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ARTICLE

Evaluation of Tung Oil (*Vernicia fordii* (Hemsl.)) for Controlling Termites

Hangtian Li[1] Siying Li[1] Hui Lu[2] Jingjing Zhang[1] Xi Yang[2] Dayu Zhang[2] Yike Zhang[2] Yongjian Xie[2*]

1. Institute of Termite Control of Yuhang, Hangzhou, 311100, China
2. College of Advanced Agricultural Sciences, Zhejiang A&F University, Hangzhou 311300, China

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ABSTRACT

In worldwide, the use of chemical pesticides to protect wood has been greatly restricted. In recent years, a large number of researchers devoted to the search for natural, safe and non-polluting bioactive chemical compounds from plants as an alternative to synthetic organic chemical preservative. In Chinese folk, tung oil can be used as paint for wooden furniture to protect them from pests. This study aimed to evaluate the chemical compositions of raw and heated tung oil and their activity against termite. In choice bioassays, weight loss of wood treated with 5% raw or heated tung oil after 4 weeks was significantly less than that of the control group. In no-choice bioassays, there was a significant difference in termite survival and wood weight loss on raw and heated tung oil-treated wood. When tung oil-treatment concentrations increased to 5%, wood weight loss was less than 10%. There was no significant difference in termite survival and wood weight loss between raw and heated tung oil-treated wood. Survival of termites in both tung oil wood treatments was significantly lower than that in the starvation control after 4 weeks. Raw and heated tung oil significantly improved the resistance of pine wood to termites, and have the potential for the development of natural wood preservatives.

1. Introduction

Coptotermes formosanus (Shiraki) is an economically important insect pest that damages wooden structures [1]. Rust and Su [2] reported that the worldwide economic loss caused by this species is at least $40 billion annually. Ammonium copper quaternary (ACQ), copper citrate (CC), and copper chrome arsenic (CCA) have been used to treat wood and prevent termite damage, but these compounds can affect human health and the environment [3]. Therefore, there is interest in more environment-friendly, convenient, and effective wood preservatives. Some wood

*Corresponding Author:
Yongjian Xie,
College of Advanced Agricultural Sciences, Zhejiang A&F University, Hangzhou 311300, China;
Email: yjxie@zafu.edu.cn

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is naturally resistant to termites [1,4], due to their active compounds that act as toxicants [2,5]. These plant compounds include tannins [4], flavonoids [6,7], alkaloids [8,9], quinones [10-12], terpinoids [13-15], and resins [16-18]. Many studies have documented that active compounds possess antitermitic properties [13,14,19].

Tung tree, *Vernicia fordii* (Hemsl), native to China, is widely cultivated in China and other countries for its industrial values [20,21]. *V. fordii* is mainly distributed in Sichuan, Hubei, Guizhou, and Hunan provinces and Chongqing municipality, which is the main production base of tung oil in China [22,23]. Tung oil, extracted from seeds, shows rapid drying, insulation, anticorrosion, acid, and alkali resistance [24]. These properties make tung oil a valuable additive in paints, varnishes, and other coatings and finishes [25]. In addition, tung tree seeds, roots, flowers, and leaves are widely used in folk medicine. Tung oil can treat burns, scalds, and cold injuries [26]. Termites are important pests of wooden structures. Many essential oils have antitermitic activities, including oils from *Lippia spp*. [27], *Artemisia absinthium* [28], *Lippia sidoides* [29], *Chamaecyparis formosensis* [30], *Liquidambar orientalis*, and *Valeriana wallichii* [31]. Hutchins [32] reported that *Aleurites fordii* wood and meal extracts had antitermitic toxicity. Tung seed is considered a good wood preservative in Chinese and is used to treat indoor and outdoor wooden furniture to protect them against insect pests and wood-rot fungi. However, the protection against termite damage offered by tung oil treatment of wood is unclear. Therefore, the purpose of this study was to compare the chemical composition of raw and heated tung oil and to determine the toxicity of raw and heated tung oil to termites.

2. Materials and Methods

2.1 Chemicals

The fatty acid methyl ester standard was obtained from Dr. Ehrenstorfer GmbH. Methanol and isooctane were purchased from Sigma Aldrich Inc, St. Louis, MO, USA. Potassium hydroxide and sodium bisulfate were obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

2.2 Tung Oil

Tung oil can be divided into raw tung oil and heated tung oil. Raw tung oil was obtained from tung seeds using a hydraulic press at room temperature, with characters of golden yellow and strong penetration. Heated tung oil was made by heating the raw tung oil to 180 °C, brown color, low transparency, and high density. The oils were supplied by the Yiyousheng home exclusive store, Tmall.com. The colors of the raw and heated tung oil were light yellow brown and dark brown, respectively.

2.3 Esterification of Tung Oil Fatty Acids

The esterification of tung oil fatty acid was pretreated according to ISO 5509-2000. A 0.010 g oil sample and 1 mL of 1 M KOH-methanol solution were added to a round bottom flask and heated in a water bath at 75 °C for 10 min. After cooling to room temperature, 1 mL of C15:0 was added to the mixture. After transfer to a 250 mL separating funnel, 20 mL of n-heptane and 20 mL of water were added to stratify the mixture, and then we collected the ester layer and dried it with anhydrous sodium sulfate.

2.4 Gas Chromatography-mass Spectrometry (GC-MS)

GC-MS analysis was performed on a gas chromatograph Agilent 6890A interfaced with an Agilent 5975C mass spectrometer (Agilent Technologies (China) Co., Ltd.). An HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The column temperature was programmed to rise from 50 °C to 280 °C at a rate of 10 °C/min. The carrier gas was helium with a flow rate of 1 mL/min. MS readings were taken at 70 eV and a mass range of 15-500. Identification of compounds of the tung oil was based on standard samples, and NIST11.LIB (National Institute of Standards and Technology) was used for qualitative analysis.

2.5 Oil Treatment

The vacuum-soak impregnation method by Nakayama and Osbrink [33] was used with slight modifications. Masson pine (*Pinus massoniana*) was cut into wood pieces that were 23 mm (length) × 14 mm (width) × 9 mm (thickness). These were sterilized in an oven at 130 °C for 24 h and weighed, and the weights were recorded. The wood was placed into a closed pressure chamber with vacuum level of –0.098 MPa for 30 min. Then, the wood specimens were immersed with different concentrations of both raw and heated tung oil-acetone mixtures, soaked for 30 min, and removed. Excess liquid on the wood surface was dried with paper, and the blocks were dried in a vacuum oven at 60 °C for 24 h. This procedure was used to remove the acetone fraction. The oil content of the oil-treated wood (%, w/w) was determined based on the gain in weight of the untreated wood.

2.6 Termites

The colonies of *Coptotermes formosanus* Shiraki were collected from Tiantong Mountain in Ningbo, Zhejiang province. The termites were reared on masson pine blocks (200 mm ×35 mm×20 mm), water soaked in plastic containers (460 mm × 36 mm × 28 mm) at 26 ± 1 °C and 80 ± 5% RH. Termites were identified using keys for soldier identification from Scheffrahn and Su [34].

2.7 Termite Resistance Test

The choice and no-choice bioassay methods of Nakayama and Osbrink [33] were used to evaluate termite resistance of pine wood treated with tung oil.

2.7.1 Choice Bioassays

The wood specimens tested contained raw and processed tung oil contents of 1.25%, 2.5%, 5.0%, 10.0%, 20.0%, and 40.0% (w/w). The termite control group was exposed to pine treated only with acetone. Two blocks of wood were placed on a sand surface on both sides of the bottom of the container (65 mm (diameter) × 90 mm (high)) (Figure 1). A total of 150 workers and 5 soldiers were placed in the container. Three replicates of the oil treatments were performed. All the testing containers were placed in a conditioning room at 26 ± 1 °C, and after 4 weeks, the wood specimens were dried and reweighed to determine wood weight loss. ASTM [35] ratings were determined for each wood block over the same period. The ASTM rating had a scale of 10-0 with 10 being sound wood with only surface nibbles permitted, 9 light attack, 7 moderate attack with penetration, 4 heavy attack, and 0 failure.

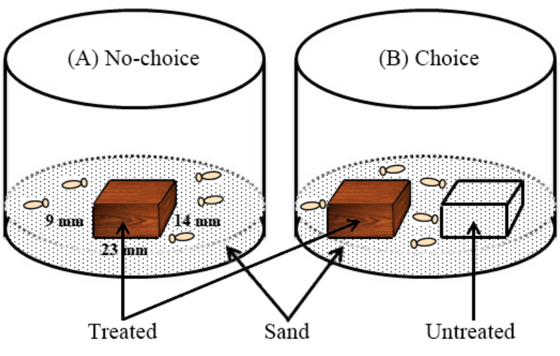

Figure 1. A test unit for evaluating termite resistance to wood treated in the laboratory. A: No-choice; B: Choice

2.7.2 No-choice Bioassays

The experimental method was the same as above, but only one piece of treated wood was placed in the middle of the container.

2.8 Statistical Analysis

For choice bioassays, Student's t test was used to compare wood specimen weight loss of untreated with treated wood specimens after 4 weeks. Differences in survival, wood specimen weight loss, and percent weight loss of wood were compared using ANOVA followed by the Student-Newman-Keuls test ($p < 0.05$).

3. Results

3.1 Chemical Compositions of Tung Oil

GC-MS analysis results of the raw and heated tung oil are shown in Table 1. The main components of raw tung oil were α-eleostearic acid (69.02%), oleic acid (12.28%), and linoleic acid (8.93%). α-eleostearic acid (65.31%), oleic acid (15.52%), and linoleic acid (9.44%) were the major components of the heated tung oil.

Table 1. Chemical composition (%) of raw and heated tung oil

No.	RI[a]	Compounds	Raw	Heated
1	1973	Palmitic acid	3.24	4.16
2	2123	Linoleic acid	8.93	9.44
3	2134	Oleic acid	12.28	15.52
4	2179	Stearic Acid	2.58	3.27
5	2482	α-eleostearic acid	69.02	65.31

[a]RI, retention index calculated on the HP-5MS column relative to C_8-C_{28} n-alkanes.

3.2 Choice Bioassays

There was significantly less wood weight loss of the blocks with 5% raw and heated tung oil after 4 weeks (Table 2) than in the untreated blocks. There was high termite survival in both tung oil treatments at 4 weeks. There were significant differences in termite survival and wood weight loss ($F = 5.406$, $df = 6, 14$, $P < 0.004$; $F = 16.374$, $df = 6, 14$, $P < 0.0001$) on raw and heated tung oil-treated wood, respectively. These results were corroborated using the ASTM rating system (Table 3).

Table 2. Comparison of weight loss between wood specimens treated with both raw and heated tung oil 4 weeks after exposure to *C. formosanus* in the feeding choice bioassays.

Treatment % (w/w)	Survival, %	Weight loss (mg) and t statistics (mean ± SD)			
		Treated	Untreated	t	*P*
raw tung oil					
40	78.0 ± 3.1d	36.7 ± 29.1	271.8 ± 37.2	8.63	0.001
20	89.8 ± 5.7ab	61.3 ± 27.8	249.4 ± 38.3	6.89	0.002
10	81.6 ± 3.0cd	85.1 ± 34.0	247.3 ± 19.7	7.16	0.002
5	81.1 ± 3.8cd	102.0 ± 60.9	243.1 ± 23.1	3.75	0.02
2.5	82.2 ± 2.7cd	191.5 ± 50.7	221.1 ± 58.9	0.66	0.55
1.25	90.4 ± 2.3ab	278.2 ± 62.2	236.3 ± 18.6	1.12	0.33
heated tung oil					
40	80.2 ± 1.1cd	33.8 ± 11.2	298.5 ± 23.9	17.34	0.0001
20	87.8 ± 2.0abc	50.1 ± 12.1	321.0 ± 67.7	6.83	0.002
10	82.0 ± 2.4cd	55.8 ± 21.5	279.2 ± 64.3	5.70	0.005
5	93.8 ± 1.7a	73.8 ± 18.0	271.7 ± 54.8	5.94	0.004
2.5	81.3 ± 2.4cd	206.2 ± 55.1	242.2 ± 40.3	0.91	0.41
1.25	90.7 ± 2.9ab	237.7 ± 28.7	257.5 ± 52.0	0.58	0.60
Control					
Acetone	85.3 ± 3.1bcd	259.4 ± 32.1	253.0 ± 35.8	0.23	0.83
Untreated	84.7 ± 2.4bcd	212.5 ± 26.9	242.5 ± 107.9	0.47	0.67

Table 3. Comparison of ASTM ratings between wood specimens treated with both raw and heated tung oil and untreated wood specimens 4 weeks after exposure to *C. formosanus* in a feeding choice bioassay.

Treatment % (w/w)	ASTM rating of wood and t statistics (mean ± SD)[a]			
	Treated	Untreated	t	*P*
raw tung oil				
40	9.7 ± 0.6	0.0 ± 0.0	29.00	0.001
20	9.3 ± 0.6	0.0 ± 0.0	28.00	0.001
10	8.8 ± 0.3	0.0 ± 0.0	53.00	0.0001
5	7.7 ± 1.2	0.0 ± 0.0	11.5	0.007
2.5	4.7 ± 1.2	3.7 ± 1.5	0.655	0.58
1.25	4.0 ± 1.0	3.3 ± 0.6	1.09	0.423
heated tung oil				
40	9.8 ± 0.3	0.0 ± 0.0	59.00	0.0001
20	9.5 ± 0.5	0.0 ± 0.0	32.91	0.001
10	9.3 ± 0.0	0.0 ± 0.0	28.00	0.001
5	8.7 ± 1.5	0.0 ± 0.0	9.827	0.01
2.5	4.0 ± 1.0	3.7 ± 1.2	1.09	0.423
1.25	5.0 ± 1.7	4.0 ± 1.5	2.01	0.184
Control				
Acetone	4.3 ± 0.6	5.0 ± 1.7	0.555	0.635
Untreated	4.3 ± 1.5	4.6 ± 2.1	0.229	0.84

[a] ASTM scale of 10-0 with 10 being sound, surface nibbles permitted, 9 having light attack, 7 moderate attack with penetration, 4 with heavy attack, and 0 failure.

3.3 No-choice Bioassays

There were significant differences in termite survival (F = 72.87; df = 6, 14, P < 0.0001; F = 39.83, df = 1, 97, P < 0.0001) and wood weight loss (F = 44.32, df = 4, 16, P < 0.0001; df = 4, 16, F = 50.97, P < 0.0001) on raw and heated tung oil-treated wood, respectively. Survival and wood weight loss in both tung oil-treatments decreased as the concentration increased (Table 4). When the raw and treated tung oil concentrations increased to 5.0%, percent weight loss of wood was less than 10% (Table 4). ASTM ratings were highest when both raw and heated tung oil content was 40% (Table 5). There were no significant differences in termite survival (F = 0.257; df = 5, 36; P = 0.932) and wood specimen weight loss (F = 0.403; df = 5, 36; P = 0.842) on raw and heated tung oil-treated wood.

Table 4. Comparison of termite survival and wood specimens weight loss between wood specimens treated with both raw and heated tung oil 4 weeks after exposure to *C. formosanus* in the feeding no-choice bioassays.

Treatment % (w/w)	mean ± SD[a]		
	Survival, %	Total wt loss, mg	Percent weight loss, %
raw tung oil			
40	26.7 ± 2.4e	36.5 ± 4.5d	1.9 ± 0.2d
20	45.1 ± 2.1d	96.5 ± 20.5bc	5.4 ± 1.1cd
10	47.3 ± 1.3cd	138.8 ± 15.0b	6.9 ± 0.8cd
5	48.7 ± 6.6cd	168.4 ± 28.4b	10.2 ± 1.7c
2.5	55.3 ± 2.7bcd	256.1 ± 29.6a	17.3 ± 2.0b
1.25	63.1 ± 5.2b	281.7 ± 22.8a	17.0 ± 1.4b
heated tung oil			
40	27.8 ± 2.0e	54.3 ± 23.8cd	2.6 ± 1.2d
20	49.1 ± 5.6cd	105.8 ± 12.4bc	6.1 ± 0.7cd
10	51.8 ± 6.0cd	141.4 ± 6.1b	8.4 ± 0.4c
5	55.3 ± 2.7bcd	163.4 ± 9.6b	8.9 ± 0.5c
2.5	58.4± 2.8bc	280.9 ± 21.2a	18.2 ± 1.4b
1.25	65.8 ± 8.4b	294.7 ± 30.0a	23.5 ± 2.4a
Control			
Acetone	84.7 ± 4.8a	284.4 ± 67.0a	24.5 ± 5.7a
Untreated	86.2 ± 2.8a	312.2 ± 45.7a	24.6 ± 3.6a
No food	83.1 ± 5.4a	-	-

[a] Means ± SD followed by the different letter within a column are significantly different ($P < 0.05$; using Student-Newman-Keuls test).

Table 5. Comparison of ASTM ratings between wood specimens treated with both raw and heated tung oil and untreated wood specimens 4 weeks after exposure to *C. formosanus* in a feeding no-choice bioassay.

Treatment % (w/w)	ASTM rating of wood (mean ± SD)[a]	
	raw tung oil	heated tung oil
40	9.7 ± 0.6a	9.3 ± 0.6A
20	8.3 ± 1.5ab	8.0 ± 1.0B
10	6.7 ± 2.5b	6.3 ± 0.6C
5	4.6 ± 0.6c	4.3 ± 1.5D
2.5	0.0 ± 0.0d	0.0 ± 0.0E
1.25	0.0 ± 0.0d	0.0 ± 0.0E
Acetone	0.0 ± 0.0d	0.0 ± 0.0E
Untreated	0.0 ± 0.0d	0.0 ± 0.0E

[a] ASTM scale of 10-0 with 10 being sound, surface nibbles permitted, 9 having light attack, 7 moderate attack with penetration, 4 with heavy attack, and 0 failure.

4. Discussion

Biodegradation of wood by fungi and termites is a serious problem for wooden structures world-wide [15]. The alkaline copper quat (ACQ) [36], boron-fluorine-chromium-arsenic (BFCA salts) [37], copper azole (CA) [38], copper chrome arsenate (CCA) [39], chlorotalonil (CTL) [36], copper- and zinc-salicylate [40], quaternary ammonium compounds (QACs) [41], siloxane [42], sodium fluoride (NaF) [43-45], and zinc borate [46] have been used to protect wood against termite damage. Besides these, nanoparticles from zinc oxide (ZnO) [47,48], CuO and B_2O_3 [49], and magnesium fluoride (MgF_2) [50] have provided promising levels of protection. Wood preservatives should ideally be environmentally friendly; thus, there is interest in safer alternative wood protection methods. Secondary plant compounds in some species of wood play a major role in the protection of wood against termite attack [51-55]. Here, we evaluated the chemical composition of tung oil and tested their activity against termite.

The major components of tung oil studied in this paper are similar to previous reports on this species. Raw tung oil, extracted from seeds, contains 60%~80% α-eleostearic acid and is used for the production of biodiesel, dyes, inks, and resins [20,56,57]. The α-eleostearic acid has a potential role in human health products such as those with antibacterial [58], antitumor [59], anti-neuroinflammatory [26], antioxidative [60], and antiobesity [61] activity. It also provides a reference on the safety of tung oil used in commercial applications. In addition, α-eleostearic acid can prevent *Anthonomus grandis* damage to cotton bolls [62]. Thus, high anti-termitic properties of tung oil could be due to the high toxicity of its major component α-eleostearic acid. Similarly, Xie et al. [15] showed that the major constituents of *Syzgium aromaticum*, eugenol, against *Reticulitermes chinensis* for 1 d, 3 d and 5 d had LC_{50} values of 38.0 μg/g, 12.1 μg/g, and 9.2 μg/g, respectively. Yang et al. [63] also demonstrated that the high toxicity of *Mentha spicata* EO against *Reticulitermes dabieshanensis* was attributed to its major components, carvone, limonene and dihydrocarvone.

In choice experiments, there was no significant difference in termite survival and wood weight loss between raw and heated tung oil-treated wood. Therefore, raw tung oil can be directly used for wood preservation to reduce the processing cost of heated tung oil. Under starvation conditions for 4 weeks, termite survival was significantly higher than that of raw and heated tung oil-treated wood, indicating that raw and heated tung oil were feeding deterrents for *C. formosanus*. Similar results were obtained showing that tung wood and meal have antitermitic properties [32]. In addition, Nakayama and Osbrink [33] showed that *A. moluccana* oil-treated wood was resistant to *C. formosanus*. Taylor et al. [64] observed that removal of the methanol-soluble heartwood components of *Thuja plicata* and *Chamaecyparis nootkatensis* reduced their resistance to termite attack. Syofund et al. [65] observed that wood extracts used as preservatives improved the resistance of less durable wood to termite attack by 50% compared to the controls. Brocco et al. [66] found that ethanol extracts of *Tectona grandis* heartwood increased the resistance and mortality against *Nasutitermes corniger* in both choice and no-choice tests. Similar results were reported by Hassan et al. [67], who showed that *Tectona grandis* and *Cedrus deodara* extracts imparted termite resistance to non-durable wood species.

5. Conclusions

In this study, we investigated the resistance of pine wood (*P. massoniana*), treated with raw and heated tung oil, to the termite, *C. formosanus*. Our study demonstrated that both tung oil treatments significantly improved the resistance of pine wood to termites. When both tung oil-treated concentrations were 5.0%, the weight loss of wood was less than 10%. There was no significant difference in termite survival rate and wood weight loss between the two tung oil treatments. Therefore, raw tung oil can be directly used for wood preservation to reduce the processing cost of heated tung oil. In addition, the use of tung oil for wood preservative treatment can expand the demand for tung oil and increase local economic income.

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Conflict of Interest

There is no conflict of interest.

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