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FORESTRY SCIENCE

Evaluation of Antitermite Properties of Wood Extracts from *Pongamia pinnata* (L.) Pierre (Leguminosae) against Subterranean Termites

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Abstract: Termiticide, repellent and antifeedant activities of extracts from *Pongamia pinnata* wood were evaluated against *Coptotermes heimi* (Wasmann) at three different concentrations preceded by a preliminary choice and no-choice tests for natural resistance of tested wood. Termites' mortality was determined in each case of extract and solvent treated Whatman filter paper. Finally, wooden blocks of poplar (19×19×19 mm) were treated with extracts and respective solvents and exposed to termites in the field for 28 days. Minimum mean weight loss was observed in dried *P. pinnata* (6.38%), followed by fresh *P. pinnata* in choice tests. In no-choice tests, dried *P. pinnata* was comparatively resistant with a weight loss of 12.37%, followed by fresh *P. pinnata* and *P. deltoides*. In toxicity bioassay, ethyl acetate-based wood extracts caused the highest mortality (41.66%), followed by petroleum ether, hexane, and water extracts at 10 mg/ml concentration. Similarly, ethyl acetate-based extracts showed maximum repellency (100%) followed by petroleum ether extracts at 10 mg/ml and ethyl acetate at 5 mg/ml after 60 min of termite exposure. Minimum wood losses were observed in woods treated with ethyl acetate extracts compared to control and other treatments in field experiments.

Key words: choice test, extracts, no-choice test, repellency, termites, toxicity.

INTRODUCTION

Naturally durable wood species possess material characteristics that can often delay biodeterioration by fungi and insects (Kadir et al. 2014). Heartwood extractive content has been shown to be an important contributor to the inherent durability of these wood species (Kirker et al. 2013). Previous studies showed that removal of extracts from durable woods make them susceptible to termites' attack and other wood deteriorating agents and suggested that the extracts have a vital impact on wood durability (Kirker et al. 2013, Hassan et al. 2016, 2018a, b, 2019a, b).

On the other hand, susceptible wood species can be made durable by the application of certain synthetic preservatives. Synthetic wood preservatives, despite their advantages, pose environmental concerns because of their toxicity and persistence (Ahmed et al. 2014, 2016, 2017, Fatima et al. 2015). There is a need for wood preservation strategies with lower environmental impacts and reduced effects on non-target species. Under this supposition, extracting toxic fractions from naturally durable wood species and using them as wood preservatives for susceptible wood is one strategy to reduce environmental and health hazards (González-Laredo et al. 2015). Chemically, these extracts include flavonoids, alcohols, terpenes, alkaloids, glycosides, esters, stilbenes, phenols, polyphenols, and water-soluble compounds having insecticidal, antifungal and antimicrobial activities (Kaur et al. 2016, Hassan at al. 2017).

Pongamia pinnata (L.) is an evergreen medium-sized leguminous tree bearing seeds having 30-40% oil in them. Nineteen biologically active constituents have been isolated and identified from this tree being used in pharmaceutics (Kumar & Singh 2002). Leaf extracts of *P. pinnata* possessed excellent antimicrobial activity against *Micrococcus* sp. (Chopade et al. 2008). Ethanolic and methanolic leaf extracts of *P. pinnata* possessed anti-inflammatory, anti-diarrhoeal and anti-ulcer activities (Sagwan et al. 2012). Petroleum ether leaf extracts of *P. pinnata* exhibited ovicidal and anti-lice activity by delaying the emergence of nymphs (Samuel et al. 2009). In combination with *Kigelia africana* (Lam), Benth, bark, and leaf extracts of *P. pinnata* has a synergistic effect in causing mortality of *Aedes aegypti* (Linnaeus) (Al-Solami et al. 2014). Methanol seed extracts of *P. pinnata* deterred gravid females of *Helicoverpa armigera* (Hübner) from egg-laying and larvae feeding on the substrate. The adults feeding on the treated subject showed a conspicuous reduction in egg-laying capacity, and lower hatchability (Reena et al. 2012). Methanol seed extracts of *P. pinnata* possessed excellent insecticidal activity against *Nilaparvata lugens* (Stål) (Hiremath et al. 1997). Seed extracts of *P. pinnata* also contain certain active compounds effective against subterranean termite, *Odontotermes obesus* (Rambur) (Verma et al. 2011). However, extracts from the wood of *P. pinnata* have not been tested as wood preservatives against subterranean termites. This tree is locally known as Sukhehein or Sukh-Chain in Urdu, Karanja or Karanj in Hindi, and Indian beech in English (Sangwan et al. 2010). In the present study, the toxicity of *P. pinnata* heartwood extracts was tested against a subterranean termite, *Coptotermes heimi* (Wasmann), via exposing termites to heartwood compounds extracts on the filter paper. Extracts were removed from wood through cold extraction using four solvents, and their toxicities were tested and then were used to preserve non-durable wood against subterranean termite species

MATERIALS AND METHODS

Selection of woods and sample preparation

A small log of *P. pinnata* wood was obtained from Patron Potato Processing Factory, Depalpur, Pakistan, that was cut from its main log. The size of this small log was 55 × 200 cm having more than 70% of heartwood. *Populus deltoides* (30 × 100 cm) was purchased from the Timber Market of Hujra Shah Muqeem, Okara, Pakistan. For choice and no-choice tests, wooden stakes of 10cm (t) × 5cm (r) ×2 cm (l) size from *P. pinnata* and *P. deltoides* (fresh) were prepared by using an electric saw from freshly cut air-dried logs. All the stakes were free of knots and showed no infection by molds, stains, or wood-destroying fungi and insects. To prepare extracts, a small part of *P. pinnata* heartwood was converted into shavings using a fine electric planer.

Preparation of extracts

Wood shavings were air-dried under shade in Termite Research Laboratory before extraction. Four solvents (water, ethyl acetate, hexane and petroleum ether) were used to extract wood shavings. We followed a method adopted by Ahmed et al. (2018) to prepare extracts. A total of 115g of air-dried wood shavings were added in a reagent bottle containing 2.5 liters of each solvent separately. The bottles were shaken at regular intervals for 20 days. The obtained extract-solvent mixture was then filtered using a muslin cloth, and the filtrate of each solvent was evaporated through a rotary evaporator. After evaporation, stock solutions were made (10 mg/ml) to prepare different concentrations (2.5mg/ml, 5mg/ml and 10mg/ml) in their respective solvents to test the termiticidal activities in different experiments.

Collection of termites

Termite workers were collected from the wood buried in the ground and corrugated cardboard in PVC monitors installed at the PARS, Jhang Road, University of Agriculture, Faisalabad, and from Botanical Garden, Forman Christian College, Lahore (Ahmed et al. 2014). Termites collected from different places were brought to the laboratory and identified using available key (Ahmad 1955, Akhtar 1975). Termite was identified as *Coptotermes heimi* (Wasmann) and was kept in Petri plates (90mm×15mm) containing moist filter papers in the laboratory at 26 ± 2°C, and 80% R.H.

Natural resistance of *P. pinnata* wood in choice and no-choice field tests

Stakes of *P. pinnata* heartwood (10cm x 5cm x 2cm) were installed in the field to determine its natural resistance against subterranean termites along with *P. deltoides* stakes (10cm x 5cm x 2cm) as a positive control (Ahmed et al. 2014). In the choice test, each of *P. pinnata* sun-dried, *P. pinnata* fresh, and *P. deltoides* (fresh) stakes were tied together with the help of copper wire in different combinations (*P. deltoides* + fresh *P. pinnata* + *P. deltoides, P. deltoides* + dried *P. pinnata* + *P. deltoides,* fresh *P. pinnata* + *P. deltoides* + fresh *P. pinnata,* dried *P. pinnata* + *P. deltoides* + dried *P. pinnata,* and fresh *P. pinnata* + *P. deltoides* + dried *P. pinnata).* These tied wooden stakes were offered to pre-invited termites by burying them underground in the field for 28 days to examine the weight losses in termites' highly infested site at PARS, Jhang Road, University of Agriculture, Faisalabad. In the no- choice test, wooden stakes from sun-dried *P. pinnata,* fresh *P. pinnata,* and *P. deltoides* (fresh) were buried underground separately for 28 days to examine the weight losses. There were three replicates for each treatment in each test, and the experiment was laid out in Randomized Complete Block Design.

Mortality bioassay on treated filter paper

We followed the method described by Hassan et al. (2017) with some modifications for filter paper toxicity bioassay. Briefly, the experiment was conducted in small plastic trays with Whatman filter paper No. 42 of 9 cm diameter. Filter papers were treated with extracts at three different concentrations (2.5mg/ml, 5mg/ml, and 10mg/ml) along with control treatments (with respective solvents and water). After drying of filter papers in the fume hood, 50 termite workers were released in the plastic tray. There were three replications for each treatment. The mortality was recorded every 3 hours up to 24 hours by counting the live number of termites in all treatments. Mortality and weight loss of filter paper were calculated at the end of the experiment.

Repellent and Antifeedancy Tests

Whatman filter papers (9 cm diameter) were sliced into two halves; one-half of filter paper was treated with 1 ml of each concentration of extracts, and the other half was treated with the respective solvent only. One-half was treated either water or solvent for water and solvent control while the second half was left untreated. After drying the filter paper under the fume hood, both halves (extracts treated and only respective solvent/water treated control) were rejoined by placing the adhesive tape underside of the filter paper halves. These rejoined filter papers were then placed in Petri dishes (diameter; 9.1 cm), and 50 active termites were released in the center of the rejoined filter paper. The number of termites on treated and untreated halves were recorded after 60 minutes. To calculate percent repellency following formula was used (Kadir et al. 2014).

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

Where Nc is the total number of termites in the control halve while Nt is the total number of termites on the treated filter paper halve. Antifeedancy indices (Absolute coefficient of antifeedancy A) were determined on the basis of weight loss of filter paper during filter paper bioassay using the following formula (Hassan et al. 2018a).

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

Where KK= Weight loss of filter paper in control treatment, EE= Weight loss of treated filter paper.

Efficacy of wood extracts of *P. pinnata* against subterranean termites on *P. deltoides* wood in field tests

Weighed and conditioned (33°C and 62±3% R.H.) blocks of *P. deltoides* (45 mm×19 mm ×19 mm) were treated by dipping in extracts, and vacuum pressure treatment at different concentrations (2.5, 5 and 10 mg/ml) of *P. pinnata* heartwood extracts separately. For control treatments, blocks were treated with respective solvents only. *P. pinnata* blocks were dipped in a 500-ml beaker with each extract concentration for 72 hours for dipping treatment. While for vacuum pressure treatment, three blocks for each treatment were placed in a beaker with the respective concentration of extracts. The beaker containing blocks was held under vacuum for 60 min, and then the pressure was applied at 40 psi for 60 min. Then blocks were blotted, dried using paper towels, weighed, and re-conditioned at 33°C, and 62±3% R.H. Treated blocks were then offered to pre-invited termites by burying them underground at the site mentioned above. All treatments were replicated three times. Data on weight loss were taken after 28 days, and mass losses of the wooden blocks was determined by using the following formula (Ahmed et al. 2014).

$$Weight\ loss = \frac{W_1 - W_2}{W_1} \times 100$$

Where W_1= Initial weight of the block, W_2= Final weight of the block.

Statistical analysis

Mortality, repellency, antifeedancy, and weight loss data were analyzed using a factorial design. Data obtained were analyzed by using Minitab16 and Statistix statistical software.

RESULTS

Natural resistance of *P. pinnata* wood against subterranean termites in choice and no-choice field tests

Weight loss of *P. pinnata* wood offered to termites in choice, and no-choice test is shown in Table I. Overall, *P. pinnata* wood was resistant to termites when compared with *P. deltoides* wood (positive control). Weight loss was significantly higher (21.79%) in the case of *P. deltoides* even with sun-dried *P. pinnata* combination and fresh *P. pinnata*. Whereas the lowest weight loss (6.38%) was recorded in sun-dried *P. pinnata* in the choice test. In a no-choice test, dried *P. pinnata* was moderately resistant against termites, and significantly lower weight loss (12.37%) was recorded.

Filter paper toxicity test

Mortality of termites after 24 hours of exposure to different extracts at three concentrations is shown in Fig. 1. Mortality was significantly higher (41.66%) in termites fed on filter paper treated with ethyl acetate extract at 10 mg/ml concentration. However, at lower concentrations of ethyl acetate extracts, mortality differed

Table I. Comparison of mean weight losses of fresh *P. pinnata*, sun-dried *P. pinnata*, and *P. deltoides* after 28 days exposure to subterranean termites in choice and no-choice field tests.

Wood types	Weight loss (%)					
	Choice test					No-choice test
	P+F+P	P+D+P	F+P+F	D+P+D	P+D+F	
Fresh *P. pinnata*	10.73±1.55[b]	-	8.57±2.17[b]	-	7.66±2.17[b]	16.05±2.97[B]
Dried *P. pinnata*	-	6.38±1.51[c]	-	8.46±1.54[c]	8.88±1.39[c]	12.37±1.75[C]
Fresh *P. deltoides*	20.84±1.15[a]	20.56±0.38[a]	20.10±0.15[a]	21.79±0.92[a]	20.82±0.30[a]	35.61±7.56[A]

Means sharing different letters are significantly different from one another F=13.76, 9.42, $p<0.05$. P= *P. deltoides*, F= fresh *P. pinnata*, D= dried *P. pinnata*.

non-significantly with other extracts except water extract, the latter recorded the lowest mortality. After feeding on filter paper, the mortality of termites treated with water extract was statistically similar with hexane and petroleum ether extract at 10 mg/ml. Minimum mortality (<12%) was observed in termites fed on filter paper treated with only solvents or water (control treatment).

Repellency test

Termites were significantly very repellent towards ethyl acetate extract of *P. pinnata* wood at the highest concentration (10 mg/ml) compared to its solvent control that differed significantly from petroleum ether (96%) and n-hexane (86.67%) extracts. At 2.5 mg/ml concentration, ethyl acetate extracts' repellency was similar to n-hexane and water extracts. The lowest repellency was seen in water extract at all concentrations where an almost equal number of termites were present on extract and water treated halves of filter paper. Repellency in water extract had a significant difference with petroleum ether and hexane extracts in addition to ethyl acetate extracts (Fig. 2).

Anti-feedant test

Antifeedant activities of different concentrations of *P. pinnata* n-Hexane, petroleum ether, ethyl acetate and water extracts are shown in Fig. 3. Results of this test were not very much different from the repellency test at the highest concentration, whereas behavior was not evident at lower concentrations. Extracts at low concentrations showed non-significant antifeedant values with water extracts which was not the case in the repellency test (Fig. 3).

Field trials

Maximum weight loss (51%) was observed in the control treatment, while minimum percent weight loss (14%) was observed in wooden stakes treated with ethyl acetate extracts (10 mg/ml). Controls in each extract case had significantly high weight loss when compared with the corresponding solvent under the dipping experiment (Fig. 4). Weight loss in wooden stakes treated with extracts under vacuum pressure application presented the similarity with dipping experiment (Fig. 5); however, numerically, weight loss was less than that of dipping experiment. Overall, ethyl acetate extracts showed more protection of *P. deltoides*, followed by petroleum ether, hexane, and water extracts.

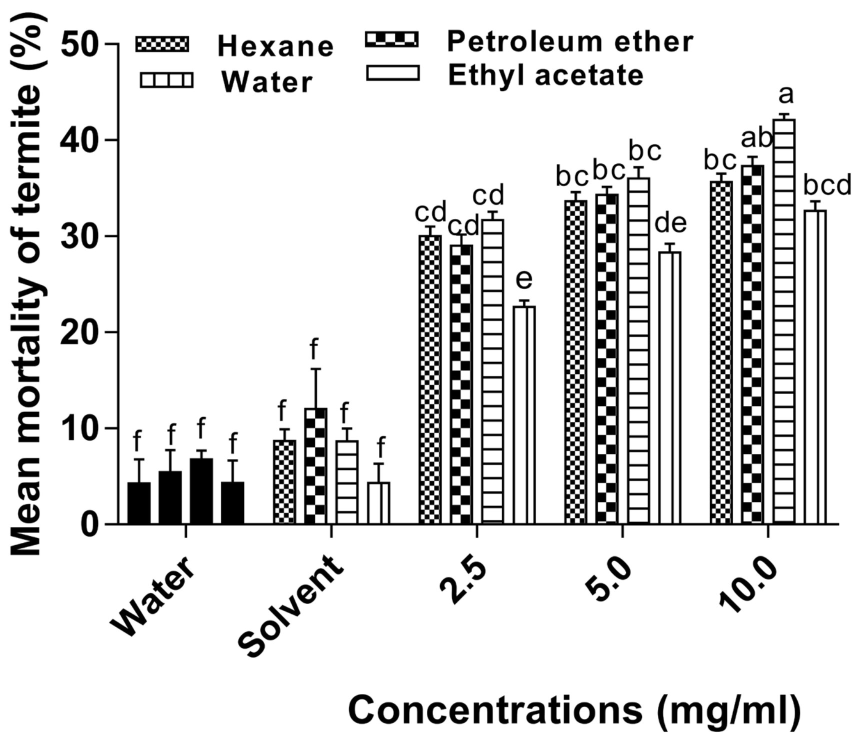

Figure 1. Mortality of termites exposed to different concentrations of n-Hexane, petroleum ether, ethyl acetate, and water extracts of *P. pinnata* wood. Means sharing different letters are significantly different from one another. F=6.60, p<0.05.

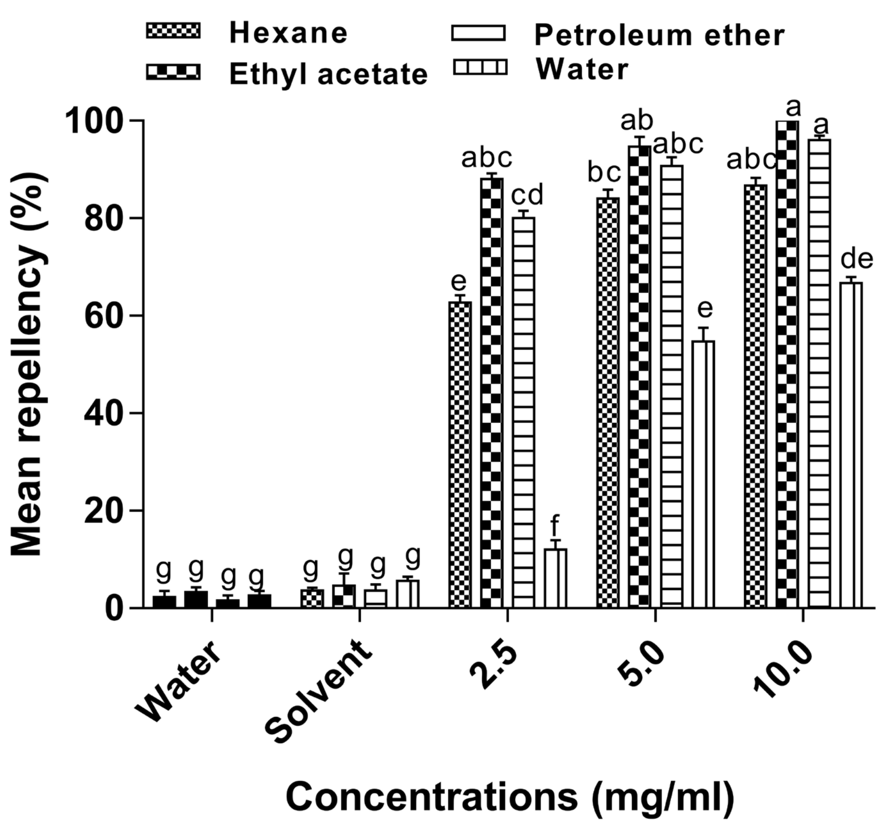

Figure 2. Repellent effect of n-Hexane, petroleum ether, ethyl acetate, and water extracts of *P. pinnata* wood against termites. Means sharing different letters are significantly different from one another. F=14.32, p<0.05.

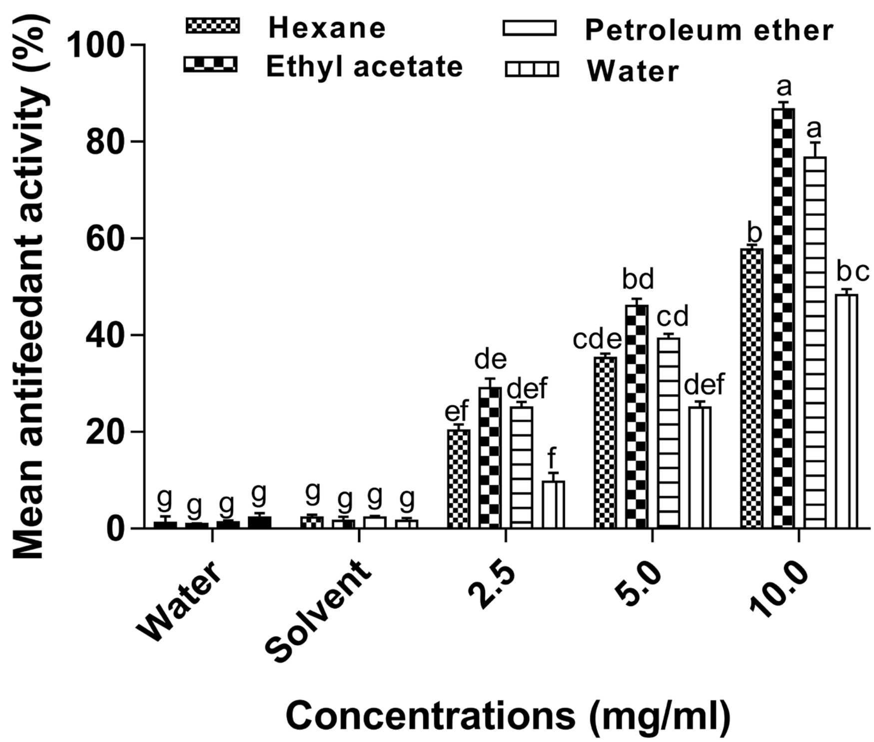

Figure 3. Antifeedant activities of different concentrations of n-Hexane, petroleum ether, ethyl acetate, and water extracts of *P. pinnata* wood against termites. Means sharing different letters are significantly different from one another. F=3.03, p<0.05.

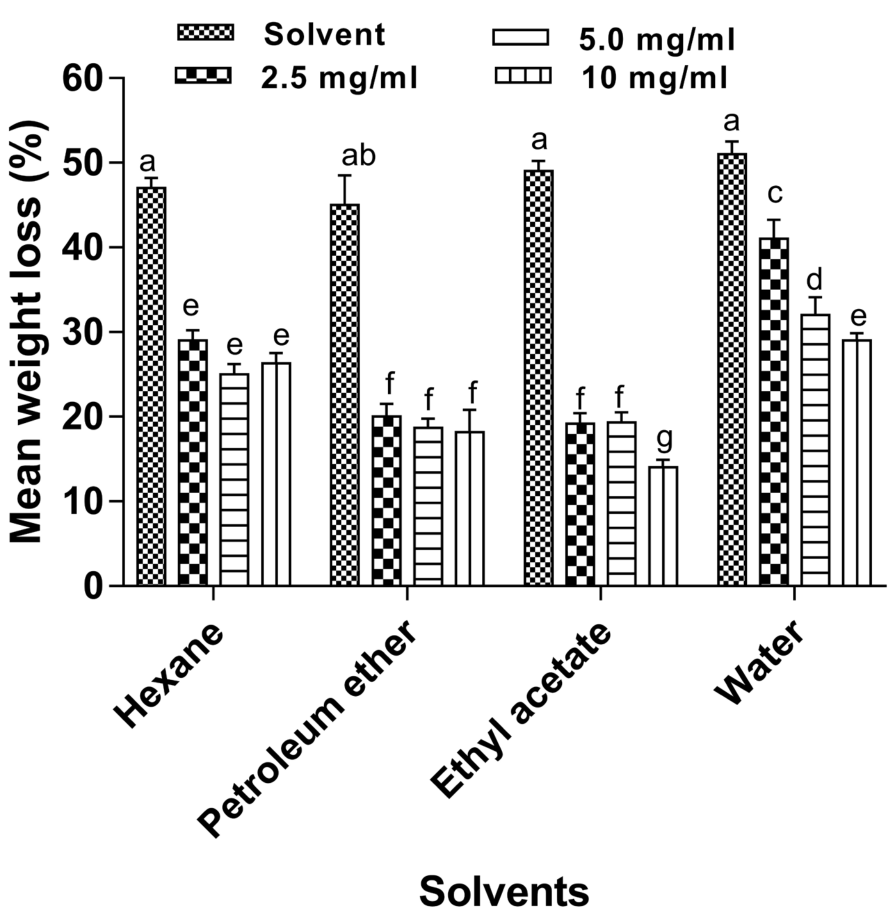

Figure 4. Mean weight loss of *P. deltoides* stakes dipped in different concentrations of n-Hexane, petroleum ether, ethyl acetate, and water extracts and exposed to termites in the field. Means sharing different letters are significantly different from one another. F=44.37, p<0.05.

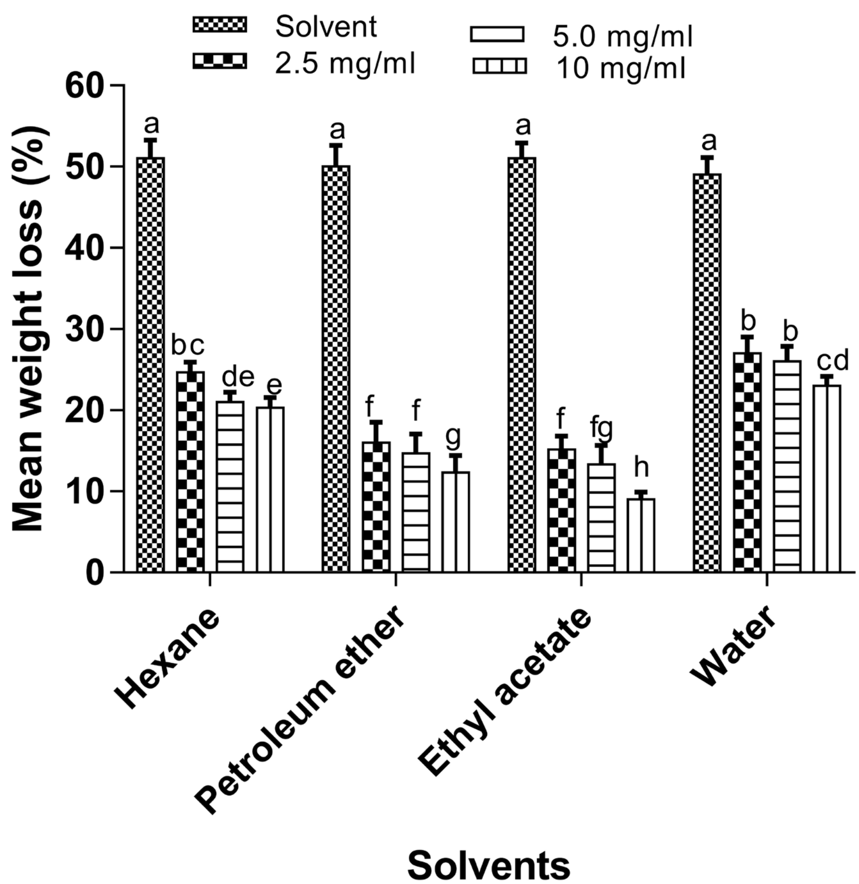

Figure 5. Mean weight loss of *P. deltoides* stakes pressure treated with different concentrations of n-Hexane, petroleum ether, ethyl acetate, and water extracts and exposed to termites in the field. Means sharing different letters are significantly different from one another. F=49.39, $p<0.05$.

DISCUSSION

Natural resistance of dried *P. pinnata* wood was confirmed under field conditions using choice and no-choice tests. Wood extracts in organic solvents exhibited higher toxicity, repellent, and antifeedant activities than other solvents and water, especially at the highest concentration. Wooden stakes treated with ethyl acetate extracts had significantly lower weight loss as compared to other solvent extracts. Leaf and seed extracts of *P. pinnata* have been explored earlier as the insecticide, particularly *Pongamia* oil has been implicated in several bioassays with insects other than termites (Hiremath et al. 1997, Pavela 2009, Samuel et al. 2009, Reena et al. 2012, Al-Solami et al. 2014). Yet, the termiticidal potential of non-edible oil seedcakes, including *Pongamia* (karanja) and their crude active component (karanjin), has not been evaluated previously against *Coptotermes heimi*. In previous studies, Crude extracts of *P. pinnata* caused 83.3% mortality of *Odontotermes obesus* (Rambur) after 2 hours of exposure while all termites were dead after 4 hours in Petri plates (Sharma et al. 2011). Verma et al. (2011) observed that the petroleum ether extracts of *P. pinnata* and *J. curcas* seeds caused 100% mortality of *Odontotermes obesus* after 6 hours of exposure. Previous studies showed that stem wood and other parts of *Pongamia* contain a larger amount of flavonoids such as kaempferol, desmethoxykanugin, tetra-o-methyl fisetin, karanjin, and kanugin. These flavonoids usually involved in the protection of wood against harmful abiotic as well as biotic factors. Plant flavonoids affect insect cytochrome P-450 dependent steroid hydroxylases and act as antifeedant and digestibility reducers in insects (Rangaswami et al. 1942, Murti & Seshadri 1945, Mitchell et al. 1993, Mierziak et al. 2014). Previous studies showed that *P. pinnata* extracts are quite effective against pests of stored grains and field crops and household insect pests (Kumar & Singh 2002).

Pongamia pinnata oil also increased termites' resistance of treated wood compared *J. curcas* and *S. glauca* oils in the previous studies. Up to only 10% deterioration was observed in wood specimens treated with pure and copperized *Pongamia* oil (Venmalar 2017). Results showed significantly less weight loss of *P. deltiodes* than the control treatment; however, complete protection of treated wood was not found (Ahmed et al. 2014, Abbas et al. 2016).

In the field experiments, *P. deltoides* wooden stakes impregnated with extracts through dipping and vacuum pressure techniques resulted in low weight loss compared to control treatments. The reduction in weight loss due to ethyl acetate extract was 82%. These results are in agreement with those described by Antwi-Boasiako & Damoah (2010), Syofuna et al. (2012), Kirker et al. (2013), Adeduntan (2015), Hassan et al. (2016) and Adedeji et al. (2017), but extracts were of different wood species.

Results of this study indicated that *P. pinnata* heartwood extract had a significant negative impact on termite activity. These wood extracts were repellent, termiticidal, and antifeedant to tested termite species. Tested wood extracts provided resistance of non-durable *P. deltoides*. These are foundational studies to establish the biological relevance of *P. pinnata* heartwood extract. Future studies will provide a targeted investigation of extract components to isolate bioactive properties from ethyl acetate extracts against termites.

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Sohail A conceived the idea and supervised the experiments. Muzammal HT performed the experiments in the lab and field. Babar H contributed with the data analysis and wrote the manuscript. All authors have read and approved the final manuscript.