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ANTITERMITIC ACTIVITIES OF SHISHAM (*DALBERGIA SISSOO* ROXB.) HEARTWOOD EXTRACTIVES AGAINST TWO TERMITE SPECIES.

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ABSTRACT

Shisham (*Dalbergia sissoo*) heartwood extractives were investigated for antitermitic activities against *Heterotermes indicola* and *Reticulitermes flavipes*. Heartwood extractives were removed from wood shavings by soxhlet extraction using (2:1) ethanol: toluene as the solvent system. Filter paper bioassays were conducted against both species to observe concentration dependent feeding response and mortality of termites. Results indicated that the highest termite mortality occurred at 10 mg/ml with a LC_{50} at 5.54 and 3.89 mg/ml against *H. indicola* and *R. flavipes*, respectively. Shisham extractives showed more repellency and antifeedant activity against *H. indicola* compared to *R. flavipes*. In choice and no-choice feeding bioassays with extracted and un-extracted Shisham wood blocks, increased wood loss due to termite feeding was observed on extracted blocks compared to un-extracted blocks. Higher termite mortality was also observed after feeding on un-extracted blocks compared to extracted blocks. Results also showed that extractives from Shisham imparted resistance to vacuum-pressure treated Southern pine (SYP, *Pinus taeda* L.) and Cottonwood (CW, *Populus deltoides*) against both species. These results suggest that Shisham extractives have antitermitic properties and may be potentially useful in the development of environment friendly termiticides.

Keywords: Shisham, antitermitic, *Dalbergia sissoo*, extractives, *Reticulitermes flavipes*, *Heterotermes indicola*

1. INTRODUCTION

Termites are major pests of wood structures and wood products globally, causing billions of dollars in damage (Su and Scheffrahn 1990). *Heterotermes indicola* (Wasmann) and *Reticulitermes flavipes* (Kollar) are considered major termite pest species in South East Asia and the United States, respectively. *Heterotermes indicola* is a very common wood destroying termite, particularly in areas north of 20°N latitude which includes India, Pakistan, and Afghanistan (Maiti 2006). In addition to causing extensive damage to wooden structures in houses, this species is also reported to destroy paper, clothes and other stored products with cellulosic components. These termites feed internally within wood, but leave an outer shell intact to protect them from desiccation. In domestic infestations, termites create galleries which can be seen running along walls and ceilings in order to reach wooden materials (Roonwal and Chhottani 1989). Out of 50 described species known to occur in Pakistan, 11 have been recorded damaging manmade wooden structures (Akhtar 1983). One of the most important termites in urban and rural areas of Pakistan is *H. indicola*, which is widely distributed throughout Pakistan

(Sheikh *et al*. 2008). *R. flavipes* is the most widely distributed termite species worldwide, and is found in the entire eastern region of North America as far north as Ontario, Canada, and south to Key Largo, Florida. It is considered to be one of the most destructive termite pest species (Su and Scheffrahn 1990, Su *et al*. 2001).

Susceptible woods that are vulnerable to termites are typically preserved with chemicals to enhance their service life by decreasing and/or repelling attack by timber deteriorating agents (Evans 2003). Preservative chemical treatments include synthetic inorganic and organic chemical agents such as creosote, CCA (Chromated Copper Arsenate), tri-butyl-tin oxide or pentachlorophenol. However, these synthetic compounds are costly and frequently injurious to the workforce and the environment. In addition, the presence of toxic compounds complicates disposal of treated wood materials at the end of use (Ofori and Bamfo 1994, Wood Preservation Canada 2008, Asamoah *et al.* 2011). This has prompted an increased interest in finding less toxic alternatives to wood protection.

The heartwood of some tree species has been shown to be resistant to degradation by biological agents, including termites and fungi. This resistance, or durability, is generally due to the build-up of extractives in the heartwood (Kityo and Plumptre 1997). Extractives are non-structural wood components and generally constitute a minor part of wood. They can be comprised of terpenoids, alkaloids, stilbenes, flavonoids, and other chemical groups. Even though there are resemblances in the occurrence of wood extractives within families, there are distinct differences in extractive composition even among closely related wood species (Hillis 1978, Walker 1993, Haygreen and Bowyer 1996). Several studies have shown that after removal of extractives, durable wood loses its natural resistance and makes them more susceptible to decay (Ohmura 2000, Taylor *et al.* 2002, Oliveira *et al.* 2010, and Kirker *et al.* 2013). Organically based wood extracts have a presumably low health hazard and are easier to detoxify and dispose of without harming the environment (Barnes 1992, Chen *et al.* 2004, Kirker *et al.* 2013). The use of natural extractives in wood for the management of termites has been previously studied. By removing extractives from a durable wood species the durability could be transferred and distributed more uniformly into a non-durable species, such as southern yellow pine or cottonwood. It has been stated that the extractives exhibit toxicity and repellency against many species of termites (Rudman and Gay 1961, Thevenon *et al.* 2001, Lukmandaru and Ogiyama 2005, Asamoah *et al.* 2011, Ragon *et al.* 2008, Dungani *et al.* 2012, Tascioglu *et al.* 2012, Kirker *et al.* 2013, Kadir *et al.* 2014, Kadir *et al.* 2015).

In the current study, extractives of the durable wood species, Shisham (*Dalbergia sissoo* Roxb) were used to treat two non-durable wood species, Southern Yellow Pine (SYP, *Pinus taeda* L.) and cottonwood (CW, *Populus deltoides*), and resistance against termite feeding was evaluated. Previous studies have indicated that the heartwood extractives of Shisham contains flavanoid and neoflvanoid such as dalbergin, dalbergiquinols and dalbergiquinones. Phytochemical analysis of Shisham reported the presence of alkaloids, carbohydrates, saponins, stilbenes, glycosides and steroids (Mukerjee *et al.* 1971, Seshadari 1972). The extracts from stem bark have been reported to have a good antioxidant activity (Aly *et al.* 2013). Shisham is classified as a class 1 (very resistant) wood species, being highly resistant to decay and termite attack (Scheffer and Morrell 1998). In Pakistan it is locally known as Shisham or Rosewood. It is an economically important multipurpose deciduous tree species belonging to family Papilionaceae (Singh *et al.* 2011). Under favorable conditions, it is a medium to large-sized tree which grows up to 30 m tall. Wood of Shisham is very hard and is best suitable for furniture (Hossain and Martin 2013). In addition, different parts of this plant have medicinal importance (Asif and Kumar 2011). Introduced in mid 1800s, this plant grows naturally in Sub-Himalayan Tarai tract ranging from Bangladesh to Afghanistan (Khan 2000). At present, it is cultivated throughout the South Asian subtropical countries such as Pakistan, Nepal and India. All plantations of Shisham are established as irrigated plantations, major ones are Changa Manga, Kundian, Perowal, Bahawalpur, Daphar, Chichawatni and Kamalia. In addition, it is commonly planted on roadsides, rail sides and waterways, and also around boarders of the agricultural fields (Sah *et al.* 2003). The area under such plantation in Punjab is 154,886 ha, with an average annual production of 28,000 m^3 (Khan and Khan 2000). During processing of this timber 30-40% of wood is considered leftover or waste, and has no commercial value. The current work addresses the possible use of Shisham heartwood extracts in improving the termite resistance property of southern pine and cottonwood.

2. EXPERIMENTAL METHODS

2.1 Wood Material and Preparation of Extractives

Logs of Shisham were obtained from the Timber Market located at Faisalabad, Pakistan. After air drying in Mississippi (USA), logs of Shisham, southern pine, and cottonwood were cut into large boards from and evened with a planer then cut into 19×19×19 mm blocks. Shavings from this process were used for the preparation of extractives. Air dried wood shavings were soxhlet extracted using 300 ml of ethanol: toluene (2:1) as solvent according to ASTM D1105-96 ″Standard Test Method for Preparation of Extractive-Free Wood″ with minor modifications (ASTM 2014). Shavings (12 g) were added to soxhlets with a small amount of cotton placed below and above, to contain the shavings, and extracted for a total of six hours. The resulting aliquot was evaporated at reduced pressure using a rotary evaporator (BUCHI, Rotavapor R-114) and extraction yield was calculated per gram of wood shavings (Ordonez *et al.* 2006). Extractives were then prepared in stock solutions (100 mg/ml) and stored in small vials at 4°C.

2.2 Preparation of Extractive Free Wood

Heartwood blocks of Shisham were extracted according to ASTM D1105-96 with the following modifications. Conditioned blocks (33°C, 62±3%) were numbered and weighed prior to being placed in the soxhlets and extracted for six hours using ethanol: toluene (2:1). Blocks were then washed with alcohol 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 with 3L of distilled water and water was changed after every hour.

2.3 Toxicity and Repellency against Termites

All tests against *R. flavipes* were conducted in Starkville MS, USA while tests against *H. indicola* were conducted in NIFA (Nuclear Institute for Food and Agriculture) Peshawar, Pakistan. Oven dried (60°C) and weighed Whatman No. 1 filter paper (42.5 mm diameter) was treated with five different concentrations (1.25, 2.5, 5.0, 7.5, and 10.0 mg/ml) of extractives. Concentrations were prepared from stock solution using ethanol: toluene as a solvent and 200 µl of this solution was applied to each filter paper. Treatments were done in triplicate 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 (*R. flavipes* and *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. ImageJ software (Developed by Wayne Rasband, Bethesda, Maryland) was used to calculate the area of filter paper consumed by the termites.

For repellency tests, the method outlined by Kadir *et al.* (2014) was used. 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. After drying under a fume hood, both halves (treated and control) were reconnected using adhesive tape that was placed on the underside of the two filter paper halves. The reconnected filter paper was then placed in a 9.1 cm diameter petri plate and 50 active termites were released to the center of each plate. Assessment of repellency was performed after 1, 2, 3, 4 and 12 h by counting number of termites on treated and un-treated filter paper. The following formula was used to calculate percent repellency:

$$\text{Repellency (\%)} = (Nc - Nt)/(Nc + Nt) \times 100$$

Where *Nc* is the number of termites in control while *Nt* is the number of termites on the treated filter paper.

Antifeedant indices were determined on the basis of weight loss of the filter paper (Absolute coefficient of antifeedancy (A), which was calculated using following formula:

$$A\ (\%) = [(KK - EE)/\ (KK + EE)] \times 100$$

Where KK is weight loss of the filter paper in the control treatments and EE is weight loss of the treated filter paper. All treatments were divided into four classes according to antifeedant values (Dungani *et al.* 2012).

2.4 Choice and No Choice Test on Extractive Free Wood

Extracted and un-extracted Shisham heartwood blocks were subjected to choice and no-choice feeding tests according to a modified AWPA E1-15 standard (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%), weighed and placed on top of the damp sand with one block in each jar. For the choice test, each jar received one extracted and one un-extracted block. The experiment was replicated five times. A total of 400 termites (396 workers and 4 soldiers) were released in each jar and jars were kept in the conditioning chamber at 27°C and 75±2% relative humidity for 28 days. After four weeks, the number of live termites were counted. Blocks were brushed to remove sand, conditioned for one week, and re-weighed to determine weight loss. All blocks were rated visually using 0-10 scale as described in the AWPA standard.

2.5 Transfer of Durability Test

Weighed and conditioned (33°C, 62 ± 3% R.H.) SYP and CW sapwood blocks (19×19×19 mm) were pressure treated with different concentrations (2.5, 5 and 10 mg/ml) of Shisham extractive. 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 40 psi for 60 min. After pressure treatment, blocks were blotted dry using paper towels, weighed, and re-conditioned at 33°C and 62±3% RH.

A termite bioassay was conducted according to a modified AWPA E1-15 (AWPA 2015). Modifications were the test block size of 19×19×19 mm and the amount of water added was 27ml. Screw top jars were filled with 150 grams sand along with 27 ml distilled water and left for two hours to equilibrate. After two hours, treated and control blocks were added to the jars so that each jar received only a single block. All treatments were replicated five times. A total of 400 termites (396 workers + 4 soldiers) were then added into each jar and jars were placed in the conditioning room at 27°C and 75±2 %R.H. for 28 days. After four weeks, the number of live termites were counted. Test blocks were brushed to remove sand and conditioned for one week prior to being re-weighed to determine weight loss. All blocks were rated visually using 0-10 scale as described in the AWPA standard.

2.6 GS-MS Characterization of Extractives

Analyses were performed by coupling gas chromatography (GC) and mass spectrometry (MS) via an Agilent 7890B GC. An Agilent 19091S-433UI HP-5ms Ultra Inert column was used. The HP-5ms capillary column was of 0°C-325°C (350°C) 30m x 250 um x 0.25um. The temperature of the gas chromatography column was programed from 50-270°C. Solvent delay was set at 3-6 minutes. The temperature of ion source in the mass spectrometer was held at 230°C, the quad temperature was 150°C. For all extracts the sample size injected was 1µL. The Agilent 7890B GC was equipped with a split less injector at 270°C and an electron capture detector (µECD) at 250°C. Injection was done in the split less mode. For Dalbergia sissoo a dual ramp up was used where the starting temperature was 45°C ramped to 165°C at 4°C/min then ramped to 280°C at 4.5°C/min and held for 35 minutes (Aly *et al*. 2013). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. All mass spectra were recorded in the electron impact ionization (EI) at 70 electron volts. The mass spectrometer scanned from m/z 3-700 at a rate of 2 scans per second. An integrator automatically calculated peak area. Compounds were identified using the NIST14 library (Mankowski *et al*. 2016).

2.7 Statistical Analysis

Percentage mortality data and weight loss of SYP and CW was subjected to one way analysis of variance (ANOVA) using the MINITAB 17 program. Means were separated at the 5% significance level using Tukey's HSD test.

3. RESULTS AND DISCUSSION

3.1 Toxicity and Repellency Determination

The effect of Shisham heartwood extractives on termite mortality is shown in Fig. 1. Extractives showed concentration dependent mortality against *H. indicola,* with all treatments significantly different from each other (F= 107.09; $p < 0.005$; $df = 6$). In case of *R. flavipes*, it was not linearly dependent on concentration (F= 7.38; $p < 0.005$; $df = 6$), however all treatments were significantly different from control treatments. Shisham extractives showed antitermitic activity with an LC_{50} of 5.5 mg/ml (n=50; $\chi^2 = 6.48$; FL 95% = 5.22-5.86) against *H. indicola* after 15 days of exposure. Maximum mortality was 81.3 % at the highest extractive concentration (10 mg/ml), while 16% mortality was found at the lowest extractive concentration (2.5 mg/ml). The percentage of filter paper consumed was found to be lower in treated filter paper groups than the controls, and results showed a strong correlation between the amount of filter paper consumed and termite mortality (r = -0.94; $p < 0.005$). While in the case of *R. flavipes,* extractives showed LC_{50} of 3.89 mg/ml (n=50; $\chi^2 = 186.5$; FL 95% = 3.41-4.36) after 15 days of exposure of filter paper to termites. Maximum mortality was 72% at the highest extractive concentration (10 mg/ml), while 60% mortality was found at the lowest extractive concentration (2.5 mg/ml). Filter paper consumed (%) was less correlated (r = -0.88, $p < 0.005$) to mortality and concentrations of extractives.

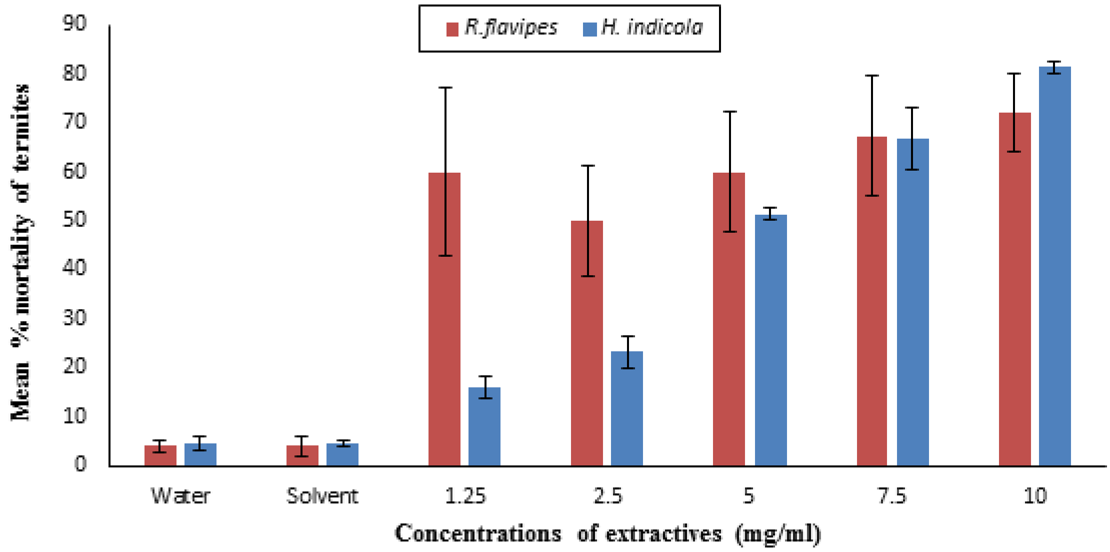

Fig 1: Effect of different concentrations of Shisham extractives on percent termite mortality

A similar trend was also found between mortality of termites and percent weight loss of filter paper after feeding trials. Minimum consumption (7.8, 5.4%) was found at the highest concentration (10 mg/ml) where there was maximum mortality of *H. indicola* and *R. flavipes* (Fig. 2). Mortality and percent weight loss of filter paper were found to be highly correlated (-0.87, $p < 0.005$) after feeding of *H. indicola* and also correlated (-0.87, $p < 0.005$) in case of *R. flavipes.* All treatments were significantly different at a 5% confidence level. Several other researchers have found dose dependent mortality of termites after feeding on extractive treated filter paper against *Incisitermes marginipennis*, *Coptotermes gestroi* and *Coptotermes curvignathus* Holmgren (González *et al.* 2013, Se Golpayegani *et al.* 2014, Kadir *et al.* 2014 Kadir *et al.* 2015).

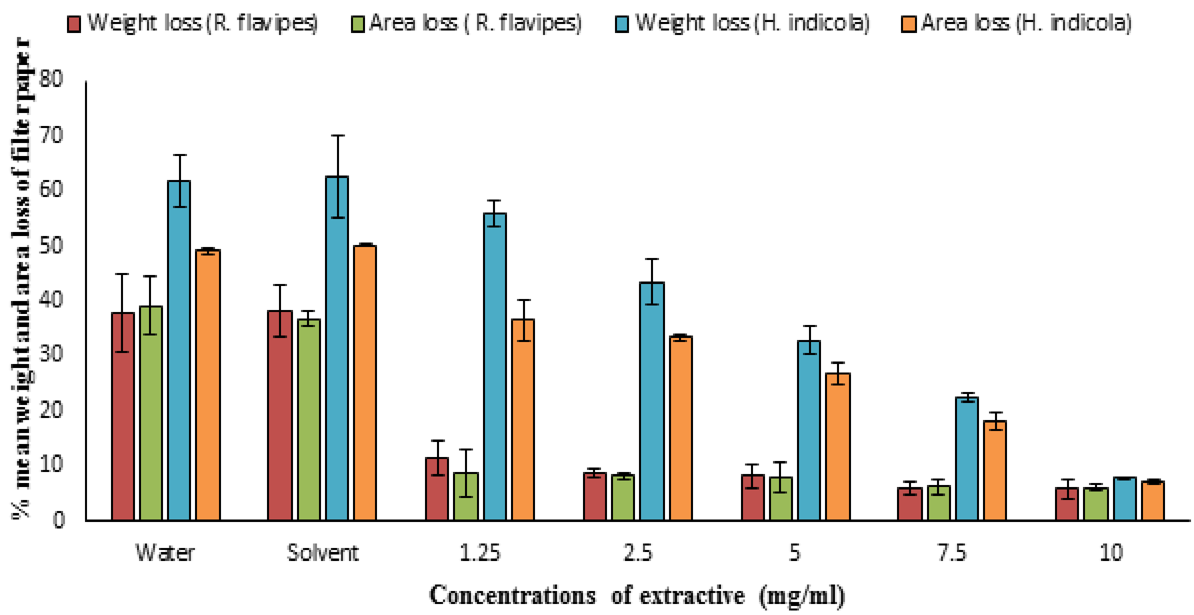

Fig 2: Consumption (%) and weight loss (%) of filter paper treated with different concentrations of Shisham extractives after 15 days exposure to termites

In order to determine the repellent properties of Shisham heartwood extractives, simple laboratory experiments were setup using both *H. indicola* and *R. flavipes* (Table 1) using similar methods to other repellency studies (Smith 1979, Sharma *et al.* 1994). In these experiments heartwood extractives showed concentration dependent repellency against *H. indicola*. At the highest concentration (10 mg/ml) 73% of termites were repelled towards control filter paper section compared to lowest concentrations (1.25 mg/ml) in which more termites were found on the extractive treated filter paper section (22.7%). Shisham heartwood extractives did not show concentration dependent repellency against *R. flavipes*. Results for *R. flavipes* showed that the 2.5 mg/ml concentration had maximum repellency (83.2 %), but higher concentrations (5, 7.5 and10 mg/ml) were found to be attractants for *R. flavipes*. In comparison with other studies, Shisham extractives were more repellent to both termite species than heartwood extractives of *Madhuca utilis* (39 to 59%) and *Neobalanocarpus heimii* (9 to 68%) at the highest concentrations, but Shisham extractives showed lower activity compared to bark extractives of both species (Kadir *et al.* 2014). However, our results of repellency test were similar to Kharkwal *et al.* 2014, who found that a chloroform extraction possesses the highest potential to extract repellent compounds from Shisham and showed 93% repellency against *Microcerotermes beesonii*.

Table 1: Antifeedant and repellent activity (± SE) of Shisham heartwood extractives against two termite species.

Conc. of extractives	*Heterotermes indicola*			*Reticulitermes flavipes*		
	Anti-feedancy (A %)	**Activity level**	**% repellent activity**	**Anti-feedancy (A %)**	**Activity level**	**% repellent activity**
1.25 mg/ml	10.16±2.12	Minimal activity	22.7±22.7	50.8±17.5	Strong activity	22.9±22.9
2.5 mg/ml	22.40±2.41	Minimal activity	32.0±18.5	60.35±8.16	Strong activity	83.2±3.33
5 mg/ml	40.64±5.83	Moderate activity	58.0±7.57	61.5±13.9	Strong activity	57.6±11.8
7.5 mg/ml	83.39±6.86	V. Strong activity	60.67±4.33	71.05±6.22	Strong activity	61.6±6.97
10 mg/ml	88.13±5.80	V. Strong activity	73.33±7.26	70.5±11.1	Strong activity	62.7±15.5

Heartwood extractives of Shisham proved to be an antifeedant for both termite species tested. The antifeedant activity of extractives was 10.16 to 88.13% and 50.8 to 70.5% against *H. indicola* and *R. flavipes*, respectively. These activities were higher compared to an earlier study on Purkwakarta teak extracts (Dungani *et al.* 2012). These results suggest that the concentration of extractive is a critical factor for termite mortality and antifeedancy (Dungani *et al.* 2012). This is most likely due to the concentration of phenolic compounds which are strong antifeedants and act as natural protectants for the living trees (Gupta *et al.* 1972, Morimoto *et al.* 2006, Ates *et al.* 2015).

3.3 Choice and No Choice Test with Extractive Free Wood

Extracted and un-extracted blocks of Shisham were exposed to termites in choice and no- choice tests. Percent average weight loss due to feeding (Fig. 4) and mortality (Fig. 3) was observed after 28 days of exposure. For *H. indicola,* 92% termites were dead in the no-choice test with un-extracted blocks, while on extracted blocks, mortality was 74.6%. With the choice test where both extracted and un-extracted blocks were offered to the termites, mortality was 77%. Mortality of *R. flavipes* was 84.5% in the choice test, while on extracted blocks (no-choice test) mortality was similarly 85%. However, on un-extracted blocks in the choice test mortality was 91%.

In the choice test, *H. indicola* generally avoided the un-extracted block (>15%WL) and consumed more of the extracted block, while in the no-choice test weight losses for extracted blocks were 13%. In the case of un-extracted blocks, consumption was nearly 2% in both the choice and no choice test after 28 days. A similar trend was observed after feeding of *R. flavipes*.

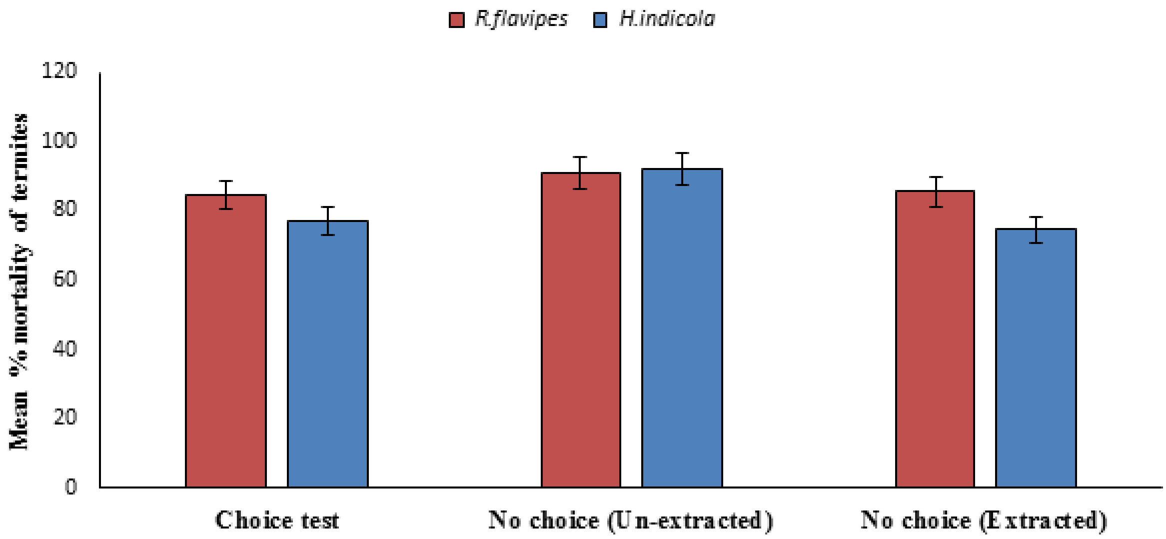

Fig 3: Percent termite mortality in choice and no choice bioassay on un-extracted and extractive free wood.

Consumption of extracted Shisham blocks was higher in both the choice and no choice test compared to un-extracted blocks. In choice tests termites consumed 10% of the extracted compared to 1% of un-extracted blocks. In no-choice tests, consumption was 11% and 3% on extracted and un-extracted blocks, respectively. This was presumably due to the presence of certain chemicals (e.g. flavonoids in the heartwood of Shisham). Qureshi *et al.* 2012 also observed less feeding and protozoicidal activities of Shisham against *H. indicola* and *Coptotermes heimi*.

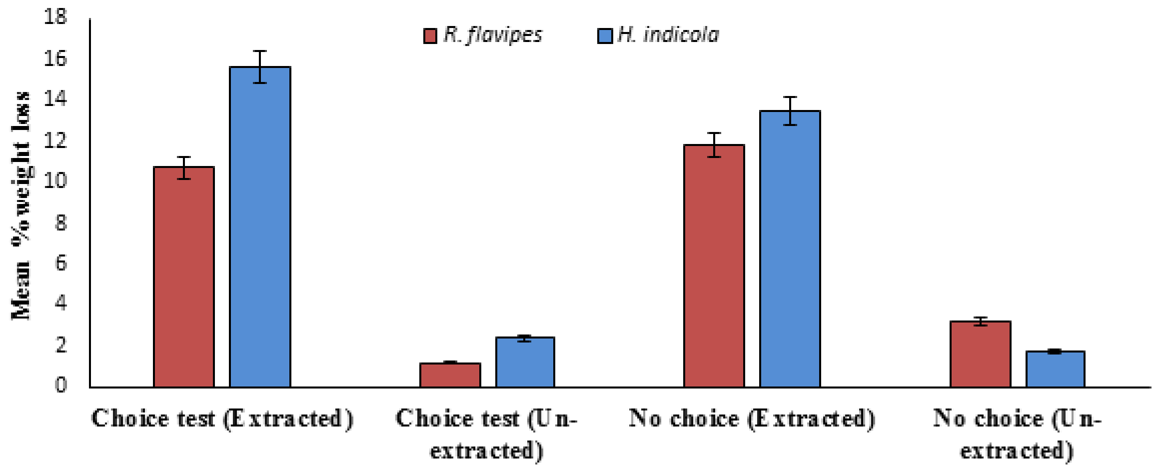

Fig 4: Wood consumption by termites in choice and no choice bioassays for extracted vs extractive free wood.

3.4 Transfer of Durability Test

Shisham heartwood extractives were lethal to termites at each concentration and caused 84% and 83% mortality of *H. indicola* at maximum concentration (10 mg/ml) after feeding on treated SYP and CW respectively (Fig. 5). At the lowest concentration (2.5 mg/ml) the mortality was lowest for both types of treated wood. All treatments differed significantly from each other and also from control treatments. Similar results were found after *R. flavipes* feeding on treated non-durable woods (Fig. 5). Maximum mortality (88 and 93%) of *R. flavipes* was found at 10 mg/ml after feeding on extractive treated SYP and CW, respectively after 28 days of exposure. While minimum mortality was observed after feeding on woods treated with lowest concentrations of extractives. These results suggest that Shisham heartwood extractives can increase the resistance of susceptible wood species to termites.

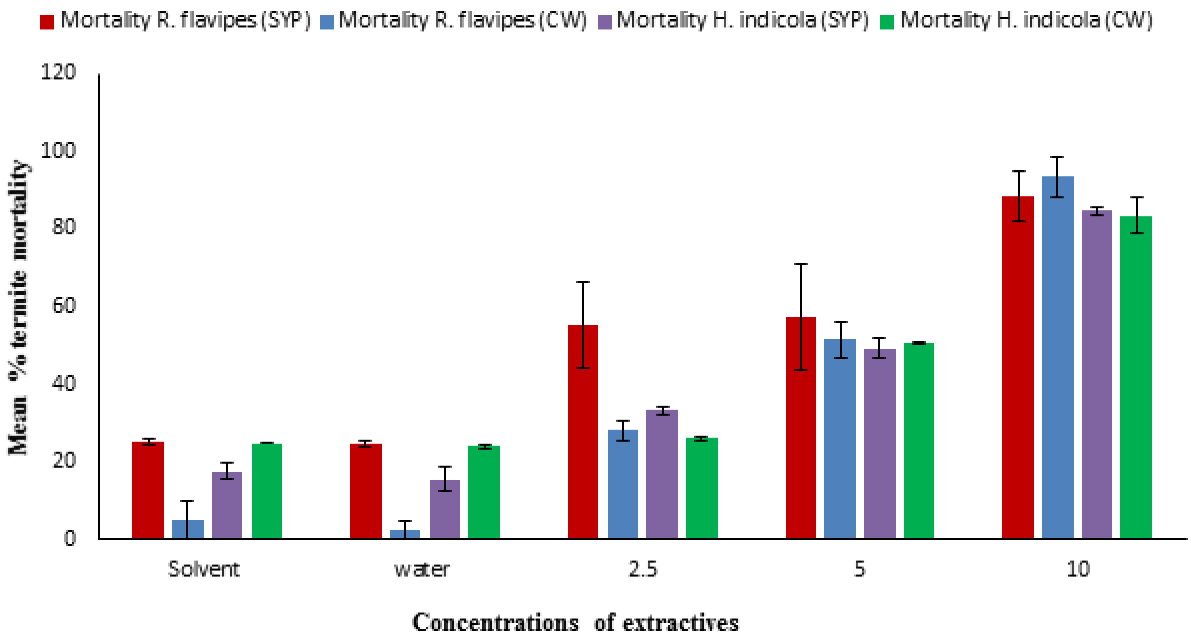

Fig 5: Mean percentage mortality of termites after feeding on vacuum-impregnated SYP and CW blocks at 3 concentrations (mg/ml) of Shisham extractives.

Average weight loss of treated and un-treated SYP and cottonwood exposed to *H. indicola* and *R. flavipes* is shown in Fig. 6. Feeding by *H. indicola* caused 28 and 25 % weight loss to SYP controls treated with solvent and water respectively, while cotton wood showed a weight loss of 42% for both solvent and water treated blocks. SYP and CW treated with highest concentrations of Shisham extracts showed 4 and 9% weight loss, respectively. Weight loss of control treatments of SYP was statistically higher than all other treatments (F =5.55; df 4, 20; p< 0.005). A similar trend was found in cottonwood (F= 41.49; df 4, 20; p< 0.005). Feeding of *R. flavipes* reduced the weight of SYP and CW treated with solvent and water up to 26 and 37%, respectively. Conversely, SYP and CW treated with highest concentrations of Shisham heartwood extractives showed minimum weight loss (5 and 12% respectively) at the highest concentration (10 mg/ml). Weight loss was found to be inversely related to the extractive concentrations. Weight loss of control treatments of SYP was statistically higher than all other treatments (F =17.23; df 4, 20; P< 0.005). A similar trend was found in cottonwood (F= 32.45; df 4, 20; P< 0.005).

Results from the current study are comparable to that reported by Yamaguchi *et al.* (2002) who found treatment of sugi with mimosa tannin had lower weight loss and higher termite mortality.

E-1 visual ratings (not given) showed very low feeding of SYP and cotton wood at 10 mg/ml concentration of Shisham wood extractive. In contrast, control blocks failed with an average rating of zero. Comparison between extractive-treated SYP and cotton wood showed Shisham wood extractives to be more effective for the protection of SYP than cottonwood by both species of termites. Although overall, pressure treatment of susceptible woods with extractives was effective in both tests against both termite species.

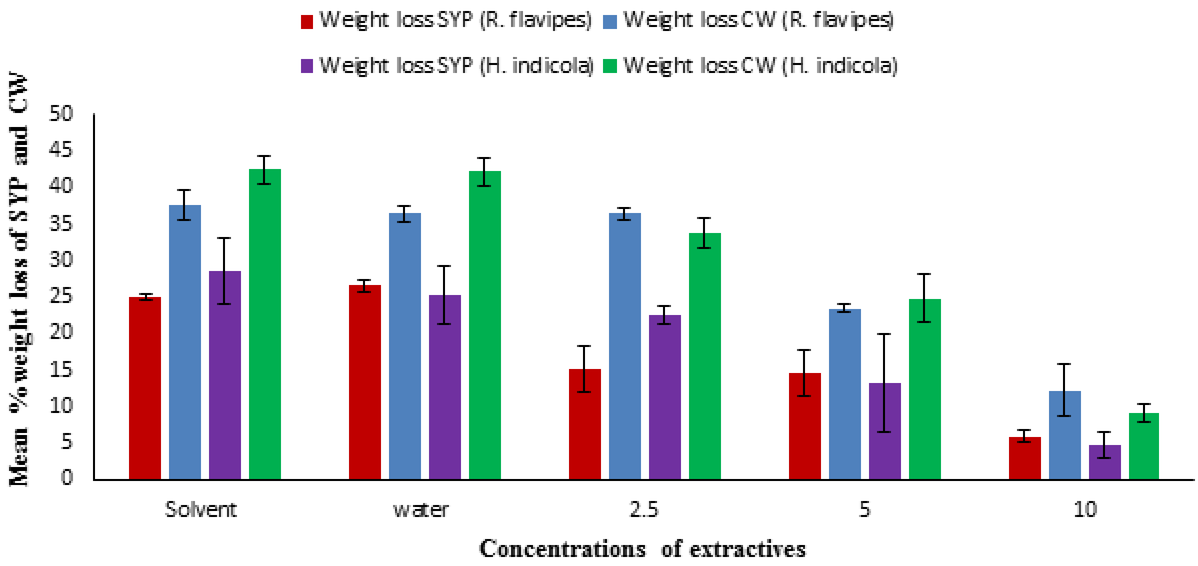

Fig 6: Mean percentage weight loss of SYP and CW blocks vacuum impregnated with Shisham extractives after feeding of termites.

Similar results were also found by Tascioglu *et al.* (2012) who saw minimum feeding with highest mortality of termites for mimosa and quebracho extract-treated scots pine at a 12% concentration level. Our results were also similar to Agatha *et al.* (2012) who found extractives from *Milicia excelsa*, *Albizia coriaria* and *Markhamia lutea* can increase termite resistance of susceptible woods up to 50% compared to control treatment.

3.5 Chemical Constituents of Extractives

Chemical characterizations of Shisham extractives from the 2:1 EtoH: Tol residues yielded several notable compounds. The top five most common compounds, which comprised 79% of the total sample are shown in Table 2. The most abundant compound was Trimethoxyresveratrol (40.6% of total). Trimethoxyresveratrol is a stilbene with published medicinal (Dias *et al*. 2013) and free radical scavenging activities (Shang *et al*. 2009). In wood protection, stilbenes are regarded as potential sources of heartwood durability (Schultz and Nicholas 2000) and are present in many other examples of naturally durable woods (Hart and Shrimpton 1979). Additional information on the chemical constituents of Shisham extractives will be presented elsewhere (Mankowski et al. 2016) at this meeting.

Table 2: Chemical compounds characterized from heartwood extractives of Shisham (RT=Retention Time, MW=Molecular weight, Q=quality, %S=percent of sample).

Compound Name	Retention Time [min]	Molecular Weight	Quality	% of Sample
1,2,9-trimethoxy-Dibenzcycloheptane	42.33	284.141	70	3.09
Trismethoxyresveratrol	44.49	270.126	64	40.6
6,8-dimethyl-Benzanthracene	45.44	256.125	64	11.41
2-(3,4-dimethoxyphenyl)-Indane-1,3-dione	57.58	282.089	58	7.25
1,3-Diamino-8-n-butyl-5,6-dihydrobenzoquinazoline	60.03	268.146	59	17.09

4. CONCLUSIONS

Heartwood of Shisham extracted with ethanol: toluene (2:1) yielded 9.1% extractives. The results of this study show a positive correlation between extractive concentration and termite resistance which strongly suggests that these extractives can play a role in protecting non-durable woods from feeding by subterranean termites. In addition, this indicates that durability can potentially be transferred from a durable to a non-durable wood species. The highest concentration was highly effective on both non-durable woods against both termite species, and field trials of SYP and CW sapwood treated with Shisham extractives are now under way. This series of bioassays has been useful in determining appropriate concentrations to expose in the field as well as assisting in the identification of active components and the optimization of the extraction procedure.

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