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Section 1

Biology

Effects of teak, *Tectona grandis* Linn, heartwood extractives against *Heterotermes indicola* (Isoptera: Rhinotermitidae)

Babar Hassan^{1*}, Sohail Ahmed¹, Mark Mankowski², Grant Kirker³, Rebecca E Ibach³ and Muhammad Misbah ul Haq⁴

¹ Department of Entomology
University of Agriculture Faisalabad
Jail Road
38000, Punjab, Pakistan

²USDA-FS Forest Products Laboratory
Wood Durability and Protection
201 Lincoln Green
Starkville, MS, 37959, USA

³USDA-FS Forest Product Laboratory
Wood Durability and Protection
One Gifford Pinchot Drive
Madison, WI, 53726-2398, USA

⁴Nuclear Institute for Food and Agriculture (NIFA)
Peshawar, Pakistan

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Babar Hassan^{1*}, Sohail Ahmed¹, Mark Mankowski², Grant Kirker³, Rebecca E Ibach⁴ and Muhammad Misbah ul Haq⁵

¹University of Agriculture Faisalabad, Pakistan sialuaf@gmail.com; saha786_pk@yahoo.com

²USDA-FS Forest Product Laboratory, Starkville, MS, USA memankowski@fs.fed.us

³USDA-FS Forest Products Laboratory, Madison, WI, USA gkirker@fs.fed.us

⁴USDA-FS Forest Products Laboratory, Madison, WI, USA ribach@fs.fed.us

⁵Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan misbah_nifa@yahoo.com

ABSTRACT

Heartwood extractives from *Tectona grandis* were investigated for antitermitic activities against *Heterotermes indicola* in laboratory experiments. Extractives were removed from wood shavings by soxhlet extraction using an ethanol: toluene (2:1) solvent system. Termite feeding and mortality followed a concentration dependent response. The highest termite mortality occurred at an extractive concentration of 10 mg/ml. The calculated LC₅₀ based on the concentration dependant response was 3.2 mg/ml. Extractives showed high repellency and antifeedant activity against *H. indicola*. Tests in which extractives were used to vacuum-pressure treat southern pine and cottonwood, showed that *T. grandis* extractives imparted resistance to these non-durable species. At the highest concentration of extractives tested, complete mortality was observed after termites were exposed to extractive-treated southern pine and cottonwood. Compared to solvent controls, minimum weight losses of 3.6 and 3.5% were observed for extractive-treated southern pine and cottonwood, respectively, at the 10mg/ml concentration after 28 days of exposure. Termites exposed to a subset of extractive treated then leached southern pine and cottonwood showed high mortality. These results suggest that *T. grandis* extractives have antitermitic properties and may be potentially useful in the development of environmentally friendly wood preservative.

Keywords: *Tectona grandis*, Preservative, lower termite, extractive transfer, *Heterotermes indicola*

1. INTRODUCTION

Species of *Heterotermes* (Rhinotermitidae) commonly infest structures and account for significant damage attributed to wood in service in regions where they occur. *Heterotermes* belongs to the same family as the more well-known subterranean termites, *Reticulitermes* and *Coptotermes*, but have not been reported on as extensively. *Heterotermes* primarily occur in the warm Neotropics including the southwestern United States, the Indian subcontinent, Australia and the Arabian Peninsula. *Heterotermes indicola* (Wasmann) is one of the most economically important and destructive subterranean termite pest species in Pakistan. It has become a major structural pest of wood in service in this country and has been ranked as the most destructive termite species in the city of Lahore (Dugal *et al.* 2015). It has also been reported to attack paper, clothes and other cellulosic materials. This species is considered to be particularly tenacious

since it remains active throughout the year (Dugal *et al.* 2015, Misbah ul haq *et al.* 2015, Hassan *et al.* 2017). Earlier studies showed that *H. indicola* attacks a wide range of wood species including *Eriobotrya japonica* (Thunb.) Lindl. *Acacia* spp., *Populus euramericana* Guinier, *Melia azedarach* L., *Mangifera indica* L., *Putranjiva roxburghii* Wall, *Moringa oleifera* Lam., *Heterophragma adenophyllum* (Wall. ex G.Don) and several other woods. *Dalbergia sissoo* Roxb, *Pinus roxburghii* Sarg., *Syzygium cumini* (L.), *Cedrus deodara* (Roxb.) G. Don were found to be resistant against this species in field and laboratory tests (Dugal *et al.* 2015, Hassan *et al.* 2016, Afzal *et al.* 2017).

Tectona grandis Linn (Teak) is a tropical hardwood species belonging to the Verbenaceae family and is highly valued due its natural durability, texture, and aesthetic properties (Krishnapillay 2000, Khera & Bhargava 2013). It is one of the most important plantation species in tropical forestry and is native to Thailand, Indonesia, India and Myanmar (Bhat *et al.* 2005). Teak is well-known for its natural resistance to native and non-native termites. In preference tests, it has been shown to be less favoured by species of *Coptotermes*, *Globitermes*, *Microceratotermes*, *Macrotermes*, and *Reticulitermes* (Ngee *et al.* 2004, Thulasidas and Bhat 2007, Lukmandaru and Takahashi 2008). Termite resistance of *T. grandis* to *Heterotermes indicola*, however, has not been previously studied.

Studies using other subterranean termite species have shown that resistance is due to the presence of certain bioactive compounds in teak heartwood (Lukmandaru and Takahashi 2009, Dungani *et al.* 2012). These bioactive compounds have been identified as anthroquinones and tectoquinones (Haluk *et al.* 2001, Mankowski *et al.* 2016, Ismayati *et al.* 2016). Several other phytochemical compounds; saponins, steroids, alkaloids, flavonoids, methyl-anthra-1,4-quinone, tecomaquinone, methylquinizarin, tectonoelin, lapachol, dehydroxy- α - lapachone have also been separated and identified from the heartwood and leaves of the teak plant (Khan and Mlungwana 1999, Ohmura *et al.* 2000, Gupta and Singh 2004, Lacret *et al.* 2012). Previous studies also showed that vacuum-pressure treating non-durable wood species with the heartwood extractives of durable wood species can increase the durability of the non-durable wood (Adegeye *et al.* 2009, Kirker *et al.* 2015). The need for further investigation into this concept of transferable durability using natural compounds is of interest in the development of more environmentally friendly wood preservative systems (Asamoah *et al.* 2011a). The teak heartwood used in this study was characterized in a previous study and found to contain Squalene, 2-methyl-9, 10-Anthracenedione, 1-Methyl-3, 4-dihydroisoquinoline, are known to have strong antifungal and antitermitic activities (Mankowski *et al.* 2016). Chemicals present in the extractives that effect and eliminate symbionts from the termite gut have been explored previously in this species (Hassan *et al.* 2017). However, no studies exist which examine the resistance of *T. grandis* and the toxicity of its extractives against *H. indicola*. In the current study, we report the repellent and antifeedant activity along with toxicity of heartwood extractives of *T. grandis* against feeding by this termite in choice and no-choice feeding tests. The potential of heartwood extractives as a wood preservative for non-durable wood species was also examined.

2. EXPERIMENTAL METHODS

2.1 Wood Samples and Extraction. Heartwood of marine grade *Tectona grandis* was acquired from a supplier in the United States (McIlvain, Pittsburgh, PA). For extractive removal, wood

was converted into wood shavings using a planer. After air drying to ~10-12% MC in the laboratory, shavings (12 g) were soxhlet extracted using 300 ml of an ethanol: toluene (2:1) solvent mixture according to ASTM D1105-96 "Standard Test Method for Preparation of Extractive-Free Wood", with minor modifications (ASTM 2014, Hassan *et al.* 2017). The solvent containing the extractives was placed in a tared round bottom flask, and evaporated to dryness at reduced pressure using a rotary evaporator. The dried residue was re-weighed and then re-dissolved with solvent (ethanol: toluene) for a final concentration of 100.0 mg/ml (w/v) based on the dry weight of the residue.

2.2 Preparation of Extractive-Free Wood. ASTM Standard (D1105-96) was followed to prepare extractive free blocks of *T. grandis*, with some modifications. *Tectona grandis* was cut into 19×19×19 mm blocks in Mississippi (USA) with an electric saw. Conditioned blocks (33°C, 62±3% RH) were numbered and weighed prior to being placed in soxhlets and extracted for six hours using the ethanol: toluene (2:1) mixture. Blocks were then washed with ethanol to remove excess toluene and secondarily extracted for six hours in ethanol (95%) alone. Ethanol-extracted blocks were air dried overnight and then boiled for six hours in 3.0 litres of distilled water with water changes every hour.

2.3 Filter Paper Bioassay. Oven dried (60°C) Whatman No. 1 filter paper (42.5 mm diameter) was weighed and treated with five different concentrations (1.25, 2.5, 5.0, 7.5, and 10.0 mg/ml) of heartwood extractives. Concentrations were prepared from stock solution using ethanol-toluene as a solvent. Each filter paper was then treated with 200 µl of each solution concentration. Treatments were done in replicates of three along with a control treatment which was treated with ethanol: toluene alone. After treatment, filter papers were oven dried at 60°C for 12 hours and weight gain after treatment was calculated. A total of 50 termites (*H. indicola*) were released into jars containing 20 grams of sand, 3.6 ml water and treated filter papers, and maintained in an incubator at 27°C and 75% RH for fifteen days. At the end of the test, termite mortality was calculated by counting the number of live termites. Filter papers were cleaned, oven dried at 60°C for 12 hours, and weight loss was calculated. A vacuum desiccator was used to equilibrate the weight of filter paper after drying. Image J software (Developed by Wayne Rasband, Bethesda, Maryland) was used to calculate the area of filter paper consumed by the termites.

2.4 Repellency and Antifeedant Bioassays. We followed the method outlined by Kadir *et al.* (2014) to test for repellency. Whatman No.1 filter paper (9 cm in diameter) was sliced into two equal halves; one-half was treated with 1 ml of each concentration of extract and the second half was treated with solvent only (Fig.1). For the control treatment, one paper half was treated with solvent and other half with water. After drying under a fume hood, both halves (treated and control) were re-joined using adhesive tape that was placed on the underside of the two filter paper halves. The re-joined filter paper was then placed in a 9.1 cm diameter Petri plate and 50 active termites were released in the centre of the plate. Assessment of repellency was performed after 1, 2, 3, 4 and 12 h by counting a number of termites on treated and untreated filter paper. Percent repellency and antifeedancy were calculated using the method described by Hassan *et al.* 2016.

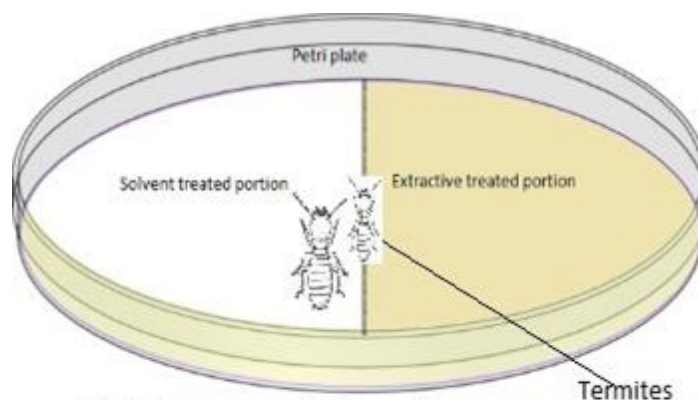


Fig. 1 Apparatus setting for repellency test

2.5 Choice and No-Choice Test of Extractive-Free Wood. Extracted and un-extracted *T. grandis* heartwood blocks were exposed to termites in choice and no-choice feeding tests according to the AWP A E-1 test (AWPA 2015). Screw top jars were filled with 150 grams sand along with 27 ml distilled water and held for two hours to equilibrate. For the no-choice test, extracted and un-extracted blocks were conditioned (33°C, 62±3% RH), weighed and placed on a small square of foil on top of the damp sand with one block in each jar. For the choice test, each jar contained one extracted and one un-extracted block. Each experiment used five replications. A total of 400 termites (396 workers and 4 soldiers) were released into each jar, which were kept in an incubator at 27°C/75±2% relative humidity for 28 days. After 28 days, the number of live termites was counted. Blocks were brushed to remove sand, conditioned for one week, and re-weighed to determine weight loss.

2.6 Termite Bioassay of Southern Pine and Cottonwood Treated With Extractives. Weighed and conditioned (33°C, 62 ± 3% RH) southern pine (SP) and cottonwood (CW) sapwood blocks (19×19×19 mm) were pressure treated with different concentrations (2.5, 5 and 10 mg/ml) of teak extractives. For controls, blocks were treated with solvent only (ethanol-toluene) or water. Blocks were pressure treated by placing five blocks in a 300 ml beaker containing the treatment solution in a vacuum-pressure chamber. Blocks were held under vacuum for 30 min and after that pressure was applied at 260 kPa (40 psi) for 60 minutes. After pressure treatment, blocks were blotted dry using paper towels, weighed, and re-conditioned at 33°C and 62±3% RH. The termite bioassay was conducted according to AWP A E1 with modifications as described in section 2.5.

2.7 Leach Resistance of Extractive Treated Southern Pine and Cottonwood Against Termites. In order to determine leaching of extractives from treated blocks, we followed the AWP A E11 standard with modifications. After conditioning, five blocks were submerged in 300 ml of deionized water (pH 7) in a 500 ml vessel and were subjected to vacuum to soak the blocks. The vessel was subject to mild agitation with water changes after 6, 24 and 48 hours then every 48 hours thereafter. The leaching process was continued for 14 days. No-choice tests against *H. indicola* were run according to the AWP A E1 standard. Wood weight loss and termite mortality was recorded after exposure to termites after 28 days. The bioassay method was the same as described in section 2.5.

2.8 Statistical Analysis. Probit analysis (Finney 1971) was used to calculate the lethal concentration (LC₅₀) of the extractives using the Polo-PC software. All other data was analysed using a one-way analysis of variance (ANOVA) to determine if there was any significant variation between treatments. A Student's t-test was used to separate mean termite mortality and weight loss of leached and un-leached wood blocks using MINITAB 17. All means were separated at the 5% significance level using the Tukey HSD test.

3. RESULTS AND DISCUSSION

The results for mortality, antifeedant and repellency tests against *H. indicola* are shown in Table 1.

Table 1: Mortality, antifeedant and repellent activity ($\pm$ SE) of *T. grandis* heartwood extractives against *H. indicola*

Conc. (mg/ml)	Mean (%) mortality $\pm$ S.E	Mean (%) repellency $\pm$ S.E.	Absolute coefficient of anti-feedancy (A %)	
			A (%)	Activity level
Control	2.33 $\pm$ 0.33 ^d	5.60 $\pm$ 0.52 ^d	-	-
1.25	3.33 $\pm$ 0.88 ^d	21.66 $\pm$ 0.33 ^c	6.95 $\pm$ 0.50	Minimal activity
2.50	59.00 $\pm$ 3.51 ^c	34.00 $\pm$ 2.00 ^c	15.3 $\pm$ 1.10	Minimal activity
5.00	75.00 $\pm$ 0.33 ^b	58.00 $\pm$ 7.57 ^b	21.5 $\pm$ 0.99	Moderate activity
7.50	80.66 $\pm$ 1.2 ^b	63.67 $\pm$ 1.33 ^{ab}	35.54 $\pm$ 0.11	Moderate activity
10.0	95.33 $\pm$ 0.66 ^a	80.33 $\pm$ 2.4 ^a	76.93 $\pm$ 0.51	V. Strong activity

The data indicate that termite mortality is dependent on extractive concentration. All concentrations tested performed differently from one another except 5.0 and 7.50 mg/ml ($F=646.93$; $p < 0.005$; $df=5, 12$). Teak heartwood extractives showed antitermitic activity with an LC₅₀ of 3.21 mg/ml ($n=50$; $\chi^2=141.83$; Slope $\pm$ SE = 2.53 ± 0.17 ; $FL\ 95\% = 2.99 - 3.4$) after 15 days of exposure. The highest mortality rate (95.3%) was observed at an extract concentration of 10 mg/ml. Results also show that when exposed to filter paper treated with 1.25 mg/ml, 21.6% termites moved to untreated filter paper. As the concentrations increased fewer termites were observed to stay or move to the treated filter paper. Both the control treatment and the solvent treatment alone did not significantly repel termites. However, at the 10 mg/ml extractive concentration, most of the termites (80.3%) were present on untreated filter paper indicating the strong repellent activity of heartwood extractives. *Tectona grandis* heartwood extractives showed minimal to strong antifeedant activity at the different concentrations. The highest absolute coefficient of antifeedancy of 76.9% (very strong activity) was observed at the high extract concentration of 10 mg/ml. While the lowest absolute coefficient of antifeedancy of 7.0% (minimum activity) was observed at the lowest concentration of extractives (1.25 mg/ml) (Table 1).

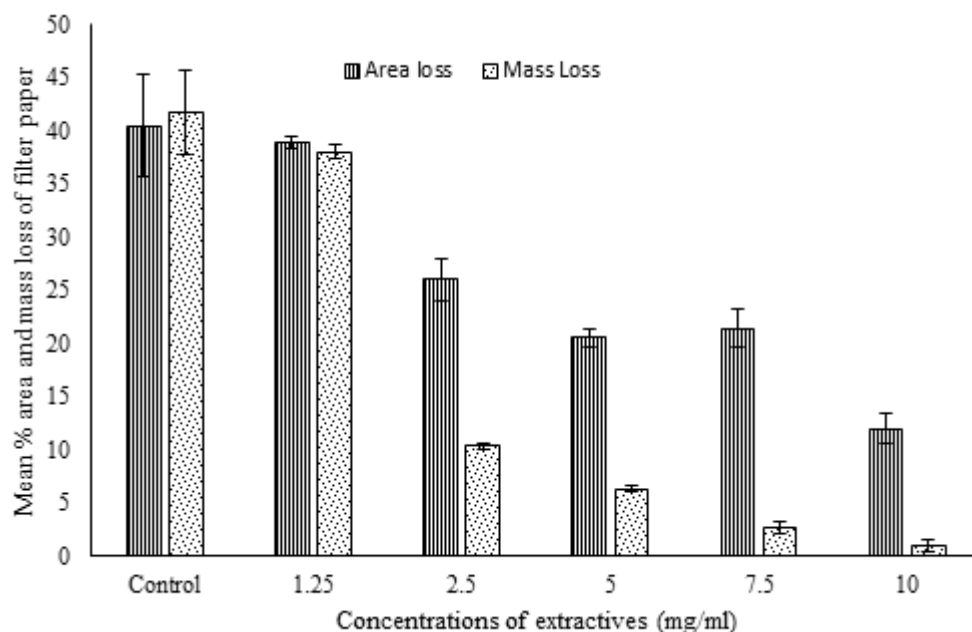


Figure 2: Percentage of filter paper area consumption and weight loss (%) treated with different concentrations of *T. grandis* extractives after 15 days exposure to *H. indicola*.

At the end of the 15 day exposure time, the average consumption of filter paper was significantly lower compared to the controls (Fig. 2). Results showed a strong correlation between the amounts of filter paper consumed (area loss) and termite mortality. A maximum consumption (area loss) of 40.4% was observed in control treatments and a minimum consumption (11.9 %) was recorded for the high extractive concentration of 10 mg/ml. There were statistically significant concentration effects on termite feeding ($F = 22.68$; $p < 0.005$; $df = 5, 12$). A parallel trend was observed for termite mortality and percent weight loss of filter paper. Minimum weight loss (1%) was found at the highest concentration (10 mg/ml) where there was maximum termite mortality (Fig. 2). Area loss was greater than weight loss as we counted area loss as area where the termites had just grazed the surface without eating all the way through the filter paper.

The heartwood of *T. grandis* is well known for its natural resistance to biodegradation and this natural durability is largely due to the presence of quinones in the heartwood (Lukmandaru and Takahashi 2009, Niamke *et al.* 2011). Recent reports on the environmental impacts of plantation teak production indicate that wood durability can differ due to site of origin (Haupt *et al.* 2003) and stand management practices (Bhat and Florence 2003). Although the origin and growth characteristics of wood were not considered, extraction yield was calculated per gram of wood shavings (Ordonez *et al.* 2006) and the mean extractive content was 5.5%. Lukmandaru 2011 found 1.9-2.7% average ethyl acetate-soluble extractive and 1.8-3.7% n- hexane soluble contents from the heart wood of *T. grandis*. Bhat *et al.* (2010) found 9.7 to 13.1% in outer and inner portion of heartwood of *T. grandis* using ethanol as a solvent. This difference is probably due to solvent selection and solubility of extractives.

Results of our filter paper bioassay were similar to Dungani *et al.* (2012) who tested the effectiveness of various extractives of teak heartwood by using discs of treated filter paper against attack from the subterranean termite *Coptotermes curvignathus* and showed that

extractions in acetone: water (9:1) at a concentration of 2-10% were the most effective. Paper discs treated with ethanol, chloroform and acetone extractives of *T. grandis* heartwood under no-choice feeding tests showed limited feeding activity and increased toxicity to the subterranean termite, *Reticulitermes speratus* (Kolbe) (Ismayati *et al.* 2016). These and similar results have confirmed dose-dependent mortality of termites after feeding on teak extractive treated filter paper against *Incisitermes marginipennis* (Latreille), *Coptotermes gestroi* (Wasmann) and *H. indicola* (González *et al.* 2013, Se Golpayegani *et al.* 2014, Kadir *et al.* 2014, 2015, Hassan *et al.* 2016). Extractives of *T. grandis* were found to be lethal to termites due to the presence of quinones and tectoquinones (Sandermann and Simatupang 1966). Our results are also in agreement with Lukmandaru and Takahashi (2008) who found several repellent chemicals in the bark and heartwood of *T. grandis* to *R. speratus* (Kolbe). Anthraquinone (24%) was found to be the major chemical responsible for repellency to *Cryptotermes brevis* Walker after GC-MS characterization (Wolcott 1947). Other authors have also reported that quinones have repellent and toxic properties against termites (Ganapthy *et al.* 2004, Dungani *et al.* 2012). The antifeedant activity of extractives obtained from *T. grandis* was 6 to 76.9%, which is similar to an earlier study on Purkwakarta teak extracts (Dungani *et al.* 2012).

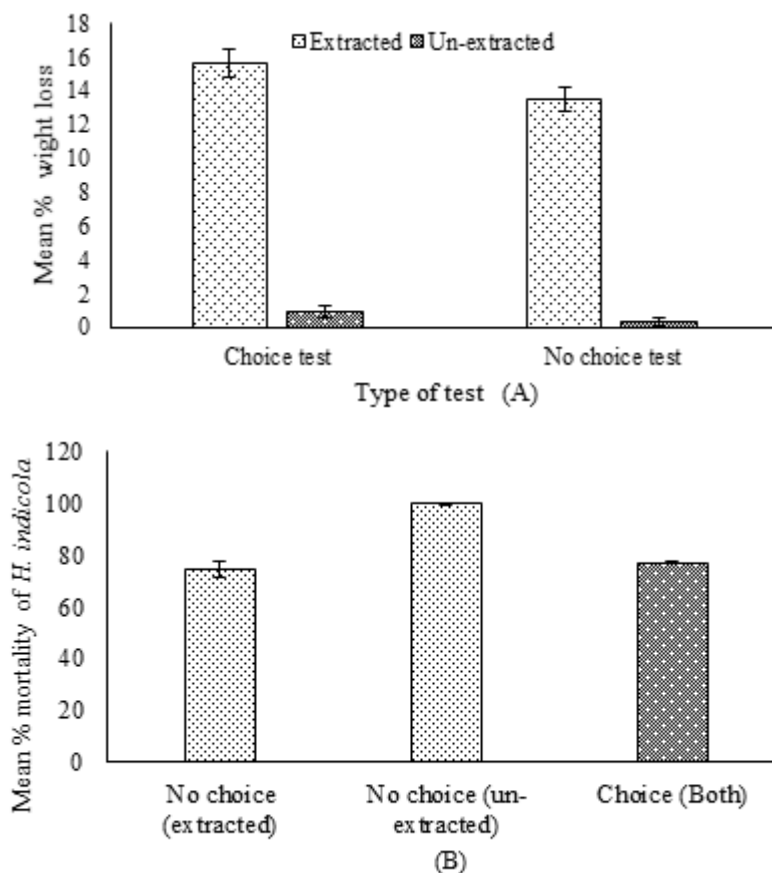


Figure 3: Wood consumption (a) and mortality (%) (b) of *H. indicola* in choice and no-choice bioassay on extractive free wood.

Mortality and wood consumption rates from the bioassays using un-extracted and extractive-free wood of *T. grandis* are shown in Figure 3a-b. In the choice test, termites generally did not feed on the un-extracted wood, instead consuming more of the extracted wood (15.6%), while in the no-choice test weight losses for extracted wood were similar (13.5%) to the choice test. In the case of un-extracted wood, consumption was less than 1% in both the choice and no-choice tests after 28 days of exposure. Complete mortality (100%) was observed in the no-choice test with un-extracted wood while on extracted wood mortality was 76%. In no-choice test, exposure to the extracted and un-extracted wood resulted in a mortality of 74 and 100% respectively, while in choice test, where both extracted and un-extracted wood were offered together termite's mortality was 77%.

Solvent and water extracted blocks of *T. grandis* wood resulted in a relatively high termite mortality. This indicates that the extractive removal efficiency of the solvent system we used was not complete in removing all toxic heartwood components. This durability may also be attributed to high wood density and hardness. Wood density can affect wood permeability, hence the solvents used may not have been able to remove all of the compounds sequestered in the heartwood. This is in agreement with Peralta *et al.* (2003) who deduced that wood density was an important factor of natural resistance of wood to termites. Natural resistance of wood could be attributed to hardness, extractive contents and specific gravity of wood (Arango *et al.* 2006). Previous studies show that there is a significant correlation between specific gravity, cellulose, density, hardness, total bioactive ingredients in wood and destruction of wood by *H. indicola* (Rasib *et al.* 2014). It has been suggested that an increase in density and specific gravity of wood is directly proportional to the resistance level. Similarly, hardness, lignin content and other bioactive ingredients also resulted in higher resistance; whereas cellulose content increased the feeding preference of *H. indicola*. It was also observed that *D. sissoo* and *S. cumini* were highly resistant to *H. indicola*, due to hardness and presence of natural bioactive compounds (Manzoor *et al.* 2009, Shanbhag and Sunararaj, 2013, Rasib *et al.* 2014, Afzal *et al.* 2017). Solubility of the extractives may have also affected the removal of the extractives from the wood. Results supporting this were found by Taylor *et al.* (2006) who observed that most extractives in the heartwood of *Thuja plicata* Donn ex D. Don and *Cupressus nootkatensis* D. Don were methanol soluble and their removal reduced the durability of those wood pieces to fungal decay and termite attack.

Tectona grandis heartwood extractives were lethal to termites at all concentration tested and caused 98.8% mortality of *H. indicola* at the maximum concentration (10 mg/ml) after feeding on treated southern pine. At the lowest concentration, mortality was 25.0 % ($F= 2390$, $p < 0.05$; $df= 4, 20$; Fig. 4). A similar trend for termite mortality was found after feeding on cottonwood treated with extractives. At the maximum concentration (10 mg/ml), there was 100% termite mortality while at the lower concentrations, mortality was significantly lower ($F= 432.10$, $p < 0.05$; $df= 4, 20$; Fig. 4). The average weight loss of treated and un-treated southern pine and cottonwood exposed to *H. indicola* is shown in Fig. 5. Solvent and water treated southern pine controls lost 28 and 25%, respectively, while cottonwood showed a weight loss of 42.5 and 42.2%, respectively for solvent and water treated blocks. At the highest concentration, weight loss of southern pine and cottonwood were reduced up to 3.6 and 3.5%, respectively.

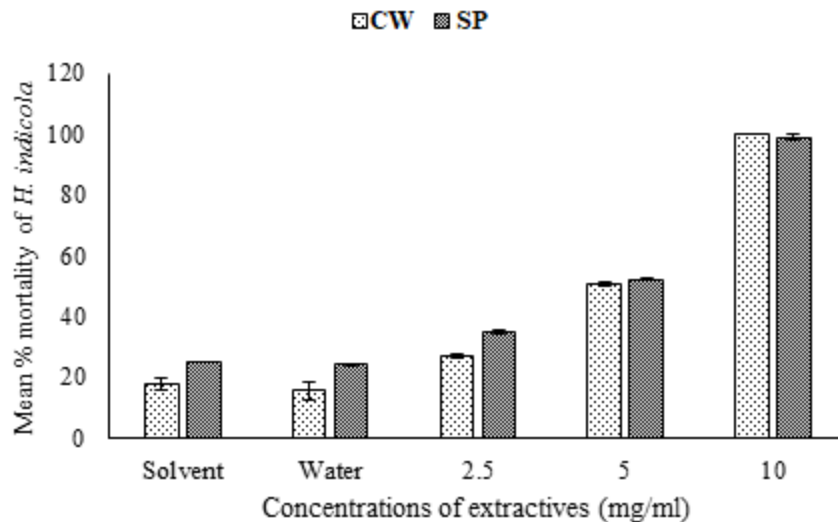


Figure 4: Mean percentage mortality of *H. indicola* after feeding on vacuum-impregnated wood blocks of southern pine and cottonwood.

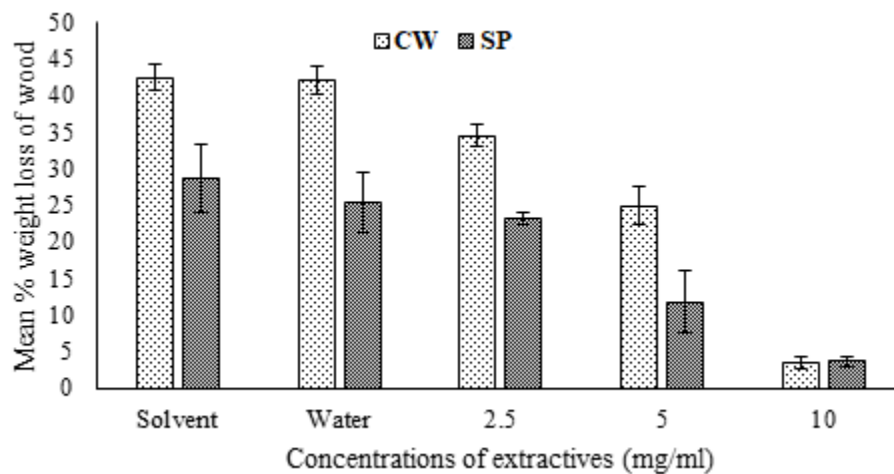


Figure 5: Mean percentage weight loss of vacuum-impregnated southern pine and cottonwood exposed to *H. indicola*.

Southern pine and cottonwood treated with *T. grandis* heartwood extracts did not show any significant difference in mortality of *H. indicola* after feeding on leached and un-leached samples. However, leached specimens of both southern pine and cottonwood became more susceptible to attack by *H. indicola* and there was a significant difference in weight losses of both woods after termite exposure (Table 2).

Table 2: Mean mortality of *H. indicola* and weight losses of Southern pine and Cottonwood after leaching and un-leaching tests of extractives.

Wood species	Treatments	Mean Mortality (%)			
		Un-leached	Leached	t	p
SP	Solvent	24.73 ± 0.51	27.52 ± 0.92	-3.47 ^{NS}	0.35
	Water	27.10 ± 0.75	27.08 ± 0.85	0.01 ^{NS}	0.99
	<i>T. grandis</i>	93.98 ± 3.16	83.26 ± 3.79	1.73 ^{NS}	0.15
CW	Solvent	34.95 ± 1.03	36.20 ± 2.29	-0.91 ^{NS}	0.41
	Water	31.84 ± 3.24	32.10 ± 5.07	-0.04 ^{NS}	0.96
	<i>T. grandis</i>	79.58 ± 2.57	73.30 ± 12.2	0.50 ^{NS}	0.63
Wood species	Treatments	Mean Weight Loss (%)			
		Un-leached	Leached	t	p
SP	Solvent	25.37 ± 0.39	24.44 ± 1.05	-1.10 ^{NS}	0.35
	Water	26.81 ± 0.82	24.60 ± 0.47	-4.43 ^{NS}	0.99
	<i>T. grandis</i>	5.53 ± 1.17	10.52 ± 0.58	5.56 ^{**}	0.15
CW	Solvent	35.52 ± 1.59	34.00 ± 1.16	-4.16 ^{NS}	0.41
	Water	37.53 ± 2.00	36.29 ± 1.08	-0.46 ^{NS}	0.96
	<i>T. grandis</i>	7.36 ± 1.41	17.91 ± 2.08	3.20 ^{**}	0.63

t, student's t-test value; p-p-value; ** - significant; NS - non-significant

Previous work has shown that the removal of extractives decreased the durability in different wood species (Agatha *et al.* 2012, Kirker *et al.* 2013). By pooling extractives from a durable species, this durability may be transferred and distributed more evenly into a non-durable species, such as southern pine or cottonwood. Utilization of extractives can also include non-marketable portions of the tree, which allows for more efficient utilization of the resource and a potential secondary value added product obtained from teak harvest. Thévenon *et al.* (2001) treated blocks of *Pinus sylvestris* L. with extractives of *T. grandis* and solutions of commercialized lapachol and tectoquinone and found protection of non-durable wood against fungi through these treatments. Similar results were also found by Tascioglu *et al.* (2012) who found minimum feeding and high termite mortality from mimosa and quebracho extract-treated scots pine at a 12% concentration level. In another study, Asamoah *et al.* 2011b treated five less used timber species in Ghana with the extractives of *T. grandis* and *Distemonanthus benthamianus* (Bonsamdua) and found that *D. benthamianus* extractives improved the durability of these woods more than *T. grandis*. The results of our study were also similar to Brocco *et al.* 2015. These authors treated *Pinus sp.* with *T. grandis* extractives in ethanol and hot water and showed treated wood to be resistant to feeding by *Nasutitermes corniger* Motschulsky. In our tests, leaching the specimens did not reduce resistance against *H. indicola* as there was no significant difference between mortality in termites on leached versus un-leached samples. However there was a significant difference in weight loss.

4. CONCLUSIONS

The results from our experimental assays indicates that *T. grandis* extractives could be a potential wood preservative against attack by *H. indicola*. Transferring durability using toxic *T. grandis* heartwood extractives to non-durable wood species improved the resistance against *H. indicola*. We showed that crude extracts had significant effects on termite mortality and feeding. Future studies should examine single extractive component isolates to determine if they are susceptible alone or act in synergy with other heartwood components. This would provide a more detailed understanding of the response of termites to teak extractives.

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6. REFERENCES

- Adegeye, A O, Ogunsanwo, O Y, Olajuyigbe, S O (2009): Antifungal activities of heart wood extract (HWE) of teak (*Tectona grandis*) against two white rots in woods of *Gmelina arborea* and *Triplochiton scleroxylon*. *Academic Journal of Plant Sciences*, **2** (4): 279-285.
- Afzal, M, Qureshi, N A, Rasib K Z, Hussain, I (2017): Resistance of commercial and non-commercial woods against *Heterotermes indicola* Wasmann (Blattodea: Rhinotermitidae) in laboratory and field conditions. *Pak. J. Zool.*, **49**(3):785-792.
- Agatha, S, Banana, A Y, Nakabonge, G (2012): Efficiency of natural wood extractives as wood preservatives against termite attack. *Maderas Ciencia y tecnología*, **14**(2): 155-163. DOI: <http://dx.doi.org/10.4067/S0718-221X2012000200003>.
- American Wood Protection Association (2015): AWP A E1-15 Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites. AWP A Book of Standards pp. 387-395. American Wood Protection Association. Birmingham, Alabama.
- Arango, R A., Green, F, Hintz, K, Lebow, P K, Miller, R B (2006): Natural durability of tropical and native woods against termite damage by *Reticulitermes flavipes* (Kollar). *International Biodeterioration & Biodegradation*, **57**(3): 146-150.
- Asamoah, A, Atta-Boateng, A, Frimpong-Mensah, K, Antwi-Boasiako, C (2011a): Efficacy of extractives from parts of Ghanaian pawpaw, avocado and neem on the durability of alstonia. *African Journal Environmental Science Technology*, **5**(2): 131–135. DOI: 10.5897/AJEST10.210.

Asamoah, A, Frimpong-Mensah, K, Antwi-Boasiako, C (2011b): Efficacy of *Tectona grandis* (Teak) and *Distemonanthus benthamianus* (Bonsamdua) water extractives on the durability of five selected Ghanaian less used timber species. *Pakistan Journal of Chemistry*, **1**(1): 28-31. DOI: 10.15228/2011.v01.i01.p05.

ASTM- International, & American Society for Testing & Materials. (2014): Annual book of ASTM standards. American Society for Testing & Materials. West Conshocken, PA. Vol. 4.10, p. 430-432.

Bhat, I U H, Khalil, A, Shuib, N S, Noor, A M (2010): Antifungal activity of the heartwood extracts and their constituents from cultivated *Tectona grandis* against *phanerochaete chrysosporium*. *Wood Research*, **55**: 59-66.

Bhat, K, Thulasidas, P, Florence, E M, Jayaraman, K (2005): Wood durability of home-garden teak against brown-rot and white-rot fungi. *Trees*, **19**(6): 654-660. DOI: 10.1007/s00468-005-0429-0.

Bhat, K M, Florence, E M (2003): Natural decay resistance of juvenile teak wood grown in high input plantations. *Holzforschung*, **57**(5): 453-455. DOI: 10.1515/HF.2003.067.

Brocco F V, Paes J B, da Costa L G, Brazolin S (2015): Potential of teak heartwood extractives as a natural preservative against *Nasutitermes corniger* termite. *Proceedings IRG Annual Meeting IRG/WP 15-30666*.

Dugal, F M, Latif, M U (2015) : Comparative study of resistance and feeding preference of 24 wood species to attack by *Heterotermes indicola* (Wasmann) and *Coptotermes heimi* (Isoptera: Rhinotermitidae, Termitidae) in Pakistan. *Sociobiology*, **62**(3): 417-425.

Dungani, R, Khalil, H P S A, Naif, A, Hermawan, D (2012): Evaluation of antitermitic activity of different extracts obtained from Indonesian teakwood (*Tectona grandis*). *Bioresource Technology*, **7**(2): 1452–1461.

Finney, D J (1971): *Probit analysis*, 3rd edn. Cambridge University Press, Cambridge. New York.

Ganapaty, S, Thomas, P S, Fotso, S, Laatsch, H (2004): Antitermitic quinones from diospyros sylvatica. *Phytochemistry*, **65**(9): 1265-1271.

González, D R, Martínez-Muñoz, R E, Ron-Echeverría, O A, Flores-García, A, Macias-Rodríguez, L I, Pacheco, M M M (2013): Dissuasive effect of an extract aqueous from *Enterolobium cyclocarpum* (Jacq) Griseb on the drywood termite *Incisitermes marginipennis* (Isoptera: Kalotermitidae) (Latreille). *Emirates Journal of Food and Agriculture*, **25**(7): 524-530. doi: 10.9755/ejfa.v25i7.15987.

Gupta, P K, Singh, P A (2004): Naphthoquinone derivative from *Tectona grandis* Linn. *Journal of Asian Natural Products Research*, **6**(3): 237-240. DOI: 10.1080/10286020310001653192.

Haluk, J, Roussel, C, Thévénon, M-F (2001): Importance des quinones dans les propriétés antifongiques du teck (*Tectona grandis*). *Les cahiers Scientifiques du Bois*, **2**: 77-83.

Hassan B, Mankowski M E, Kirker, G T, Ahmed, S, Misbah ul haq, M (2016) : Antitermitic activities of Shisham (*Dalbergia sissoo* Roxb.) heartwood extractives against two termite species. *Proceedings IRG Annual Meeting*. IRG/WP 16-10856.

Hassan, B, Mankowski, M E, Kirker, G, Ahmed, S (2017): Effects of heartwood extractives on symbiotic protozoan communities and mortality in two termite species. *International Biodeterioration & Biodegradation*, **123**: 27-36.

Haupt, M, Leithoff, H, Meier, D, Puls, J, Richter, H, Faix, O (2003): Heartwood extractives and natural durability of plantation-grown teakwood (*Tectona grandis* L.) – a case study. *European Journal of Wood and Wood Products*, **61**(6): 473-474. DOI: 10.1007/s00107-003-0428-z.

Ismayati, M, Nakagawa-Izumi, A, Kamaluddin, N N, Ohi, H (2016): Toxicity and feeding deterrent effect of 2-methylanthraquinone from the wood extractives of *Tectona grandis* on the subterranean termites *Coptotermes formosanus* and *Reticulitermes speratus*. *Insects*, **7**(4): 63. Doi: 10.3390/insects7040063.

Kadir, R., Awang, K., Khamaruddin, Z. & Soit, Z. (2015). Chemical compositions and termiticidal activities of the heartwood from *Calophyllum inophyllum* L. *Anais da Academia Brasileira de Ciências*, **87**(2): 743-751. DOI: <http://dx.doi.org/10.1590/0001-3765201520140041>.

Kadir, R, Ali, N M, Soit, Z, Khamaruddin, Z (2014): Anti-termite potential of heartwood and bark extract and chemical compounds isolated from *Madhuca utilis* Ridl. H. J. Lam and *Neobalanocarpus heimii* King P. S. Ashton. *Holzforschung*, **68**(6): 713–720. DOI: 10.1515/hf-2013-0101.

Khan, R M, Mlungwana, S M (1999): 5-hydroxylapachol: A cytotoxic agent from *Tectona grandis*. *Phytochemistry*, **50**(3): 439-442. DOI: [http://dx.doi.org/10.1016/S0031-9422\(98\)00478-6](http://dx.doi.org/10.1016/S0031-9422(98)00478-6).

Khera, N, Bhargava, S (2013): Phytochemical and pharmacological evaluation of *Tectona grandis* Linn. *International Journal Pharmacy and Pharmacology Science*, **5**(3): 923-927.

Kirker, G., Blodgett, A., Arango, R., Lebow, P. & Clausen, C. (2013). The role of extractives in naturally durable wood species. *International Biodeterioration & Biodegradation*, **82**: 53-58. DOI: <http://dx.doi.org/10.1016/j.ibiod.2013.03.007>.

Kirker, G T, Bishell, A, Lebow, P (2015): Laboratory evaluations of durability of southern pine pressure treated with extractives from durable wood species. *Journal of Economic Entomology*, **109**(1): 259-266.

Krishnapillay, B (2000): La sylviculture et la gestion des plantations de teck. *Unasyuva*, **51**: 14-21.

Lacret, R, Varela, R M, Molinillo, J M, Nogueiras, C, Macías, F A (2012) : Tectonoelins, new nor lignans from a bioactive extract of *Tectona grandis*. *Phytochemistry Letters*, **5**(2): 382-386. <http://dx.doi.org/10.1016/j.phytol.2012.03.008>.

Lukmandaru, G, Takahashi, K (2008): Variation in the natural termite resistance of teak (*Tectona grandis* Linn. Fil.) Wood as a function of tree age. *Annals of Forest Science*, **65**(7): 708-708. DOI: 10.1051/forest: 2008047.

Lukmandaru, G, Takahashi, K (2009): Radial distribution of quinones in plantation teak (*Tectona grandis* L.f.). *Annals of Forest Science*, **66**(6): 1-9. DOI: 10.1051/forest/2009051.

Lukmandaru, G (2011): Variability in the natural termite resistance of plantation teak wood and its relations with wood extractive content and color properties. *Journal of Forestry Research*, **8**: 17-31. DOI: <http://dx.doi.org/10.20886/ijfr.2011.8.1.17-31>.

Mankowski, M, Boyd, B, Hassan, B, Kirker, G (2016): GC-MS Characterizations of termiticidal heartwood extractives from wood species utilized in Pakistan. *Proceedings IRG Annual Meeting IRG/WP 16-10857*, 16 pp.

Manzoor, F, Zameer, K, Malik, S A, Cheema, K, Rahmin, A (2009): Comparative studies on two Pakistani subterranean termites (Isoptera: Rhinotermitidae, Termitidae) for natural resistance and feeding preferences in laboratory and field trials. *Sociobiology*, **53**(1): 259-274.

Misbah-Ul-Haq, M, Khan, I A (2015): Study of trail-following behavior of subterranean termite, *Heterotermes indicola* (Isoptera: Rhinotermitidae) after exposure to various concentrations of insect growth regulators. *Journal of Entomology and Zoology Studies*, **3**(5): 406-411.

Ngee, P S, Tashiro, A, Yoshimura, T, Jaal, Z, Lee, C Y (2004): Wood preference of selected Malaysian subterranean termites (Isoptera: Rhinotermitidae, Termitidae). *Sociobiology*, **43**(3): 535-550.

Niamké, F B, Amusant, N, Charpentier, J P, Chaix, G, Baissac, Y, Boutahar, N, Adima, A A, Kati-Coulibaly, S, Jay-Allemand, C (2011): Relationships between biochemical attributes (non-structural carbohydrates and phenolics) and natural durability against fungi in dry teak wood (*Tectona grandis* L. F.). *Annals of Forest Science*, **68**(1): 201-211. DOI: 10.1007/s13595-011-0021-2.

Ohmura, W, Doi, S, Aoyama, M, Ohara, S (2000): Antifeedant activity of flavonoids and related compounds against the subterranean termite *Coptotermes formosanus* Shiraki. *Journal of Wood Science*, **46**(2): 149-153. DOI: 10.1007/BF00777362.

Ordoñez, A A L, Gomez, J D, Vattuone, M A, Isla, M I (2006): Antioxidant activities of *Sechium edule* (Jacq.) swart extracts. *Food Chemistry*, **97**: 452-458. DOI: 10.1016/j.foodchem.2005.05.024.

Peralta, R C G, Menezes E B, Carvalho, A, Menezes E. (2003): Feeding preferences of subterranean termites for forest species associated or not to wood decaying fungi. *Floresta Ambient.*, **10**: 58-63.

Rasib, K Z, Ashraf, H, Afzal, M (2014): Feeding preferences of *Odontotermes obesus* (Rambur) (Isoptera: Termitidae) on different commercial and non-commercial woods from Lahore, Pakistan, under laboratory and field conditions. *Zoology and Ecology*, **24**(4): 369-379.

Sandermann, W, Simatupang, M H (1966): Zur chemie und biochemie des teakholzes (*Tectona grandis* L. Fil). *Holz als Roh-und Werkstoff*, **24**(5): 190-204.

Se Golpayegani, A, Thévenon, M-F, Gril, J, Masson, E, Pourtahmasi, K (2014): Toxicity potential in the extraneous compounds of white mulberry wood (*Morus alba*). *Maderas Cienc. y Tecnologia*, **16**(2): 227-238. DOI: 10.4067/S0718-221X2014005000018.

Shanbhag, R R, Sundararaj, R, Erbilgin, N (2013): Physical and chemical properties of some imported woods and their degradation by termites. *Journal of Insect Science*, **13**: 63.

Tascioglu, C, Yalcin, M, Troya, T d, Sivrikaya, H (2012): Termiticidal properties of some wood and bark extracts used as wood preservatives. *Bioresource Technology*, **7**(3): 2960–2969. DOI: 10.15376/biores.7.3.2960-2969.

Taylor, A M, Gartner, B L, Morrell, J J, Tsunoda, K (2006): Effects of heartwood extractive fractions of *Thuja plicata* and *Chamaecyparis nootkatensis* on wood degradation by termites or fungi. *Journal of Wood Science*, **52**: 147-153. DOI: 10.1007/s10086-005-0743-6.

Thévenon M-F, Roussel C, Haluk J-P (2001). Possible durability transfer from non-durable wood species. The study case of teak wood. *Proceedings IRG Annual Meeting*, IRG/WP 01-10392, 6 pp.

Thulasidas, P K, Bhat, K M (2007): Chemical extractive compounds determining the brown-rot decay resistance of Teak wood. *Holz als Roh- und Werkstoff*, **65**: 121–124. DOI: 10.1007/s00107-006-0127-7.

Wolcott, G N (1947): The permanence of termite repellent. *Journal of Economic Entomology*, **40**: 124–129.