

TERMITICIDAL PROPERTIES OF SOME WOOD AND BARK EXTRACTS USED AS WOOD PRESERVATIVES

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The feasibility of using naturally extracted solutions as wood preservative chemical was tested. Extracts extracted from mimosa (*Acacia mollissima* Willd.), quebracho (*Shinopsis lorentzii* Griseb.), and *Pinus brutia* Ten. bark were used to treat sapwood of Scotch pine (*Pinus sylvestris* L.), beech (*Fagus orientalis* L.), and poplar (*Populus tremula* L.) at two different retention levels (6% and 12% weight/weight) against the subterranean termite *Reticulitermes grassei* Clement (Blattodea: Rhinotermitidae). The lowest mass loss and highest termite mortality rates were recorded for mimosa and quebracho extract treated woods at the 12% concentration level. Pine bark extract seemed to be ineffective as a wood preservative chemical even at the highest retention level. The results suggest that mimosa and quebracho extracts can be utilized as an environmentally-sound alternative wood preservative chemicals for indoor applications against *Reticulitermes grassei*.

Keywords: Bark extract; Wood extract; Termite resistance; *Reticulitermes grassei*; Mimosa; Quebracho

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INTRODUCTION

Subterranean termites are an economically important timber pest worldwide. They cause extensive damage to lignocellulosic material in temperate and tropical climates (Ragon *et al.* 2008).

Protection of wood and wood-based products against biological agents (*e.g.* termites, insects) requires various synthetic chemicals worldwide. However, synthetic chemicals create environmental problems and negatively affect many beneficial insects and organisms (Abudulai *et al.* 2001). Extractives isolated from naturally resistant heartwood and some plant species may provide alternatives in pest control because of their bioactive chemicals. In addition, plant extractives are biodegradable and they seem to help resolve environmental problems caused by synthetic pesticides (Kim *et al.* 2006; Ahmed *et al.* 2007 and Rodrigues *et al.* 2010).

The utilization of natural extracts for pest control has long been studied in the field of wood preservation. It has been reported that the extractives retain repellency and toxicity against some termite species (Rudman and Gay 1961; Carter and de Camargo 1983; Ragon *et al.* 2008; Manzoor *et al.* 2011). Hashimoto *et al.* (1997) correlated the lower extractive content with reduced termite resistance.

Termiticidal resistance of wood species varies depending on many factors including natural durability, density, and extractive types and quantities (Carter and

Smythe 1974; Akhtar 1981). Extractives from naturally durable species can be isolated and utilized to increase the durability of non-durable and non-refractory wood species (Schultz and Nicholas 2000; Thevenon *et al.* 2001).

Similar studies have been reported indicating that some wood, plant, seed, and fruit extracts were utilized to increase natural durability of wood species such as bald cypress (*Taxodium distichum*) heartwood extract (Scheffrahn *et al.* 1988), southern catalpa (*Catalpa bignonoides*) heartwood extract (McDaniel 1992), red louro (*Sextonia rubra*) wood extract (Rodrigues *et al.* 2011), cinnamon (*Cinnamomum cassia*) bark extract (Lin *et al.* 2007), pepper (*Piper sarmentosum*) extract (Chieng *et al.* 2008), water pepper (*Polygonum hydropiper*) leaf extracts (Rehman *et al.* 2005), and birbira (*Milletia ferruginea*) seed extract (Jembere *et al.* 2005).

This paper compares the effectiveness of extractives obtained via wood and bark extraction against termite activity for woods used for indoor applications. The focus here is on indoor applications because no leaching test was performed in the study. Though termites live outdoors, they can easily reach indoor environments via crawl spaces under houses.

EXPERIMENTAL

Wood Material

Specimens were cut 20 × 20 × 10 mm (radial × tangential × longitudinal directions) in size from randomly selected first grade Scotch pine (*Pinus sylvestris* L.), beech (*Fagus orientalis* L.), and poplar (*Populus tremula* L.) sapwood lumber showing no spiral grain, knots, splits, or discoloration with minimum variation in density. The specimens were conditioned at 20 ± 2 °C and 65 ± 3% relative humidity until they reached stable weight before the subsequent treatments. The oven-dried densities of species were 0.48 gr/cm³, 0.74 gr/cm³, and 0.43 gr/cm³ for Scotch pine, beech, and poplar, respectively. A total of 63 specimens were prepared according to 3 × 3 × 2 experimental design, with 18 specimens (excluding controls) for each wood species, extractive chemical, and retention level, respectively.

Extractive Solutions

Mimosa (*Acacia mollissima*) and quebracho (*Shinopsis lorentzii*) extractives were obtained as fine powder from nearby leather plants in Turkey. Pine barks (*Pinus brutia*) were collected from a nearby forest in Duzce. Air-dried samples then were oven-dried at 100 °C before coarse grinding. The coarse-ground bark particles were ground further with a laboratory scale Wiley mill to obtain fine particles to pass through a 60-mesh screen for the extraction process. All three powder extracts were mixed with distilled water at 6% and 12% by weight and extracted on a hot plate with a magnetic stirrer at 100 °C for 20 minutes. After cooling, the solution was filtrated for subsequent treatment.

Treatment

A vacuum treatment was used for impregnation. The wood blocks were placed into cylindrical containers according to their intended treatments. After adding extractive solutions, a vacuum of 6.10⁻³ MPa was applied for 20 minutes using glass desiccators. At the end of each treatment, the specimens were removed at ambient atmospheric pressure.

The treated wood blocks were immediately weighed to determine gross solution uptake. Retention of extractive material was calculated (kg/m^3) as follows,

$$R = \frac{(M_1 - M_0) \times C}{V} \times 10 \text{ kg/m}^3 \quad (1)$$

In this equation, M_0 is the weight before treatment (g), M_1 is the weight after treatment (g), C is the concentration of solutions, and V is the volume of wood blocks (m^3).

The treated specimens were stored at 20 ± 2 °C and $65 \pm 3\%$ relative humidity for two weeks before the subsequent termite resistance test.

Termite Resistance Test

Termite resistance tests were conducted in the Wood Protection Laboratory, Forest Products Department of INIA-CIFOR, Madrid, Spain. Wood specimens were exposed to *Reticulitermes grassei* Clement (Blattodea: Rhinotermitidae) according to the EN 117 (2005) procedure with minor modification in specimen size. A test specimen was placed at the center of the cylindrical test container. A total of 100 workers were introduced into each test container along with 3 soldiers and 3 nymphs. Three replicates per treatment were assayed against termites. The test containers were kept at 28 °C and 85% RH for eight weeks. At the end of the exposure period, the exposed wood blocks were weighed to the nearest 0.01 g to determine the post-exposure weight. The percent mass loss (ML) and termite mortalities (TM) were calculated as follows:

$$\text{Mass loss (\%)} = [(M_1 - M_2) / M_1] \times 100 \quad (2)$$

In this equation, M_1 is the weight of specimens before termite test (g), and M_2 is the post-exposure weight (g). Termite mortality was calculated as,

$$\text{Termite mortality (\%)} = [(T_1 - T_2) / T_1] \times 100 \quad (3)$$

where T_1 is the number of termites alive at the beginning of the test, and T_2 is the number of termites alive at the end of the test

In addition, the attack by termites was rated based on visual observation. The following scale was used; (0) no attack, (1) attempted attack, (2) light attack, (3) medium attack, and (4) heavy attack.

Chemical Analyses of Extracted Solutions

Extracted solutions were analyzed for organic compounds using a Perkin Elmer Series 200 High Pressure Liquid Chromatogram (HPLC) with a UV detector and Phenomenex Kromasil C-18 column and run isocratically with 2% formic acid in DI water and acetonitrile mobile phases. The flow rate was 1 mL/min. The sample solutions were filtered through a 20 μm PTFE filter and injected manually (20 μL). The total run time was 40 minutes. The compounds were identified via matching against standards previously prepared at 3 concentration levels.

Statistical Analyses

An analysis of variance (ANOVA) test was applied to evaluate the effects of wood species, extract species, and concentration levels using SPSS software (SPSS 19,

2010). Significant differences between variables were determined by the Duncan test at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Retention

Table 1 shows the mean extract retentions of treated wood blocks as calculated from solution uptake. According to the results, it is clear that the vacuum method successfully delivered extracted solution into the solid wood blocks.

Table 1. Mean Extractive Retentions (kg/m^3) in Treated Wood Blocks as Calculated by Solution Uptake (mean of three replicates, numbers in parentheses are standard deviations)

Destination species	Source species	Concentration (%)	Retention (kg/m^3)
Scotch pine	Mimosa	6	35.19 (± 6.88) cd
		12	91.03 (± 0.89) g
	Quebracho	6	28.21 (± 1.27) ab
		12	95.90 (± 3.75) h
	Pine bark	6	26.08 (± 0.74) a
		12	90.54 (± 4.02) g
Beech	Mimosa	6	38.81 (± 3.75) d
		12	85.72 (± 2.08) f
	Quebracho	6	33.87 (± 1.36) c
		12	84.77 (± 1.47) f
	Pine bark	6	32.34 (± 2.34) bc
		12	84.57 (± 2.90) f
Poplar	Mimosa	6	50.16 (± 0.67) e
		12	101.41 (± 2.10) i
	Quebracho	6	35.01 (± 0.85) cd
		12	105.69 (± 1.13) j
	Pine bark	6	31.92 (± 0.41) bc
		12	102.18 (± 0.78) i

*Means within each column and factor followed by the same letter are not significantly different

Based on these findings, retention values were dependent on solution concentrations, extractive species, and wood species used ($p < 0.05$). As expected, destination species with lower densities resulted in higher retention values. The highest retention were calculated for poplar wood at the 12% concentration level as 105.69 kg/m^3 , 102.18 kg/m^3 , and 101.41 kg/m^3 for quebracho, pine bark, and mimosa extracts, respectively.

Termite Resistance

The mean mass losses of untreated control specimens were recorded as 22.08%, 14.18%, and 21.15% for Scotch pine, beech, and poplar, respectively, indicating that *Reticulitermes grassei* was active under the test conditions. Relatively lower mass losses for beech wood could be attributed to its density. The hardness of wood effects termite chewing ability, resulting in relatively lower mass losses (Behr *et al.* 1972). The

extractive compounds present in wood also play an important role on termite consumption rates, which affects mass losses (Yazaki and Hillis 1977).

Table 2 demonstrates mass loss, termite mortality, and visual termite attack ratings of Scotch pine wood treated with all three extracts. While the 6% and 12% retention levels of mimosa and quebracho extracts provided significant reductions in mass losses, the pine bark extract failed to protect the Scotch pine wood against *Reticulitermes grassei*. According to the statistical analyses, lower retention levels (6%) of all extracts did not result in significant reductions in mass losses when compared the untreated controls. Termite mortality (TM) values showed a similar trend with mass loss data, indicating that the highest retentions (12%) of mimosa and quebracho caused the highest mortalities. Visual ratings also supported the finding above. The highest retentions of quebracho and mimosa treated Scotch pine specimens were only rated as 0.7 and 0.3, respectively, while the control and pine bark extract treated specimens were totally destroyed by termites (Fig. 1).

Table 2. Mean Mass Loss, Termite Mortality, and Termite Attack Ratings of Scotch Pine Samples

Destination species	Source species	Concentration (%)	Mass loss (%)	Mortality (%)	Termite attack
Scotch pine	Control	-	22.08 b ¹	10.67 a	4.0
	Mimosa	6	22.34 b	29.00 a	4.0
		12	5.54 a	99.67 b	0.7
	Quebracho	6	18.61 b	33.67 a	4.0
		12	3.81 a	93.33 b	0.3
	Pine bark	6	21.71 b	36.00 a	3.7
		12	22.62 b	24.67 a	4.0

¹ mean within each column followed by the same letter are not significantly different

As shown in Table 3, both concentrations of pine bark extract were not effective to protect mass loss or increase termite mortality for beech wood.

Table 3. Mean Mass Loss, Termite Mortality, and Termite Attack Ratings of Beech Samples

Destination species	Source species	Concentration (%)	Mass loss (%)	Mortality (%)	Termite attack
Beech	Control	-	14.18 c ¹	37.33 a	4.0
	Mimosa	6	10.72 b	40.33 a	3.7
		12	7.18 a	95.00 b	0.7
	Quebracho	6	11.76 bc	34.00 a	4.0
		12	6.86 a	96.33 b	1.3
	Pine bark	6	11.45 bc	28.00 a	4.0
		12	9.98 ab	30.00 a	4.0

¹ mean within each column followed by the same letter are not significantly different

It can be concluded that a two-fold increase in mimosa and quebracho retentions significantly helped to reduce mass losses or especially to increase mortality rates. One would expect further reductions or higher mortalities if beech wood was treated even higher retentions of mimosa and quebracho.

Surprisingly, the poplar wood, which showed the highest retentions for all extracts, did not perform well in terms of mass loss values. Table 4 indicates that no extractive solutions, regardless of concentration, provided satisfactory protection against the termites except 12% quebracho extract. The highest retention of quebracho extract reduced the mass loss by approximately 39% when compared to the untreated controls.

Table 4. Mean Mass Loss, Termite Mortality, and Termite Attack Ratings of Poplar Samples

Destination species	Source species	Concentration (%)	Mass loss (%)	Mortality (%)	Termite attack
Poplar	Control	-	21.15 bc ¹	6.33 a	4.0
	Mimosa	6	22.92 c	19.00 ab	4.0
		12	15.51 ab	50.33 d	4.0
	Quebracho	6	18.48 bc	34.67 bcd	3.0
		12	12.73 a	52.33 d	2.7
	Pine bark	6	20.43 bc	25.00 abc	4.0
		12	20.58 bc	40.33 cd	4.0

¹ mean within each column followed by the same letter are not significantly different

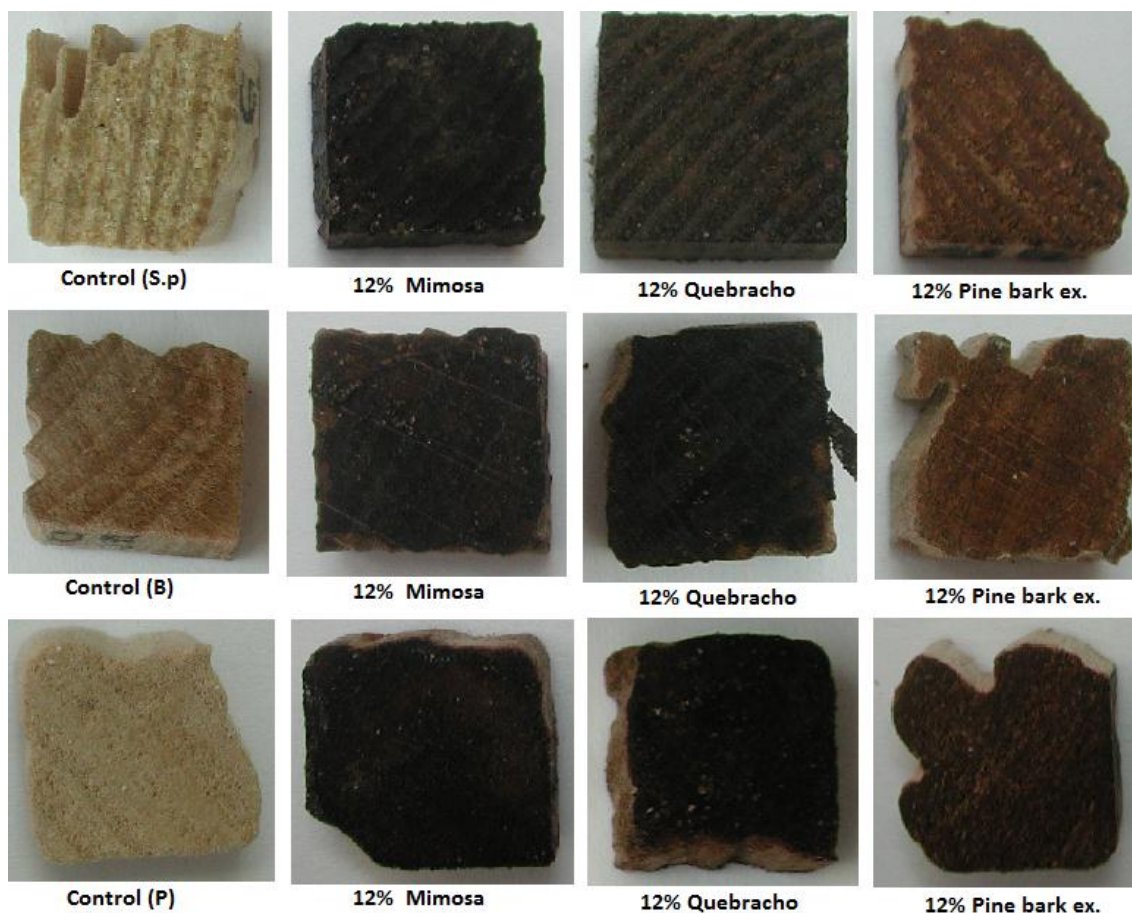
Further statistical analyses were conducted including an analysis of variance to obtain the effects of destination species (DS), source species in different concentration levels (SS+C), and their interactions on mass loss and termite mortality values shown in Table 5. It is clear that destination species, source species, and their interaction (DS and SS+C) at different concentration levels had significant differences at high confidence level.

Table 5. Analyses of Variance for the Effect of DS, SS+C and their Interactions on Mass Loss and Termite Mortality

	Source	Mean square	df	Sum of squares	F	Sig.
Mass loss (ML)	Destination species (DS)	1026.733	2	513.367	51.028	0.000
	SS+C	1382.625	8	172.828	17.179	0.000
	SS*DS+C	443.937	16	27.746	2.758	0.003
	Error	543.270	54	10.061		
	Corrected Totals	3396.5646	80			
Termite mortality (TM)	Destination species (DS)	6360.469	2	3180.235	8.154	0.001
	SS+C	46006.025	8	5750.753	14.745	0.000
	SS*DS+C	8048.642	16	503.040	1.290	0.238
	Error	21060.667	54	390.012		
	Corrected Totals	81475.8	80			

SS+C: Source species in different concentration levels

Figure 1 displays representative test specimen from each treatment and retention level to observe termite damage visually. The visual ratings are in line with mass loss and mortality data.



S.p: Scotch pine, B: Beech, P: Poplar

Fig. 1. Visual representation of termite damage on control and treated specimens

Yamaguchi *et al.* reported that Japanese sugi treated with 5 % w/w mimosa tannin showed lower mass losses and higher termite mortalities when compared to untreated control samples (Yamaguchi 2001; Yamaguchi *et al.* 2002). Quebracho colorado (*Schinosis balansae*) extract treated wood specimens at 9 to 25 kg/m³ retention level exhibited reductions in mass losses caused by *Pycnopus sanguineus* (white rot) and *Gloeophyllum sepiarium* (brown rot) fungi (Bernardis and Popoff 2009).

Chemical Identification

There were 14 components identified in extracted solutions obtained from wood and bark in this study (Table 7). In terms of quantities, rutin (124.26 g/kg) and gallic acid (103.72 g/kg) were listed as dominated compounds in quebracho extract. Similarly, p-cumaric acid (67.19 g/kg) and catechol (31 g/kg) were found as major compounds in mimosa extract. Pine bark extract, on the other hand, resulted in much lower quantities of active compounds.

Table 7. Identified Compounds and their Quantities In Extracted Solutions

Compounds	Mimosa (mg/g)	Pine bark (mg/g)	Quebracho (mg/g)	Scotch pine(mg/g)	Beech (mg/g)	Poplar (mg/g)
Gallic acid	9.943		103.716	-	-	-
Clorgenic acid	25.455	11.101	24.296	-	-	-
Catechol	30.995	2.796	7.452	86.47	-	47.09
Caffeic acid	7.500	14.227	7.974	-	-	-
Epicatechin	3.796	-	18.951	119.93	-	-
P- cumaric acid	67.189	-	4.255	-	-	-
Rutin	3.796	-	124.261	84.28	85.85	71.48
Luteolin 7-O-glucoside	14.167	5.787	45.033	30.55	21.82	22.11
Calicylic acid	12.421	-	21.234	97.72	120.37	11.48
Naringenin	-	-	50.422	-	-	-
Coumarin	-	-	-	-	-	36.24

Lower mass losses and high termite mortality rates found in quebracho extract treated wood blocks might be correlated with antitermitic properties of rutin and gallic acid. Gallic acid has been reported for its antifungal (Kishino *et al.* 1995) and antioxidant (Martinez *et al.* 2011) properties in the literature. Catechin is also known as antitermitic (Anderson 1961), insecticidal (Guerra *et al.* 1990), and antioxidant (Ruiz-Martinez *et al.* 2011). Rutin has been mentioned for its insecticide properties (Isman and Duffey 1982; Simmonds 2003; Wu *et al.* 2009).

CONCLUSIONS

1. In summary, this study demonstrated that mimosa and quebracho extract can be used as alternative wood preservatives against *Reticulitermes grassei* (Clement) for indoor applications.
2. A twofold increase in mimosa and quebracho retentions from 6% to 12% significantly reduced mass losses for all wood specimens tested. Therefore, the highest retentions of mimosa 91.03 kg/m³ for Scotch pine and 85.72 kg/m³ for beech and quebracho 95.90 kg/m³ for Scotch pine 84.77 kg/m³ for beech and 105.69 kg/m³ for poplar can be considered as threshold values against termite attack, since lower retentions failed to protect the wood specimens.
3. Pine bark extract, on the other hand, failed to protect wood specimens from termite activity regardless of concentration.

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