Science World Journal Vol. 15(No 1) 2020
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Full Length Research Article

EVALUATION OF HONEY BEE PROPOLIS AS WOOD PRESERVATIVE USING WEIGHT LOSS

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ABSTRACT
Propolis, a natural product of Honeybees was evaluated as wood preservative. The need for this study arises as a result of the toxicity of conventional preservatives. Propolis Extract (PE) was obtained through ethanol extraction. The extract, diluted with absolute ethanol was prepared into four concentration levels using volume to volume method. Test blocks of Triplochiton scleroxylon measuring 5.0 x 2.5 x 1.5 cm were oven-dried at 103°C for 18h and treated by dipping in the four concentrations for 4 minutes. The treated and untreated test blocks were incubated in culture jars containing active growth of Coriolopsis polyzona and Coniophora puteana grown on Potato dextrose agar for 16 weeks. The efficacy of PE, in inhibiting fungal growth, was evaluated using Weight Loss (WL). Data obtained were analyzed using Descriptive Statistics, 2 - way Analysis of Variance and LSD test for mean separation. Results were significant at $p<0.01$. The PE was observed to inhibit the test fungi at higher concentrations after incubation. Post mortem analysis revealed the Killing Point Concentration (KPC) of PE against C. polyzona at 50 % and Coniophora puteana at 75 %.

Keywords: Propolis, Wood preservative, *Coriolopsis polyzona, Coniophora puteana,* Weight loss

INTRODUCTION
Wood is attacked because it is an organic material containing cellulose which is food for bio deteriorating agents (Adetogun, 2011). Timber can last indefinitely if the cellulose in wood is rendered toxic and inaccessible to degrading agents. This is achieved using wood preservatives (Egbewole *et al*., 2011). Conventional preservatives contain various harmful contaminants (Goktas *et al.,* 2007). These synthetic chemicals are expensive and often harmful to workers and the environment (Venmalar and Nagaveni, 2005). The ever increasing awareness of the toxicity of conventional or synthetic preservatives to the environment has necessitated a renewed interest in the use of forest bio preservatives that are environmentally friendly (Onuorah, 2000 and Kartal *et al.,* 2004). Biocidal potentials of cashew nut shell liquid (CNSL) against wood rotting basidiomycetes and the resistance of engine oil treated wood against termite attack have been investigated (Adetogun *et al*., 2009; Omole and Adetogun, 2010). Recently, the potentials of *Moringa oleifera* Lam seed oil as bio preservative agent against fungal attack and the potentials of heartwood extracts of *Gliricidia sepium* (Jacquin) Steudel as fungicide against wood-decaying fungi have also been investigated (Ajala *et al*., 2012; Ajala, 2014). Any material can be degraded by fungi, as long as there is moisture. As fungi grow through wood, its chemical structure is altered and mass is removed (Adetogun *et al.,* 2003), manifesting as weight loss. Weight loss is therefore used in evaluating fungal degradation in the wood of *T. scleroxylon*.

MATERIALS AND METHODS

Study Area
The wood for the study was sourced from a natural forest in cocoa research institute of Nigeria (CRIN) Ibadan, Southwestern Nigeria, lying between latitude 07°25'N and longitude 3°53'E. It is approximately 12km from Ibadan city. The annual rainfall is 1257mm. The relative humidity ranges between 84.5% (June to September) and 78.8% (December to January). The mean annual temperature ranges from 21.0 to 31.3°c (Shomade 2000, cited by Ajala 2014).

Propolis Extraction and Formulation
Collected propolis was cleaned and extracted using ethanol. Test preservative was formulated using the volume to volume method (Adetogun 1998). The preservatives were tested using four concentration levels, thus: 25 %, 50 %, 75 % and 100 %. Test block samples measuring 5.0 x 2.5 x 1.5 cm were used for the study, and prepared such that the grains of the wood follow the longitudinal axis. Blocks were prepared for test by drying and sterilizing in the oven at 103 °c until a constant weight was attained after 18 hours (Adetogun *et al*., 2009 and Adetogun, 2011). The weight obtained immediately after oven drying was taken as the initial dry weight (W1) (Adetogun, 1998 and Ajala, 2014). Synthetic Potato Dextrose Agar (PDA) was used as culture medium and prepared according to Adetogun (1998) and Ajala (2014). *Coriolopsis polyzona* (Pers) RYV and *Coniophora puteana* (Schum) Fries were used as test fungi. Dipping impregnation method (FAO, 1986; Adetogun, 1998 and Olajuyigbe, 2007) was used to treat test blocks with the preservatives. The same procedure was used for blocks treated with solvent only. Control blocks were not treated with propolis. The blocks were weighed to determine the rate and level of absorption. The weight obtained was taken as the initial wet weight (W_2).

Preservative absorption by test blocks, in Kilograms per cubic meter (Kg/m^3) was calculated using the equation below, according to Adetogun (1998) and Adetogun *et. al*., (2006, 2009)

$$\text{Absorption} = \frac{t\,x\,c\,x\,10}{v\,x\,n}\,kg/m^3 \quad \text{............................ (eqn. 1)}$$

Where: t is the total absorption in kg, c is concentration of fungicide (%), v is volume of wood sample used in (cm^3), and n is no of pieces of wood samples

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Weight Loss Determination

Weight loss method was used to evaluate the efficacy of PE in protecting the wood of *T. scleroxylon* against wood rotting basidiomycetes after 16 weeks of exposure (Zabel and Morell 1993; Adetogun *et al.,* 2006; and Sarker *et al.,* 2006). The weight obtained after incubation was taken as the final wet weight (W_3). The test blocks were dried and disinfected in the oven at 103 ^{0}C until a constant weight was attained. The weight obtained immediately after this was taken as the final dry weight (W_4). Weight loss was then determined using the equation below:

$$W_L = \frac{W_3 - W_4}{W_3} \times 100 \quad \text{(eqn. 2)}$$

Where: W_L is weight loss (g), W_3 is initial dry weight (g), and W_4 is final dry weight after 16 weeks of exposure to fungi (g).

Data collected were transformed using arcsine transformation procedure (Adetogun *et al.*, 2009) and subsequently analyzed using 2-way analysis of variance (at $p < 0.01$ level of significance) and descriptive statistics. A factorial design was used to analyze the data collected after inoculation. LSD test was used for means separation.

RESULTS

Tables 1 shows the absorption rate and weight loss of wood samples treated with propolis extract at different concentrations. Absorption of preservative increases with concentration, it ranged from 6.68 Kg/m ^{3} ± 0.33 for 0 % concentration to 10.52 ± 0.400 at 50 % concentration (the threshold value). There was no absorption at higher levels (above 75 %).

Table1: Absorption rate and weight loss of *T. scleroxylon* wood treated with Propolis Extract at different concentrations

Conc of PE (%)	Absorption (Kg/m^3)	Weight Loss (%) *C. polyzona*	Weight Loss (%) *C. puteana*
Control	—	50.10 ± 1.20	46.25 ± 1.81
0	6.68±0.330	42.20 ± 0.10	36.43 ± 1.02
25	9.35±0.380	24.05 ± 1.12	26.43 ± 1.11
50	10.52±0.400	7.90 ± 1.15	15.21 ± 1.02
75	1.80±0.030	0.00 ± 1.02	8.09 ± 1.12
100	0.10±0.004	0.00 ± 1.05	0.00 ± 1.41

Source: field work, 2014

The mean percentage weight loss of samples exposed to *Coriolopsis polyzona,* decreased from 50.10 % ± 1.20, for the control, to 7.90 % ± 1.15 at 50 % concentration (Table.1).

For samples exposed to *Coniophora puteana*, the weight loss decreased from 46.25 % ± 1.81, for the control, to 8.09 % ± 1.12 at 75 % concentration. Table 2 revealed that the fungi, concentration and blocks all have significant ($p<0.01$) effects on weight loss. The table also revealed that there is significant ($p<0.01$) difference in the fungi and in the concentrations. Table 2 revealed that there is significant ($p<0.01$) difference in the weight loss of the two fungi and that concentration levels are different from one another, hence they all affected weight loss differently. It further showed that blocks 1, 2, 3 and 4 are the same; blocks 3 and 5 are the same; blocks 4 and 6 are the same.

Table 2: Averages of Weight Loss, for the two fungi

Para Meters	Solvent	25 %	50 %	75 %	100 %	Control
Weight loss	23.48 ± 6.0829^a	19.29 ± 6.1496^b	11.50 ± 7.8911^c	8.48 ± 6.1600^d	5.16 ± 4.2929^e	27.20 ± 8.8152^f
	B1	**B2**	**B3**	**B4**	**B5**	**B6**
Weight loss(arcsine)	16.06 ± 10.9906^a	17.69 ± 11.4523^b	15.41 ± 9.6746^c	15.21 ± 11.0808^d	15.51 ± 9.7910^c	15.22 ± 9.7081^d

Values along the same row with different superscripts are significantly different ($P > 0.01$).

DISCUSSION

The trend in absorption was due to the increase in viscosity at higher concentrations, thereby limiting preservative penetration into the wood. The differences in the range of absorption could have been accounted for by the differences in concentration, wood species, specific gravity, moisture content and temperature (Ajala, 2014). Olajuyigbe (2007) reported absorption range of 20.27- 24.26 Kg/m^3 for *Gmelina arborea* wood samples treated with *Tectona grandis* heart wood extract, while Ajala (2012) reported a range of 1.77- 6.19 Kg/m^3 for *Aningeria robusta* wood samples treated with Moringa seed oil.

Resistance of treated wood to fungal attack increased up to 50 % concentration, indicating a threshold value at 50 % concentration level of PE. The solvent, ethanol, had little resistance to the fungi. The greatest attack was found in the control, with 50.10 % of mean weight loss. This was followed by ethanol which had 42.20 % weight loss. There was sharp reduction in weight loss between 25 % and 50 % PE indicating preservative effectiveness at 25% PE, but not as effective as 50 % level which was the maximum effective level; the threshold level.

This result, on *C. polyzona*, is contrary to Badejo (2009) in his work on *Ceiba pentandra* and *Triplochiton scleroxylon* inoculated with *Schizophyllum commune,* a white rot fungus, who reported an increase in weight loss with increase in concentration level from 25% to 100%. This result is in consonance with the report of Adetogun (1998) that resistance was increasing with increase in the level of concentration of Obeche subjected to *Coriolopsis polyzona* and *Lenzites palisoti* (white rot fungi) after treatment with Cashew Nut Shell Liquid. This result is also in consonance with Ajala (2014) in his work on *Aningeria robusta* inoculated with *Lentinus sajor-caju* (a white rot fungus), who reported a decrease in weight loss with an increase in concentration level from control to 50 % concentration level.

The result, on *C. puteana* is at variance with Badejo (2009) who reported an increase in weight loss with concentration except for 50 % level when *Ceiba pentandra* and *Triplochiton scleroxylon* were exposed to a brown rot fungus. The preservative was more effective on *C. polyzona* than *C. puteana*. This conforms with Adetogun *et al.* (2006) on *Afzelia Africana* and *Nesogordonia papaverifera* on aspen blocks treated with heartwood extracts, Zabel and Morrell (1993) and Green and Highley (1997) that brown rot fungi cause more weight loss than white rot fungi, and at variance with Badejo (2009). This is at variance with Osman *et al* (2007) who reported that *Trametes versicolor* (a white rot) causes more degradation than *Postia placenta* (a brown rot) when scot pine (*Pinus sylvestris*) inoculated with *Postia placenta* (a brown rotter) and *Trametes versicolor* (a white rot) and treated with *Sternbergia candidum* extract, showed increase in weight loss with concentration.

Conclusion

Absorption of PE was least at higher concentrations. Control samples suffered the highest weight loss, while the lowest weight loss was recorded at 100% concentration, indicating the effectiveness of propolis in preventing weight loss (preserving wood) at higher concentrations. The weight loss is influenced by extract concentration. Propolis is able to control the test fungi at concentrations of 50 % and 75 % respectively.

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