The diminishing weight loss with an increase in preservative concentration observed in this study aligns with the findings of Adetogun (2009) study on the fungicidal activities of cashew nut extract against wood. However, this finding contrasts with the studies conducted by Ogunsanwo and Adedeji (2010) on Obeche wood treated with the bark extracts of *Erythrophleum suaveolens* against a brown rot fungus (*Fomitopsis pinicola*) and Goktas (2007a) on beech wood and Scot's pine treated with *Sternbergia candidum* extract against a brown rot fungus (*Postia placenta*). They suggested that the phenomenon could be attributed to the enhanced solubility of effective preservative components at lower concentrations, rendering them more toxic to the fungus at such levels. Furthermore, in the treatment of beech wood and Scots pine exposed to a white rot fungus (*Trametes versicolor*), Goktas (2007b) documented an increasing trend in weight loss with rising preservative concentration. This phenomenon was attributed to the presence of organic materials in certain botanicals, constituting nutrition for the fungus. Consequently, increasing the concentration only served to augment the nutritive components in the wood, subsequently attracting more fungi and leading to an increase in wood weight loss.

Table 3 also highlighted the greater virulence of brown rot fungi compared to white rot, aligning with the findings of several researchers (Green and Highley, 1997; Adetogun *et al*., 2006; Goktas *et al*., 2007b; Emerhi *et al.,* 2008; Ogunsanwo and Adedeji, 2010; Adebawo, 2019). This observed trend may be attributed to the fact that brown rot fungi proportionately degrade the major wood constituents, cellulose and hemicelluloses, while white rot fungi predominantly target lignin. Additionally, the preferential invasion of conifers by brown rot fungi could contribute to this pattern (Gilbertson, 1980). Conversely, Venmalar and Nagaveni (2005), Goktas (2007a), Badejo (2009), and Ajala (2014) reported in their studies that white rot fungi exhibited higher virulence compared to brown rot fungi. The average weight loss observed in the control samples in this study (6.98% - 15.85%) still falls within the weight loss range for resistant species (11% - 24%) as per the ASTM (1989) classification of wood resistance to bio-deterioration. Thus, it can be inferred that the wood is moderately durable against fungal attack.

CONCLUSION

This study has shown the potentials of Castor seed oil extract as an ecofriendly bio-preservative against *Pleurotus ostreatus and Sclerotium rolfsii* decay especially on *Delonix regia* wood due to the presence of phyto-chemicals therein. The method used in extraction of the oil was noticed to give a limited amount of oil which also affected the quantity we were able to work with so other methods of extraction can be looked into in subsequent studies. Also, the application method used can affect the treatment level, so further investigations should be worked upon to affirm this. *Delonix regia* wood can be inferred to be moderately durable to fungal attack as the value obtained for the average weight loss still falls within the range for resistant species. The use of bio-preservatives is seen to be cheaper and more ecofriendly, if improved upon with more research findings, its wide acceptability can be achieved especially for large or commercial scale use and this will make life easier and improve the environment for the inhabitants.

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