Termite resistance of *Citrus sinensis* oil on treated woods of *Pterygota macrocarpa* and *Aningeria robusta.*

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Abstract
With increasing concerns and global awareness on the health and environmental hazards associated with chemical preservatives, both during and beyond service life of treated woods, there has been a quest for a shift to eco-friendly, non-toxic preservatives for wood protection. This study therefore investigates the suitability of sweet orange oil, extracted from its fruit peels as wood preservative. The oil was applied on *Pterygota macrocarpa* and *Aningeria robusta* and their resistance to biodegradating agents were examined. Test specimens of *Pterygota macrocarpa* and *Aningeria robusta* were cut into sizes of 10.5 x 3.0 x 1.5 cm and prepared in accordance to British Standard of 1961. Ethanolic extraction method was used to extract oil from the dried peels of sweet orange fruit. Treated samples of the two wood species with sweet orange oil and the untreated samples were evaluated for weight loss after 8 weeks of exposure to termites. The mean values of sweet orange oil absorptions are 256.7 Kg/m^3 and 402.4 Kg/m^3 for wood samples of *Pterygota macrocarpa* and *Aningeria robusta* respectively. There are significant differences ($p < 0.05$) between weight losses of samples treated with sweet orange oil and the untreated samples of the two species. The mean values of weight loss of the untreated and the treated samples of *Pterygota macrocarpa* are 46.46% and 26.44% respectively. For *Aningeria robusta*, 63.53% and 23.70% are the mean weight loss of the untreated and the treated samples respectively. The findings of this study showed that sweet orange oil could serve as a natural anti-biodegrading compound.

Keywords: Biodegradation, *Citrus sinensis* oil, termite resistance.

Introduction
In southwest Nigeria, termite infestation on woods and wooden products, both above ground and subterranean, continue to be a major occurrence and source of bio-deterioration thereby shortening the service life of woods. While several chemical preservatives such as chromated copper arsenate and pentachlorophenol have been formulated, their toxic effects both on human health and the environment pose a serious challenge towards achieving ecologically sustainable wood protection mechanism. Clausen *et al* (2010) reported that environmental concerns about commercial wood preservatives have initiated interest in "green preservatives". It is therefore pertinent that a shift from the use of synthetic, hazardous to non-toxic, eco-friendly preservatives be embraced.

Extending the service life of wood and wooden products using natural compounds as bioactives is proving to be an attractive approach for wood protection from human health and environmental perspectives (Schultz and Nicholas, 2002; Evans, 2003; Singh and Singh, 2010). The use of natural compounds of plant origin is one of such suitable alternatives being explored for a range of options towards formulating an environmentally friendly preservative for wood protection.

Several herbaceous plant essential oils such as oils of *Theobroma cacao* (cocoa), *Tithonia diversifolia* (sunflower), *Anacardium occidentale* (cashew) and *Azadirachta indica* (neem) have been reported to possess inhibitory properties against subterranean termites (Zhu *et al.,* 2003; Raina *et al.,* 2007; Osipitan and Oseyemi, 2012, Addisu *et al.,* 2014). These essential oils are volatile oils occurring in different parts of plants such as buds, flowers, leaves, fruits, seeds and roots (Heath, 1981). They are odoriferous and are made up of a wide variety of organic compounds of many different functional groups and molecular structures.

Flowers of plants that are rich in essential oils attract insects by their odour for pollination, suggesting that these oils have a role in natural selection and preservation, while the chemical constituents of the essential oils may cause physiological reactions. In recent years, essential oil has been identified in different parts of citrus fruit while report has also shown that Citrus essentials oil can be used in cosmetics and pharmaceuticals application (Okwu and Emenike, 2006; Chede, 2012). Thus, suggesting the need for research on citrus as anti-biodegradant against termite.

The genus Citrus has many species which include sweet orange (*Citrus sinensis*), grape (*Citrus paradisi*), lemon (*Citrus limon*), lime (*Citrus auratinfolia*) and tangerine (*Citrus tangerina*) with sweet orange being the most consumed. Sweet orange is consumed by many people due to its sweet and refreshing properties, nutritional and medicinal values. An important medicinal value of sweet orange is the prevention of degenerative process, particularly lowering incidence and mortality rate of cancer, cardio- and cerebro-vascular diseases due to the various antioxidant phyto-nutrients it contained (Rapisararda *et al.*, 1999; Okwu and Emenike, 2006).

Citrus sinensis oil is produced by cells within the rind of a sweet orange fruit. A wide variety of organic compounds of many different functional groups and molecular structures has been found in the essential oils obtained from the peels of sweet orange fruit. The organic constituents present include alkaloids, flavonoids, the terpenes, alcohols, aldehydes, acids, aromatic compounds of benzenoid structure, aliphatic hydrocarbons and their oxygenated derivatives as well as nitrogen-or sulphur containing compounds (Manthey, 2004; Pultrini *et al.*, 2006; Sharma and Tripathi, 2006). According to Shahid 2003, Alkaloids bring about disturbance in the nervous system of insects thereby causing death while flavonoids can modulate the feeding behaviour of insects (Nawrot *et al.*, 1986). These compounds (alkaloids and flavonoid) functions as inducible anti-insect compounds (Dixon, 1999).

The consumption of orange fruits do generates orange peel regarded as wastes which is contributing to environmental pollution. In citrus industry in many part of the world, emphases are laid only on orange fruits marketed fresh or processed as canned juice, while fruit peels produced in great quantities during the process are mainly discarded as waste. Presently, in Nigeria the local processing of *Citrus sinensis* fruits is on the increase to meet increasing local demands for fruit juice that was previously met by large-scale importation (Odubanjo and Sangodoyin, 2002), meaning a quantum increases in the volume of its peel.

Considering the chemical composition of its oil, it will amount to huge economic waste to consider the sweet orange peels as waste littering the environment and constituting pollution. This study therefore aim at evaluating the effect of sweet orange oil as anti-biodegradant against termite in treated woods of *Pterygota macrocarpa* and *Aningeria robusta.* These species are lesser used wood species that are evolving in building and furniture making. The use of sweet orange peels can prevent the environmental menace that may arise from the huge piles of orange peel, enhance the economic value of sweet orange while providing a cheaper and safer alternative source for the preservation of wood.

Materials and Method

Test specimens

Heartwood portions obtained from clear, straight and knot-free samples of the two species (*Pterygota macrocarpa* and *Aningeria robusta*) were cut into 10.5 x 3.0 x 1.5 cm. The cuttings were arranged that the grains of the wood follow the long axis (BSI, 1961; Greaves, 1985).

Pre-treatment: Drying of wood

The test samples were oven-dried at 103˚C for 24 hours to reach constant weight. Weights were taken and recorded every 6 hours during the drying period. Moisture content (%) was determined using equation 1:

$$MC\ (\%) = \frac{M_g - M_{od}}{M_{od}} \text{ X } 100 \text{ .... Equation (1)}$$

where Mg is the green mass and Mod is the oven-dry mass.

Oil extraction

Ethanol was used for the extraction of oil as adopted by Chede (2012). Chipped, dried peels of the orange fruits were soaked in 99.7 – 100 % ethanol for 72 hours at room temperature with rigorous shaking of beakers every 24 hours. Thereafter, the extract was filtered and allowed to stand for 3 days for evaporation of excess solvent.

Oil treatment of wood

Oven dried wood samples were soaked in the extracted oil for 24 hours at room temperature. After treatment, samples were kept on dry clean slates for 72 hours for adherence of oil. Oil absorption was calculated using equation 2:

$$\text{Absorption } Kg/m^3 = \frac{10^6 \text{ x weight of preservative absorbed}}{1000 \text{ x volume of wood}} \text{ .... Equation 2}$$

Termite test

Untreated (Control) and treated wood samples were exposed to termite in ground contact. The level of biodegradation was estimated after 8 weeks in terms of weight loss using equation 3:

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

where W1 is weight before and W2 is weight after exposure to termite in grams.

Results

Oil extraction

1000 ±10mL of pure ethanolic oil extract was obtained from 900g of chipped peels of sweet orange (Plate 1). The concentration of the oil obtained was 99.9%. Table 1 presents the summary of the extraction.

Plate 1: Extracted *Citrus sinensis* oil

Table 1: Summary of oil extraction from peels of sweet orange using ethanol

Description of variable	Results obtained
Mass of dried peels	900 grams
Volume of solvent	2700 ±5ml
Extraction temperature	room temperature (20 - 25°C)
Solvent to peel ratio	3:1 (v/w)
Volume of extract	1000 ±10ml

Oil absorption

The mean values of sweet orange oil absorptions are 256.7 Kg/m^3 and 402.4 Kg/m^3 for wood samples of *Pterygota macrocarpa* and *Aningeria robusta* respectively (Table 2). The rate of oil absorption by *Pterygota macrocarpa* wood samples was lower than that of *Aningeria robusta*, thus revealing its lower permeability. Furthermore, there is a significant difference ($p < 0.05$) in the level of absorption of sweet orange oil by the two species (Table 2). The result indicates that sweet orange oil absorption varies with wood species.

Table 2: Comparison of level of absorption of the oil (kg/m^3) by *Pterygota macrocarpa* and *Aningeria robusta* with the Students t-test

Species	N	Mean	SD	t-tab	t-cal
Pterygota macrocarpa	10	256.67	32.55	2.10	10.70*
Aningeria robusta	10	402.35	28.23		

*= significant (p<0.05)

Termite test

Lower values of weight loss that ranges from 22.25% to 25.95% were obtained for treated *Aningeria robusta* while for treated *Pterygota macrocarpa*, the weight loss ranges from 21.93% to 30.89%. The control samples of *Aningeria robusta* however had higher weight loss that ranges from 48.56% to 82.45% when compared to a range of 39.20% to 50.40% for *Pterygota macrocarpa* (Table 3).

The mean values of weight loss of the untreated and sweet orange oil treated samples of *Pterygota macrocarpa* are 46.46% and 26.44% respectively. For *Aningeria robusta*, 63.53% and 23.70% are the mean weight loss of the untreated and the treated samples respectively (Table 4). There are significant differences ($p < 0.05$) between weight losses of the untreated and the treated samples of the two species (Table 4). Plates 1-4 show the weight lost by the treated and untreated samples of the two species.

Table 3: Weight loss (%) of untreated and treated samples after exposure to termites for 8 weeks

S/N	*Pterygota macrocarpa*		*Aningeria robusta*	
	Control	**Sweet orange oil treated**	**Control**	**Sweet orange oil treated**
1	46.61	25.49	56.21	23.36
2	50.40	30.89	78.58	23.99
3	50.26	26.83	48.56	22.95
4	39.20	27.08	51.84	22.25
5	45.81	21.93	82.45	25.95
Mean	**46.46**	**26.44**	**63.53**	**23.70**

Table 4: Comparison of Weight loss (%) of the Untreated and Treated Samples of *Pterygota macrocarpa* and *Aningeria robusta* using the Student- t-test Result

Species	N	Mean	SD	t-tab	t-cal
Pterygota macrocarpa					
Control	5	46.46	4.08	2.31	8.97*
Sweet Orange Oil	5	26.44	2.88		
Aningeria robusta					
Control	5	63.53	14.13	2.31	6.27*
Sweet Orange Oil	5	23.70	1.26		

*= significant ($p<0.05$)

Plate 1: Residual samples of untreated *Pterygota macrocarpa*

Plate 2: Residual samples of *Pterygota macrocarpa* treated with *Citrus sinensis* oil

Plate 3: Residual samples of untreated *Aningeria robusta*

Plate 4: Residual samples of *Aningeria robusta* treated with *Citrus sinensis* oil

Discussion

The lower rate of oil absorption obtained for *Pterygota macrocarpa* when compared with *Aningeria robusta* revealed its lower permeability to sweet orange oil absorption. The permeability of wood is determined by the amount of water or liquid that can be absorbed by wood and this further depend on the density and water diffusivity of the wood (Khazaei, 2008). Higher rate of absorption is associated with greater density (Simpson and Tenwolde 1999). This correlated relationship according to Panshin and DeZeeuw (1980) is because the cell wall substances that are responsible for density are also responsible for water or liquid uptake and dimensional changes. Thus, the greater the amount of cell wall substances presents, the higher the permeability. Average density that ranged from 455kg/m3 to 560kg/m3 and from 380kg/m^3 to 450kg/m^3 have been obtained for *Aningeria robusta* and *Pterygota macrocarpa* respectively (Ajala and Ogunsanwo, 2011; Wood Database, 2015). Thus the higher density of *Aningeria robusta* could be deduced for its higher rate of *Citrus sinensis* oil absorption.

On the other hand, the water diffusivity coefficient of wood describes the rate at which water and liquid moves from surface to the interior of wood. This movement depends on the porous structure of wood and the reactivity of its chemical components; whereby slow movement of moisture and liquid has been observed with porous wood. Higher density wood are characterized with less porous structure since they contain more solid cell wall materials and extractives in the cell lumen (Zobel and Jett 1995). Hence, the less porous structure of *Aningeria robusta* due to its higher density when compared with *Pterygota macrocarpa* could also be responsible for the higher absorption of *Citrus sinensis* oil.

Lower weight losses in the treated samples of both species indicated that sweet orange oil improved the resistance of wood against termite bio-deterioration. This supports earlier findings that natural chemicals derived from plants are possible alternatives to the synthetic insecticides for the control of termites (Sbeghen *et al*., 2002); and that essential oils may be the alternative sources of termite control agents because they constitute rich source of bioactive molecules (Sakasegawa *et al*., 2003, Cheng *et al*., 2004, Park and Shin, 2005).

Ogunsina *et al*. (2009) used hexane and ethanol extracts of *Lantana camara* (African nutmeg) and *Euphorbia laterifloria* (Enuopiri) against subterranean termite workers. Different concentrations of these plants extract tested showed maximum mortality, and anti-feedants effects on termites. 50% and 90% mortality were recorded with 0.50 g/100mL and 0.90 g/100mL of hexane extract of both *Lantana camara* and *Euphorbia laterifloria* respectively. Mokabel and Gowda (2000) tested the water extracts of some common plant products for their anti-termite properties by exposing treated bamboo baits in Karnataka, India. All the treatments showed significant reduction against termite damage. From the treatments, 92-95% protection was provided by Pongamia (*Pongamia pinnata*) seed oil, 72-88% by Neem (*Azadirachta indica*) seeds cake and oil, 71-79% by Castor (*Ricinus communis*) seeds and roots oil; and 70-78 % by Mahogany (*Swietenia macrophylla*) seeds oil. Specifically, Neem plant extracts, from its leaf, bark, trunks, twigs and branches were bioassayed on the subterranean termite, *Coptotermes curvignathus*. The termites were exposed to the plants' extractives prepared at three concentration levels (250 ppm, 500 ppm, and 1000 ppm and the solvents 0 ppm as the control) by feeding the termites with treated filter paper. The termite's bioassay test showed that Neem extractives caused high termite mortality coupled with less consumption of filter paper treated with bark extracts.

With respect to essential oils, Cheng *et al*., (2007) investigated the anti-termitic activities of 11 essential oils from the sapwood and heartwood of three species of coniferous tree (*Calocedrus macrolepis* var. *formosana*, *Cryptomeria japonica* and *Chamaecyparis obtusa* var. *formosana*) against *Coptotermes formosanus.* Results showed that at the dosage of 10 mg/ml, the essential oils had 100% mortality after 5 days of the test. Among the tested oils the heartwood essential oil of *Calocedrus macrolepis* var. *formosana* killed all termites after 1 day of test, exhibiting the strongest termiticidal property. Victoria (2010) reported that essential oil of leaves of *Adhatoda vasica* contain vasicine, vasicinone and maiontone which have high insecticidal properties to control insect pests and give significant results. Acda (2009) used oil of the Physic nut, *Jatropha curcas* L. for its repellent activity and barrier against the Philippine milk termite *Captotermes vastator*. *Jatropha curcas* oil had anti-feeding effect, increased mortality as well as induced reduction in tunneling activity in *captotermes vastator*. Singh and Sushil (2008) also used *Jatropha curcas* oil at different concentrations of 1%, 5%, 10%, 20% against *Microcerotermes beesoni* termite. All the treatments proved to be effective over control with the 20% concentration showing maximum wood protection against termites. The weight loss ranged from 18.77% to 48.80% at concentrations of 20% to 1% of *Jatropha curcas* oil formulation.

The improved resistance offered by sweet orange oil could be partly accounted for by some of the phytochemicals substances in the oil which include alkaloids, flavonoids, tannins and saponins as also reported by Okwu *et al.,* (2007). Alkaloids brings about disturbance in the nervous system of insects Shahid (2003) while flavonoids functions as inducible anti-insect compound (Dixon, 1999) and modulate the feeding behaviour of insects (Nawrot *et al.,* 1986). However, the weight loss by oil treated samples of the wood species (Plates 2 and 4), despite oil treatment, affirm the earlier reports that treatment may only reduce and not stop degradation, unless applied at extremely toxic levels (Schultz and Nicholas, 2002; Singh and Singh, 2010).

Conclusion

Based on the above findings, essential oil from fruit peel of sweet orange has the potential of extending the service life of wood. It improved the resistance of wood to biodegradation by termite. However, the weight loss by both *Pterygota macrocarpa* and *Aningeria robusta*, despite oil treatment, shows that treatment could only reduce the attack and may not totally stop degradation. It is therefore recommended that more improvement in the use of sweet orange oil as wood preservative be sought by forming a synergy with other suitable natural compounds of plant origin.

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