

Wood Preservation Based on Neem Oil: Evaluation of Fungicidal and Termiticidal Effectiveness

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Abstract

This research examined oil from the neem tree (*Azadirachta indica* A. Juss.) for its potential as an eco-friendly wood preservative. In contrast to expectations from the literature, according to which neem oil should be effective against insects and fungi, neem oil performed poorly as a preservative for *Pinus radiata* D. Don wood, which suffered significant mass losses in the bioassays. Using standard experimental procedures from the wood preservation industry, concentrations of 0.01, 0.1, 1.0, 2.5 and 5.0 percent neem oil in white spirit were bioassayed against five species of decay fungi. Additionally, concentrations of 0.01, 0.1 and 1.0 percent neem oil were bioassayed against two species of termites. It is concluded that neem oil can only be useful as a wood preservative if new, optimized formulations are sought, probably exploiting synergy with cobioicides.

Wood is a natural material containing cellulose and hemicelluloses, and can therefore act as food for a group of extremely active organisms, the xylophages, whose activity leads to both the decomposition of substratum and the reduction of material to its constituent elements. The xylophages of greatest economic importance are fungi and termites. Owing to the problems associated with wood biodeterioration, especially in tropical countries, treatment techniques using preservatives are often required if wood is to be used in construction (Cavalcante 1982, Costa 2000).

Concerns have been raised about the traditional preservatives used to protect wood because of the risk they may pose to both the environment and public health. Of particular concern are preservatives based on heavy metals such as chromium and arsenic, and those containing fluorine or creosote. Because the toxic compounds used often have low biodegradability, additional difficulties can arise in the post-use disposal of treated timber (Evans 2001, Hata et al. 2006).

A more environmentally friendly alternative may occur through the use of natural biocides, and some researchers have focused on the chemical components from the extracts of wood and other plants (Ohmura et al. 2000, Cookson et al. 2004, Rodrigues et al. 2012, Syofuna et al. 2012). This study considers the use of oil from the neem tree (*Azadirachta indica* A. Juss) as a potential eco-friendly wood preservative. Neem oil has low mammalian toxicity, while possessing biologically active compounds, such as

azadirachtin and other limonoids (Kraus 1995). Azadirachtin is a highly oxidized tetranortriterpenoid that has antifeedant and growth regulating effects on insects and fungi, although its biochemical effects at the cellular level are still not understood (Mordue and Blackwell 1993, Islam et al. 2009).

Neem oil and extracts have been reported to be active against termites, including *Zootermopsis nevadensis* (Hagen) (Ohmura et al. 2006), *Reticulitermes speratus* Kolbe (Serit et al. 1992), *Coptotermes formosanus* Shiraki (Grace and Yates 1992, Doolittle et al. 2007), and *Incisitermes*

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marginipennis (Latreille) (Arcos-Roa et al. 2001). Methanol and hexane extracts from a similar timber species, *Azadirachta excelsa* (Jack) Jacobs, were also active against *Coptotermes curvignathus* Hohngrren (Sajap et al. 2006). Neem oil impregnated into *Populus deltoides* Bartr. ex Marsh controlled *Microcerotermes beesoni* Snyder in a laboratory bioassay (Dhyani and Tripathi 2006) and was suggested as a spray treatment that could avert termite attack (Yashroy and Gupta 2005). Neem wood or mulch itself has some resistance to *C. formosanus* (Delate and Grace 1995) and in India is usually free from insect attack (Koyani et al. 2011).

Neem also produces fungicidal compounds (Locke 1995, Paul and Sharma 2002, Amadioha 2004). The outer heartwood of neem was found to be naturally durable against four species of basidiomycete decay fungi (Rao 1990). High concentrations (25%) of neem oil impregnated into *Pinus roxburghii* Sargent controlled the white-rotting fungus *Trametes versicolor* (L.: Fr.) Pilát and the brown-rotting fungus *Postia placenta* (Fr.) M. Larsen & Lombard (Dhyani and Tripathi 2006).

Neem oil is usually produced by crushing seeds and is an available commercial product in Brazil. To examine whether commercially available neem oil could be used as a wood preservative, we dissolved it in white spirit, which is a common solvent for the timber treatment industry. This formulation was assessed against termites and decay fungi using laboratory bioassays that are standard test procedures for new wood preservatives in Australia (Australasian Wood Preservation Committee [AWPC] 2007).

Materials and Methods

Termite bioassays

Solutions of 0.01, 0.1, and 1.0 percent (m/m) neem oil in white spirit were impregnated into sapwood specimens of *Pinus radiata* D. Don, 50 by 25 by 15 mm. The neem oil was obtained from a farm plantation in São Paulo state, Brazil, where it was collected from crushed seeds. Treatment was achieved by pulling a vacuum of -98 kPa for 30 minutes, adding the treatment solution under vacuum, releasing the vacuum, and then leaving the specimens to soak at atmospheric pressure for 30 minutes. The specimens were removed, blotted to remove excess solution, weighed to determine solution uptake, and wrapped in plastic bags, where they remained for 1 week to slow the rate of solvent evaporation. Specimens were then placed on trays in the laboratory to air-dry for 4 weeks. They were artificially weathered in vacuum ovens for 5 days at -98 kPa and 40°C , removed to cool in a desiccator, and weighed to determine the oven-dry mass. Additionally, the same procedures were used with test specimens that were submitted to two solvent control treatments (water alone and white spirit alone). Test specimens were also treated with the traditional preservative copper-chromium-arsenic (CCA) to the approved hazard class 2 retention of 0.32 percent (m/m) total active elements (TAE) of Cu, Cr, and As (AS 1604.1, Standards Australia 2005). There were six replicates per termite species and retention.

The test termites were *Coptotermes acinaciformis* (Froggatt) and *Mastotermes darwiniensis* Froggatt, which were obtained from the Northern Territory near Darwin, Australia. For the *C. acinaciformis* bioassay, a single test specimen was embedded in a moist matrix of *Coptotermes*

lacteus (Froggatt) mound material (50 g, 80% moisture content [MC]) within a 1.2-liter glass jar. Ten grams of *C. acinaciformis* was added to each jar. A Bakelite lid, with a central 9-mm-diameter ventilator, closed the jar. The duration of the bioassay was 8 weeks.

The *M. darwiniensis* bioassay utilized 700-mL glass jars in which a mixture of 18 g of vermiculite (5- to 10-mm particle size range) and 6 g of *Eucalyptus regnans* F. Muell. sawdust was placed. A single test specimen was then embedded upright in the vermiculite-sawdust matrix close to the inside wall of each jar. Sixty-six milliliters of water was then added to the matrix to achieve 275 percent MC. Fifteen grams of *M. darwiniensis* was added to each jar, and a metal lid, with a central 9-mm-diameter ventilator, closed the jar. The duration of the bioassay was 6 weeks.

At the conclusion of the bioassays, test specimens were removed from the jars and cleaned. Test specimens, as well as vacuum oven controls, were then vacuum oven-dried under the same conditions used to obtain the initial masses. After cooling and weighing the test specimens, the final and initial masses were compared to determine the percentage of mass loss.

Fungal bioassays

Solutions of 0.01, 0.1, 1.0, 2.5 and 5.0 percent (m/m) neem oil in white spirit were impregnated into *P. radiata* sapwood specimens 20 by 20 by 10 mm using the same procedures as described previously. The 5.0 percent concentration was close to the maximum amount of pure neem oil that could be dissolved in white spirit. For the CCA comparison, test specimens were treated to the approved hazard level 3 retention of 0.38 percent (m/m) TAE (AS 1604.1, Standards Australia 2005). Vacuum oven-drying was used to artificially weather the test specimens as described in the termite bioassay. In addition, another set of test specimens treated with 1.0, 2.5 and 5.0 percent (m/m) neem oil was leached before vacuum oven-drying. These were vacuum impregnated with water and leached with more than three times their volume of water in jars on a shaking water bath at 35°C for 5 days, with the water changed daily. After obtaining initial masses following the vacuum oven-drying procedure, all test specimens were sterilized by gamma irradiation.

The test vessels for the fungal bioassay were 250-mL screw-cap glass jars each containing 150 g of "Toolangi forest loam" soil moistened to 60 percent MC. Two poplar sapwood veneer feeder strips, previously soaked overnight in 1 percent malt extract solution, were placed on the soil in each jar. Jars were autoclaved for 2 hours. The feeder strips were inoculated with the appropriate test fungus, i.e., brown-rotting fungi, *Coniophora olivacea* (Fr.) Karst., *Fomitopsis lilacino-gilva* (Berkeley) J. Wright and Deschamps, *Serpula lacrymans* (Schum. ex Fr.) S.F. Gray, and *Gloeophyllum abietinum* (Fr.) Karst., and the white-rotting fungus *Perenniporia tephropora* (Mont.) Ryv. One set of jars was left uninoculated as a sterile control to determine whether there was any mass loss or gain not attributable to fungal attack.

After 14 days, the fungi had grown sufficiently on the feeder strips (30 days for *S. lacrymans*), whereupon sterilized test specimens were planted. There were six replicates per fungus-retention combination. Fungi and sterile controls were incubated at 25°C , with the exception of *S. lacrymans*, which was incubated at 20°C . Relative

humidity was at least 85 percent, because jars were placed in trays containing water, and each tray was enclosed in a large plastic bag. After 12 weeks of incubation, specimens were removed from the jars, weighed to determine MC, vacuum oven-dried, and weighed once more to obtain individual mass losses.

The toxic threshold value is considered to have been achieved when the mean mass loss is less than 3 percent for fungi, or less than 5 percent for termites.

Statistical analyses

Analysis of variance (ANOVA), with significance level of 5 percent, and taking the equivalence between means as null hypothesis ($H_0: \mu_1 = \mu_2 = \dots = \mu_n$), evaluated with the aid of SAS software, was used to verify the effect of treatments on mass loss of the woods. P values greater than the significance level implies accepting H_0 , rejecting it otherwise. To validate the ANOVA model, the normality data and equivalence between variances were verified, using the Anderson-Darling and Levine's tests, respectively, both at the 5 percent significance level, with consideration of normality data and equivalence between variances as the null hypothesis for the tests. When treatments proved to have a significant effect on mass loss according to ANOVA, Tukey's test was used for grouping.

Results

The mean mass losses caused by *C. acinaciformis* and *M. darwiniensis* to test specimens are shown in Table 1. Both termite species caused extensive damage to the water and white spirit controls, with mean mass losses above 90 percent for *C. acinaciformis* and above 99 percent for *M. darwiniensis*. In comparison, the specimens treated with a hazard class 2 retention of CCA had negligible mean mass loss, demonstrating excellent termite control. Almost all test specimens treated with the 0.01 and 0.1 percent solutions had mean mass losses similar to the untreated controls. However, those treated with the 1.0 percent neem solution had slightly reduced attacks compared with the controls ($P < 0.05$), with 59.9 percent mean mass loss against *C. acinaciformis* and 72.5 percent mean mass loss against *M. darwiniensis*, demonstrating slight termite resistance. Indeed, all termites exposed to the 1.0 percent neem treatment

Table 1.—Mean mass loss in *Pinus radiata* test samples subjected to bioassay against *Coptotermes acinaciformis* and *Mastotermes darwiniensis*.

Treatment ^a	Mean \pm SD mass loss (%) ^b	
	<i>C. acinaciformis</i>	<i>M. darwiniensis</i>
Water	93.6 \pm 8.2 A	99.7 \pm 0.0 A
White spirit alone	92.9 \pm 6.9 A	99.7 \pm 0.0 A
CCA	0.3 \pm 0.2 D	3.4 \pm 1.4 C
White spirit with neem, % (m/m)		
Neem, 0.01	86.6 \pm 14.1 B	99.7 \pm 0.0 A
Neem, 0.1	91.0 \pm 6.8 A	99.7 \pm 0.0 A
Neem, 1.0	59.9 \pm 11.0 C	72.5 \pm 19.6 B

^a CCA = copper-chromium-arsenic.

^b The mass losses are adjusted for vacuum oven controls. Within the same column, means followed by the same letter are not significantly different (Tukey test at 5%).

had died by the end of the bioassay, while the majority of termites in jars containing test specimens treated with the 0.01 or 0.1 percent neem solutions were alive. This result suggests that a higher concentration of neem could give a higher level of protection against termites.

Table 2 shows the data for mean mass losses caused by the decay fungi *C. olivacea*, *F. lilacino-gilva*, *S. lacrymans*, *G. abietinum*, and *P. tephropora*. The treatments with water and white spirit had no clear inhibitory effect upon the growth of all brown-rotting fungi, because mean mass losses were high, in the range 35 to 65 percent. White-rotting fungi usually cause lower mass losses in softwoods than brown-rotting fungi in bioassays, and this occurred with *P. tephropora*, where the mean mass loss was 19 percent (Table 2). These results with the untreated controls demonstrate the viability of the bioassay methods used.

The treatment with CCA gave the best protection against all test fungi, because mean mass losses were below the toxic threshold of 3 percent (Table 2). In contrast, test specimens treated with 0.01, 0.1, 1.0, 2.5 or 5.0 percent (m/m) neem in white spirit were heavily decayed and generally incurred similar mean mass losses to the untreated controls at all concentrations examined. Even the highest concentration of neem (5.0%) failed to protect *P. radiata* (Table 2). There was a small trend in unleached blocks for lower mean mass losses with higher treatment concentrations against *C. olivacea* and *S. lacrymans* (Table 2). Nevertheless, the treatments investigated had a negligible or minor ability to provide protection against fungal attack. *P. radiata* samples treated with 1.0, 2.5 and 5.0 percent neem concentrations were also examined after artificial leaching. The mean mass losses in the majority of leached specimens were not significantly different from, or significantly higher than ($P > 0.05$), the corresponding test specimens without leaching, although there were some minor exceptions against *G. abietinum* and *P. tephropora* (Table 2). Note that the distribution shows normality, and equivalence of variances was observed ($P > 0.05$), validating the results from ANOVA.

Discussion

In contrast to previous reports in the literature of neem oil being a promising source of biocides, the results from this research suggest that neem oil is ineffective as a wood preservative against termites and decay fungi at the treatment levels examined. Our studies were conducted by impregnating softwood specimens and followed the standard procedures used for evaluating the efficacy of new wood preservatives in Australia (AWPC 2007). These are quite rigorous tests, where test specimens are vacuum oven-dried (H2 tests) or leached and vacuum oven-dried (H3 tests) to simulate long-term ageing in service, a process that may have removed active neem components. These results corroborate the findings by Grace and Yates (1992), who found lower activity against termites (*C. formosanus*) compared with other insect species in filter paper trials of a commercial neem-based insecticide. Additionally, Paes et al. (2012) showed that formulations based on alcoholic solutions of neem failed to protect impregnated wooden stakes when exposed to decay fungi in soil contact.

Despite the poor results for neem oil, there may still be merit in optimizing formulations to use it as an eco-friendly wood preservative. It is worth noting that our results showed that with neem oil at 1.0 percent, attack by termites was

Table 2.—Efficacy of treatments against wood decay fungi.

Treatment ^a	Mean ± SD mass loss (%) ^b				
	<i>Coniophora olivacea</i>	<i>Fomitopsis lilacino-gilva</i>	<i>Serpula lacrymans</i>	<i>Gloeophyllum abietinum</i>	<i>Perenniporia tephropora</i>
Water	65.4 ± 1.0 A	35.0 ± 5.8 E	38.2 ± 8.2 A	52.4 ± 3.5 B	19.5 ± 4.7 A
White spirit alone	64.4 ± 2.2 A	35.6 ± 6.1 E	43.3 ± 2.8 A	54.9 ± 5.7 B	19.3 ± 5.8 A
CCA	1.3 ± 1.3 D	-0.3 ± 0.3 F	0.9 ± 0.5 D	0.1 ± 0.2 D	0.1 ± 0.2 E
White spirit with neem, % (m/m)					
Neem, 0.01	65.0 ± 2.5 A	37.7 ± 8.9 E	39.8 ± 4.4 A	51.9 ± 12.0 BC	18.5 ± 3.3 B
Neem, 0.1	60.1 ± 5.2 B	43.4 ± 2.9 C	27.2 ± 4.8 B	52.7 ± 9.0 B	16.6 ± 4.1 C
Neem, 1.0	60.0 ± 3.8 B	45.4 ± 3.8 B	27.4 ± 5.3 B	53.9 ± 6.6 B	21.0 ± 4.5 A
Neem, 2.5	59.7 ± 4.5 B	45.3 ± 3.9 B	26.4 ± 3.5 B	57.9 ± 4.7 A	20.5 ± 2.6 A
Neem, 5.0	55.7 ± 4.0 C	40.3 ± 4.5 D	19.8 ± 6.6 C	60.7 ± 5.7 A	20.3 ± 3.6 A
Neem L, 1.0	61.2 ± 4.9 B	48.1 ± 6.0 A	30.9 ± 4.2 B	48.0 ± 9.7 C	12.5 ± 2.4 D
Neem L, 2.5	62.8 ± 2.8 B	51.1 ± 3.2 A	27.2 ± 8.3 B	53.2 ± 4.7 B	16.6 ± 3.7 C
Neem L, 5.0	61.5 ± 5.0 B	45.5 ± 3.8 B	28.3 ± 4.5 B	52.2 ± 6.8 B	19.2 ± 4.1 AB

^a CCA = copper-chromium-arsenic; L = leached.

^b The mass losses are the results after 12 weeks of incubation and are adjusted for sterile controls. Within each column, means followed by the same letter are not significantly different (Tukey test at 5%).

reduced, and the termites had died by the end of the bioassay. This result suggests that a higher concentration of neem oil could give a better level of protection against termites, possibly leading to suitable levels of preservation. Other formulations of neem may be more effective, or neem may offer synergies with cobioicides (Antwi-Boasiako and Damoah 2010). For instance, Venmalar and Nagaveni (2005) formulated neem with copper and obtained control against white- and brown-rotting fungi. Certainly, further studies are warranted to obtain a better understanding of neem oil's biocidal potential.

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