Revista Brasileira de Entomologia 64(1):e201998, 2020

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution
www.rbentomologia.com

Termiticide activities of wood extractives of *Ziziphus mauritiana* (Rhamnaceae) against subterranean termites under field conditions

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ARTICLE INFO

Article history:
Received 16 September 2019
Accepted 19 December 2019
Available online 31 January 2020
Associate Editor: Eliana Cancello

Keywords:
Natural products
Wood preservation
Environment friendly
Termite
Wood extracts

ABSTRACT

Synthetic wood preservatives are the causes of large-scale environmental pollution and few have been withdrawn from the commercial markets in the past years. The present studies focused on determination of naturally present extractives of *Ziziphus mauritiana* as less toxic wood protectant against subterranean termites. Firstly, natural resistance of *Z. mauritiana* heartwood against termite was determined by exposing stakes in the field. For the preparation of extractives, air-dried *Z. mauritiana* heartwood and bark shavings were soaked in 1 liter each of ethyl acetate, hexane, petroleum ether and water in a bottle separately. Different dipping treatment times (36 and 72 hours) at 10, 20 and 30% concentrations of extractives on *Populus deltoides* wooden stakes were used and stakes were exposed to termites in submerged manner. Combination of extractives in different solvents were included as separate experiment and finally, seasoning prior to extractives application on *P. deltoides* wooden stakes was also done and stakes were arranged in three replications for each treatment. Maximum mean percent weight loss (81.1%) was observed in case of *P. deltoides* followed by boiled *Z. mauritiana* (15.24%) in termite resistance test. Stakes treated with petroleum ether extracts had minimum weight loss alone or in combination with other extract's solvent in all experiments. Extractives in other solvents followed petroleum ether non-significantly but were significantly different from their respective control treatment, which had the highest weight loss (>60%). Transferring durability using extracts of *Z. mauritiana* increased resistance of non-durable *P. deltoides* against termites and extractives could be used as wood preservatives.

Introduction

Subterranean termites are a major pest of human structures throughout tropical and sub-tropical regions, causing billions of dollars in damage to timber-in-service worldwide (Ahmed and French, 2008). Thirteen species of termites out of pool of fifty were identified as pests of constructions in rural and urban areas of Pakistan, which had caused economic losses. Subterranean termites are considered one of the most destructive termites in in Pakistan and the economic loss to timber in service by termites constitutes the greatest problem compared to other wood-destroying insects in the country (Hassan, 2017; Hassan et al., 2018c).

The trend for the management of termite using chemicals has changed over recent years because of environmental concerns. Wood extractives from resistant tree species have been realized as alternatives to water and oil borne wood preservatives since latter are being suspected for environmental concerns due to persistent effect and hazardous to applicators as well (Lee et al., 2018). Severe soil contamination has been reported because of traditional wood preservation activities using creosote, copper chromium arsenate (CCA) and pentachlorophenol (PCP) (Ottosen et al., 2002). Number of plant extractives including oils, waxes, resins, tannins and/or polyphenols, are toxic and thus not only inhibit the growth of saproxylic but also of other wood degrading organisms resulting in lower rates of wood decay. Usually heartwood is more resistant than the sapwood due to its higher lignin content, density and to the presence of certain biocide substances (Bajraktari et al., 2018). These extractives can replace chemicals currently used in wood preservation. Previously, stem wood extractives of *Albizia lebbeck, Tectona grandis, Dalbergia sissoo, Cedrus deodara, Pinus roxburghii, Morus alba* and *Morus nigra* have been demonstrated to reduce weight loss in treated wooden stakes under field conditions against subterranean termites (Hassan et al., 2017a; Ahmed et al., 2018; Hassan et al., 2019a, b, c).

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https://doi.org/10.1590/1806-9665-RBENT-2019-98

Besides extracts / extractives, plant oils have anti-feedant and growth regulating effects on termites (Islam et al., 2009).

Almost 100 species of deciduous shrubs and trees belong to Genus *Ziziphus*, Family *Rhamnaceae*, which grow well in tropical and subtropical areas of the World. Some species, like *Z. mauritiana* occurs in almost every green continent. *Ziziphus* spp. survive on extremely drought conditions and are excellent source for natural vegetation in deserts of Indo-Pak (Razi et al., 2014). Leaf phenolic extracts of *Z. mauritiana* was effective for controlling *Aedes* mosquitoes by decreasing fecundity, fertility and adult life span (Devi and Bora 2017). Betulinic acid, a terpenoid, from bark of *Z. mauritiana* showed growth inhibition of *Spodoptera litura* and *Tribolium confusum* (Lingampally et al., 2012; Badathu et al., 2014). Crude methanolic extract of *Z. jujuba* showed significant termiticide activity against *Heterotermes indicola* and proved itself as potent source of cytotoxic drugs (Ahmad et al., 2011). Oil of *Ziziphus mauritiana* has high larvicidal potency against the 3rd larval instar of *Culex pipiens* (El-Husseiny et al., 2014). Oil of *Z. jujuba* also showed significant reduction in insect sex ratio, egg hatchability in *C. pipiens* (El-Husseiny and Ei-Kholy, 2015).

However, there is little information about toxicity of *Ziziphus* wood extractives against insect pests especially termites. *Ziziphus* is not reported termite resistant but is not found susceptible to it as far as scientific literature is concerned. Evaluation of tree wood sensitivity towards the termites is not fully explored. Therefore, the objective of this study was to evaluate efficacy and toxicity of wood and bark extractives isolated in polar and non-polar solvents against subterranean termites.

Materials and methods

Wood source and preparation of samples

Heartwood logs and bark of *Ziziphus mauritiana* (Ber) and a sapwood log of *Populus deltoides* were purchased from Timber Market located at Jhang Road, Faisalabad. Wooden stakes of *P. deltoides* and Z. *mauritiana* were prepared for anti-termite bioassays under field conditions using electric saw. Wooden stakes measuring 130L x 50T x 20R mm (LTR = longitudinal, tangential and radial) were excised from heartwood of Z. *mauritiana* and sapwood of *P. deltoides* logs. Logs were with no observable sign of infection by mold or wood-destroying fungi and termite.

Confirmatory test for resistance of Z. *mauritiana*

A preliminary test to determine potential termiticide in *Z. mauritiana* wood extractives, a set of four wooden stakes were dipped into hot water in a water bath set at 80°C for 12 hours. Before dipping, moisture contents of stakes were determined by oven dry method. Dipped stakes were taken out and dried and weighed to pretreatment moisture contents. These stakes were tied using cable with fresh wooden stakes of similar dimension (130 x 50 x 20 mm LTR) from *Z. mauritiana* in the following order: fresh-boiled-fresh, boiled-fresh-boiled, fresh-boiled, fresh and boiled. Tied stakes (in triplicate) were placed in termite-infested soil pits already prepared for this purpose (Ahmed et al., 2014; Hassan et al., 2019a). Briefly, concrete soil pits (0.48×0.32 m wide and 0.40 m deep) were built inside Entomological Research Laboratories, at Post Graduate Agriculture Research Station, University of Agriculture Faisalabad, Pakistan. Pit floor was cemented leaving a border enough for the entrance of termites and to prevent rodents. Top of the pit was covered with iron sheet and soil was lined around the corners of iron top to seal it from rain. Soil pits were constructed in vicinity of subterranean termite nest as evidenced by the presence of nymphs in poly (vinyl chloride) corrugated monitoring pipes having cardboard as a bait (Ahmed et al., 2014). The wood specimens were placed horizontally on the cemented floor for experiments. Three fresh stakes of *P. deltoides* (130 x 50 x 20 mm LTR) served as positive control treatment. Wooden stakes of all kinds were sampled after 90 days and wood weight loss was determined by following formula:

$$Weight\,loss(\%) = (W1 - W2)/W1 \qquad (1)$$

Where, $W_{1=}$ Initial weight of stake; $W_{2=}$ Final weight of stake.

Preparation of Extractives

Air-dried *Z. mauritiana* heartwood and bark were converted into wood shavings using a planner separately. A total of 250 g of heartwood and bark shavings were soaked in 1 liter each of ethyl acetate, hexane, petroleum ether and water in a bottle separately which was shaken at regular intervals for 20 days. After filtration, solvents were evaporated through rotary evaporator. Three concentrations (10%, 20%, and 30%) of solid material in the respective solvents were prepared to test the termiticidal properties.

Treatment of P. deltoides wood with extractives of Z. *mauritiana*

P. deltoides wooden stakes were treated by dipping (36 and 72 hours) with different concentrations (10%, 20%, and 30%) of *Z. mauritiana* heartwood and bark extractives separately. Treated stakes were offered to pre-invited termites in soil pits (partially buried in the pits as described above) in three replications for each treatment and only solvent treated stakes were controls. Data were taken after 60 days and weight loss of stakes was determined by above mentioned formula.

Treatment of P. deltoides wood with combined extractives of Z. *mauritiana*

Extractives from four solvents were combined in the following fashion in 1:1 ratio (w/w). Ethyl acetate + petroleum ether; Hexane + ethyl acetate; Hexane + petroleum ether; Water + petroleum ether; Water + ethyl acetate; Water + hexane. Stakes of *P. deltoides* were dipped in concentration (30%) of each combined extractive of *Z. mauritiana* bark and heartwood for 36 and 72 hours. Treated stakes were offered to pre-invited termites in the pits (as described above) and weight loss was calculated after 60 days exposure.

Efficacy of extractives on oven dried woods of P. deltoides

Wooden stakes of *P. deltoides* were dried at 80°C for 7 days. Stakes were soaked in each of water, n-hexane, petroleum ether and ethyl acetate extractives for 72 hours from heartwood and bark extractives separately. Treated stakes along with control treatment were placed in soil pits infested with termites as in above experiments and after 60 days, wood weight loss was determined.

Statistical analysis

Completely Randomized design was used to conduct field experiments and data were analyzed using Analysis of variance test. Means were separated at the 5% level of significance using Tukey's HSD in GraphPad Prism software.

Results

Differences of mean percent weight loss of different combinations of fresh and boiled *Z. mauritiana* along with *P. deltoides* as positive control after 90 days of exposure to subterranean termites is presented in Fig. 1. Weight loss of *P. deltoides* (positive control) was maximum followed by boiled *Z. mauritiana*. While consumption of wood from combinations; fresh + boiled + fresh, boiled + fresh + boiled, fresh + boiled, fresh and boiled *Z. mauritiana* was significantly low (F= 135.9; p<0.01), but not significantly different from one another. Maximum mean percent weight loss (81.1%) was observed in case of *P. deltoides* followed by boiled *Z. mauritiana* (15.24%). While non-significant mean % weight losses of 1.65, 5.12, 4.53 and, 3.3.1 in fresh + boiled + fresh *Z. mauritiana*, boiled + fresh + boiled *Z. mauritiana*, fresh+ boiled *Z. mauritiana*, boiled *Z. mauritiana,* fresh *Z. mauritiana*, respectively, were recorded. *P. deltoides* and boiled *Z. mauritiana* were susceptible to termite attack as compared to woods from fresh and boiled *Z. mauritiana* combinations and fresh *Z. mauritiana* only.

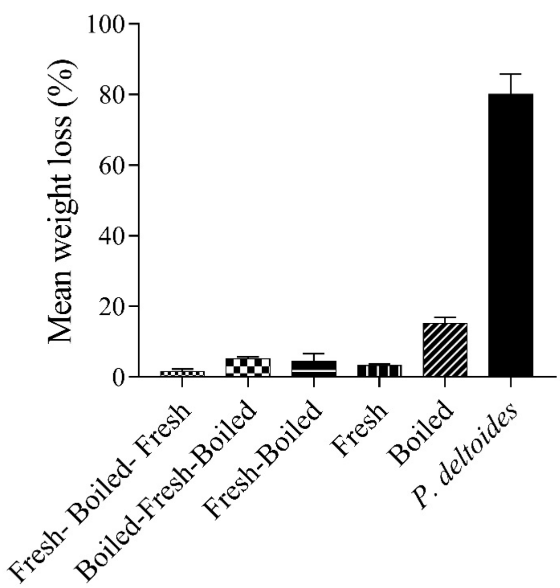

Figure 1. Comparison of mean weight loss (%) of Z. *mauritiana* and *P. deltoides* under choice and no-choice test after 60 days exposure to subterranean termites.

Regarding results of extractives treatments, there was significant difference in weight loss of *P. deltoides* wood dipped for 36 h in heartwood extractives in each concentration (F= 62.41; p<0.0001) and each type of extractives (F= 10.8; p<0.01). Similarly, wood dipped for 72 h in heartwood extractives also showed significant difference in each concentration (F= 43.25; p<0.0001) and each type of extractives (F = 3.086; p<0.04). Maximum percentage of weight loss (68.94%) was observed in control treatment of petroleum ether, which was not significantly different from other control treatments. Minimum weight loss (22.41%) was observed in wooden stakes treated with water extractives from heartwood at concentration of 30 mg ml^{-1}. Concentrations of all solvents had non-significant difference among themselves as compared to control treatments for woods treated for 36 h and 72 h. Water extractives was followed by ethyl acetate, petroleum ether and n-hexane extractives in effectiveness after 36 and 72 hours of dipping (Fig. 2 AB). There was no significant effect of dipping time on wood weight loss of *P. deltoides* after treatment with heartwood extractives (F= 0.90; p =0.78).

In case of bark extractives, there was no-significant difference in wood weight loss of *P. deltoides* after treatment for 36 h (F = 0.58; p=0.68) and 72 h (F = 0.91; p=0.44) with bark extractives in different solvents. There was no-significant difference among concentrations of each type of extractives in performance except control treatment when wood dipped for 36 h (F= 317; p<0.0001) and 72 h (F= 67.35; p<0.0001) (Fig. 3 AB). Maximum percentage weight loss (>70%) was observed in control treatments while minimum percentage weight loss (<10%) was observed in wooden stakes treated with different concentration of extractives from each solvent except at 30% concentration of petroleum ether extract. All concentrations had non-significant differences among themselves (Fig. 3 AB). There was no significant effect of dipping time on wood weight loss of *P. deltoides* after treatment with bark extractives (F= 2.06; p=0.15).

Among extractives type (heartwood vs bark), there was significant less weight loss of *P. deltoides* stakes was observed after treatment with bark extractives compared to all type of heartwood extractives (p<0.05) (data not shown).

Percent weight of poplar wooden stakes dipped in different combination (water + hexane, hexane + petroleum ether, hexane + ethyl

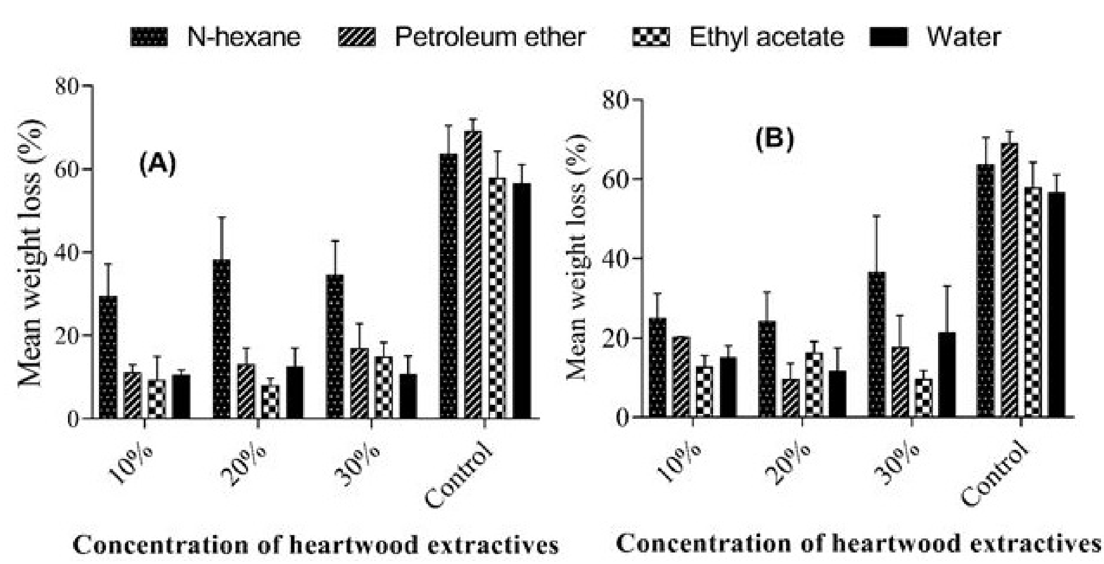

Figure 2. Comparison of mean weight loss (%) of *P. deltoides* wooden stakes dipped in different concentrations of n-hexane, petroleum ether, ethyl acetate and water solvents based *Z. mauritiana* heartwood extractives for 36 (A) and 72 (B) hours after exposure to termites.

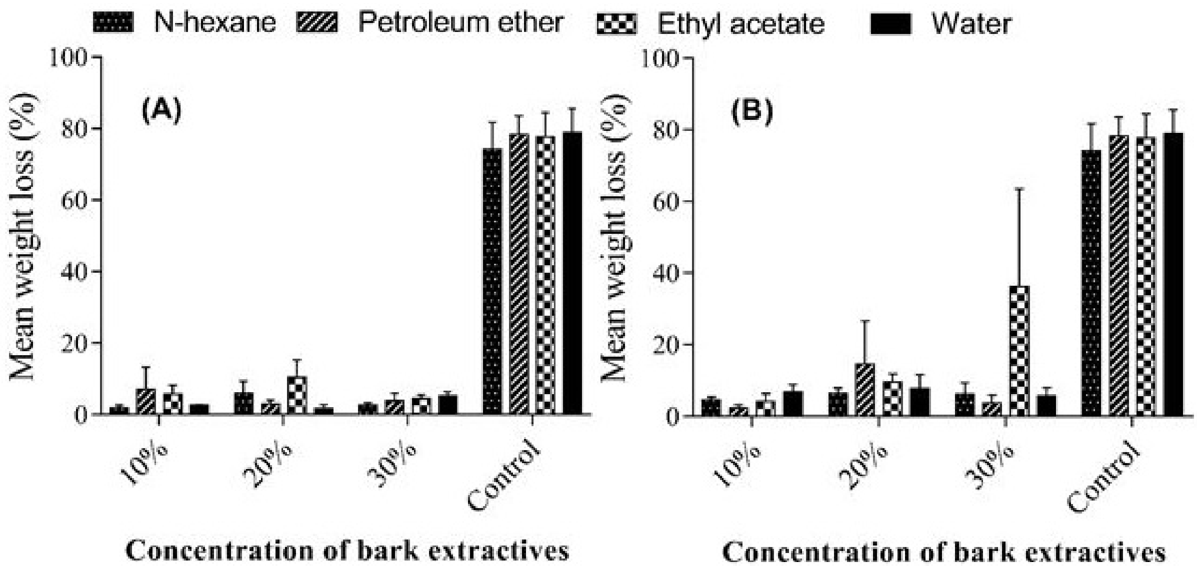

Figure 3. Comparison of mean weight loss (%) of *P. deltoides* wooden stakes dipped in different concentrations of n-hexane, petroleum ether, ethyl acetate and water solvents based *Z. mauritiana* bark extractives for 36 (A) and 72 (B) hours after exposure to termites.

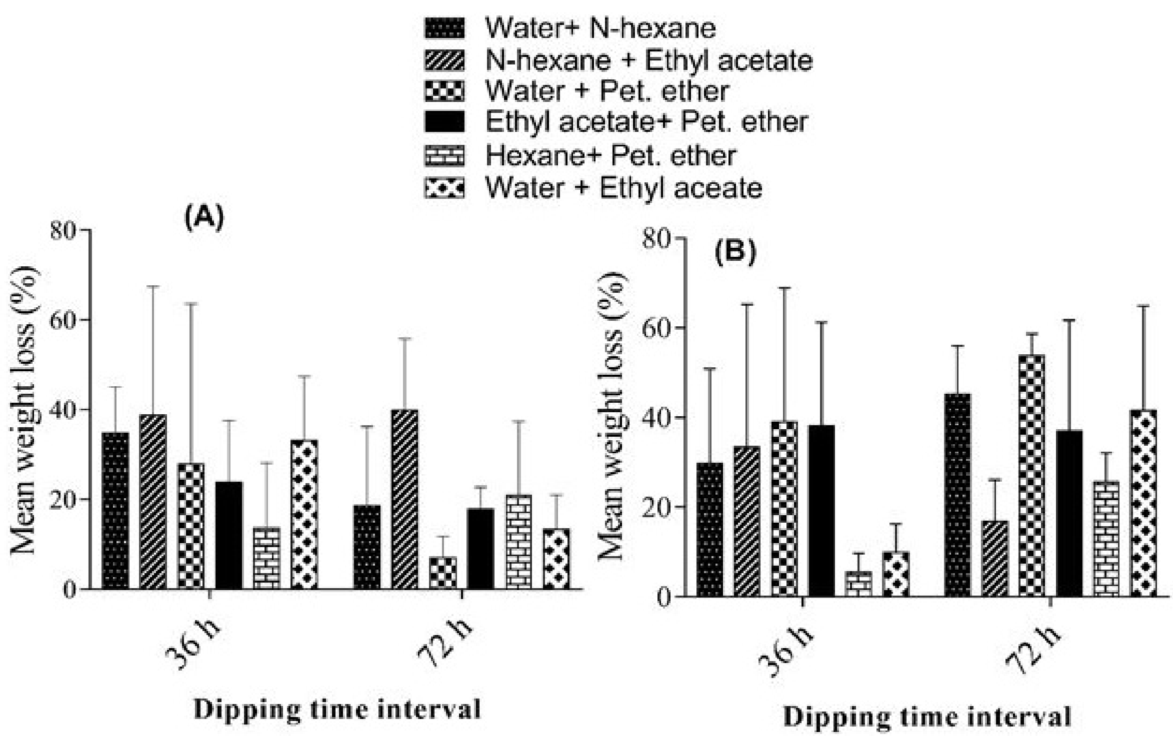

Figure 4. Comparison of mean weight loss of *P. deltoides* wooden stakes dipped for 36 h and 72 h in 30% concentrations of different mixture of *Z. mauritiana* heartwood (A) and bark (B) extractives

acetate, water + pet ether, water + ethyl acetate, and ethyl acetate + pet ether) of extracts for 36 and 72 hours exposed to termites in field for 60 days is presented in Fig. 4 AB. There was no-significant difference in wood weight loss after treatment with heartwood extractives at each concentration (F= 1.33; p=0.29) for each type of extractives at each time interval (F= 2.35; p=0.13). Similar results were observed for bark extractives at each time concentration and time interval (p>0.05). Maximum weight loss was observed in ethyl acetate $_+$ hexane based combination of extract which was not significantly different from rest of combination.

Results shows that when *P. deltoides* wood was oven dried at 80°C for seven days and then treated with heartwood and bark extractives. There was no significant difference among the different concentrations of each extractives from wood and bark except control treatment. Maximum weight loss (>70%) was observed in control treatment of each solvent while minimum weight loss was observed in wooden stakes treated with pet ether at concentration of 30 mg ml^{-1} after seasoning (80°C). Overall extract of *Z. mauritania* in hexane performed less activity against termites compared to other extractives (Fig. 5 AB).

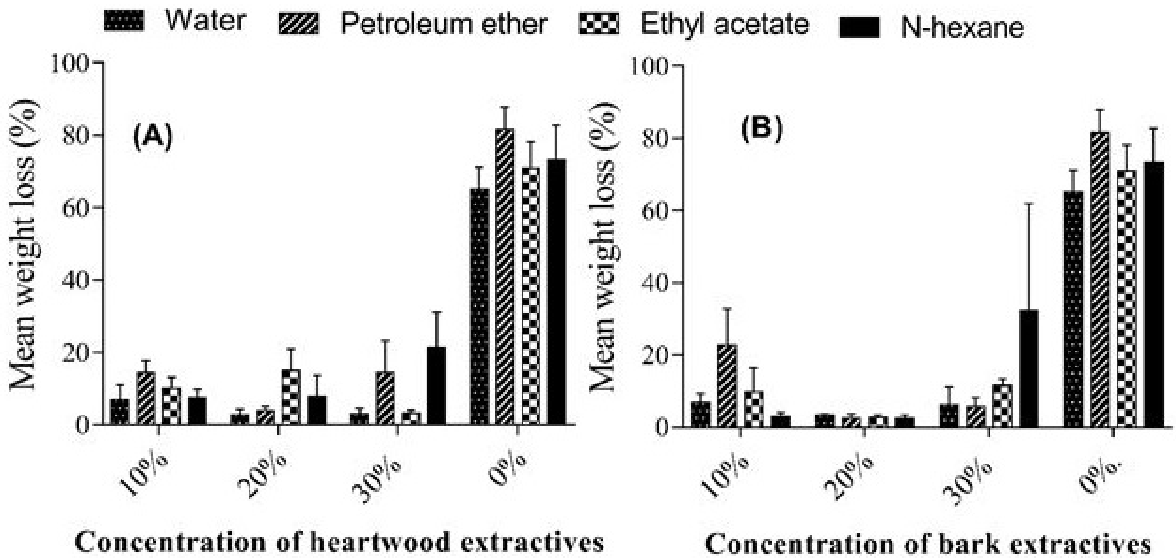

Figure 5. Comparison of mean weight loss of *P. deltoides* wooden stakes oven dried at 80°C for seven day and treated with different concentrations of *Z. mauritiana* heartwood (A) and bark (B) extractives by dipping for 72 h.

Discussion

The natural resistance of Z. *mauritiana* has been demonstrated by activities of heartwood and bark extractives in protection of a susceptible wood against termites in choice and no choice test. Maximum mean percent weight loss 81.1% was observed in case of *P. deltoides.* Weight loss of boiled *Z. mauritiana* was significantly higher compared to fresh + boiled + fresh *Z. mauritiana* 1.65%, boiled + fresh + boiled *Z. mauritiana* 5.12%, fresh+ boiled *Z. mauritiana* 4.53%, fresh *Z. mauritiana* 3.31% respectively. Dugal and Latif (2015) reported another species *Z. jujuba* resistant to *Coptotermes heimi* and *Heterotermes indicola* along with several other trees including *Mangifera indica, Erythrina suberosa, Betula utilis, Elaeis guineensis, Ficus religiosa, Heterophragma adenophyllum, Terminalia arjuna, Moringa oleifera, Putranjiva roxburghii,* and *Syzygium cumini.*

Efficacy of heartwood extractives of *Z. mauritiana* showed antitermitic activities at 10-30 mg ml^{-1} and recorded lowest weight loss in susceptible wood of *P. deltoides*. Petroleum ether and aqueous extractives had maximum termiticide activity and lowest weight loss at the highest concentration on longer period of treatment (dipping in extractive solution) as compared to other extractives in various solvents. However, this was not the case in bark extractives. There was non-significant effect of dipping time on performance as wood protectant. Purpose of seasoning was to reduce the moisture content of tested susceptible wood and then determine the toxicity of extractives against termites. Results showed that there was non-significant effect of seasoning of performance of extractives against termites.

Previous studies showed that fruits, seed and wood of *Z. mauritiana* have high concentrations of phenolic compounds such as epicatechin, vanillin, quercetin, naringenin and Kaempferol in the extracts (Memon et al., 2012; Ashraf et al., 2015). Phenolic compound has been identified as inhibitor of detoxification enzymes in the termites' gut (Hassan et al., 2018a) leading to lethal effect on termites. Many extractives present in naturally durable wood are not only toxic to termites but are also a rich source of antioxidants/radical scavengers (Hassan et al., 2018a, b), which may act synergistically to affect termite mortality. Previous studies also showed that heartwood extractives from resistant wood containing flavonoid and terpenoids were toxic to gut symbiotic communities of termites (Hassan et al., 2017b) which is another reason of extractives being lethal to termites. Beside this, extractives of several wood species act as repellant against termites (Hassan et al., 2016).

Insecticide and repellent activities of parts of *Ziziphus* spp. Other than wood have been documented. Ethanol and pet ether extracts of *Z. joazeiro* and *Z. jujube* against mosquitoes species have been reported (Omena et al., 2007; El-Husseiny et al., 2014; El-Husseiny & Ei-Kholy, 2015). Betulinic acid from the bark of *Z. jujuba* in acetone proved potent insect growth regulator and controlled *Spodopetra litura* effectively (Badathu et al., 2014). Lingampally et al. (2012) showed that a terpenoids from the bark of *Ziziphus jujube* acted as growth regulator against *Tribolium confusum*. Ahmad et al. (2011) revealed that crude methanolic extracts of aerial parts *Z. jujuba* were effective against the tested termites.

In addition, extracts of *Z. mauritiana* was also effective against microbes such as *Escherichia coli, Staphylococcus aureus, Xanthomonas axonopodis pv, Bacillus subtilis, and Staphylococcus aureus*, as well as anti-fungal activity against *Dreschlera turcica* and *Aspergillus flavus* (Najafi, 2013; Nkafamiya et al., 2013). Results of this study indicated that the transferring durability using toxic extractives from heartwood and bark of *Z. mauritiana* to non-durable *P. deltoides* improved the resistance against subterranean termites. Heartwood and bark extractives in petroleum ether had a significant negative impact on termite activity. These are foundational studies to establish biological relevance of the extractives from *Z. mauritiana*. Further studies are suggested to provide information about the active chemical(s) being toxic to termites which can be formulated wood protecting agent.

Acknowledgements

Authors are very thankful to the undergraduate students of Termite Research Laboratory, University of Agriculture Faisalabad, for their help during field experiments.

Conflicts of interest

All authors declare no conflict of interest for the manuscript entiltled "TERMITICIDE ACTIVITIES OF WOOD EXTRACTIVES OF Ziziphus mauritiana (Rhamnaceae) AGAINST SUBTERRANEAN TERMITES UNDER FIELD CONDITIONS"

Author contribution statement

We confirm that this manuscript "TERMITICIDE ACTIVITIES OF WOOD EXTRACTIVES OF *Ziziphus mauritiana* (Rhamnaceae) AGAINST SUBTERRANEAN TERMITES UNDER FIELD CONDITIONS" by Zoulfiqar et al., is original and has not been published elsewhere nor under consideration by any other journal. All authors have contributed equally to the manuscript and approve of its publication in this journal. All experiments were conducted according to the laws of the country where they have been performed. To the best of our knowledge, everybody who participated substantially in the study is not omitted from the article and all persons listed as authors qualify for authorship

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