

**REPELLENCY OF UNRIPE FRUIT EXTRACTS FROM *Melia azedarach* L. ONTO
Reticulitermes flavipes KOLLAR (ISOPTERA: RHINOTERMITIDAE)**

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Abstract

The world widespread North American native subterranean termite, Reticulitermes flavipes Kollar (Isoptera: Rhinotermitidae) is one the major biotic agents which damage wood and a severe pest in several countries, where it causes important economic losses and has strong economic and social impacts. In urban environments this termite is a pest that damage houses and wooden structures, generating considerable losses. In central Chile there have been foci of infestation with a big expansion in the last years. This study evaluated in laboratory the repellence effect of extracts from Melia azedarach L. (Meliaceae) unripe fruits onto R. flavipes workers. The extracts were elaborated from two solvents, distilled water, and ethanol. The repellence effect was determined by a direct selection test, in which termites in contact with extract treated or untreated filter paper disks were evaluated, and the results were subjected to comparison paired tests. The extracts from the unripe fruits of M. azedarach were effective as a repellent against R. flavipes workers, with the greatest repellence at the greatest concentrations (3.0, 5.6, 7.4, and 5.4, 7.5, 10.1% w/v for the ethanol and water extracts, respectively), with stronger effects of the ethanol than the water extract. The results demonstrate the repellence effectiveness of these extracts from M. azedarach onto R. flavipes, thus unripe fruits of this tree species could be considered in biopesticide preparation for integrated management of this pest, which also requires field evaluation and economic study.

Keywords: *Reticulitermes flavipes*, subterranean termites, repellency, *Melia azedarach*

1. INTRODUCTION

Reticulitermes flavipes Kollar (Isoptera: Rhinotermitidae) a subterranean termite native to North America that has spread worldwide, has a strong social and economic impact. This xylophage insect feeds on the cellulose in the wood with the help of protozoa and bacteria found in its digestive tract (Pearce 1997). The importance of the microbiome in the biology of termites is evidenced by their unique adaptations in behavior to process proctodeal food and allowing continuous transfer of fecal material between individuals in the nests to maintain the colonies (Boucias et al. 2012), which are organized into various castes. The king and queen establish the nest, and the fertile female lays hundreds of eggs from which nymphs emerge. These develop to workers, soldiers, supplementary kings and queens, and reproductive winged adults (Edmonds et al. 2011). Recently, Funaro et al. (2018) have expanded understanding of chemically mediated actual recognition in this termite species and helped to understand the evolution of insect societies. In urban environments this termite is a pest that damage houses and wooden structures, generating considerable losses. The wood damage is characterized by internal galleries to keep the humidity and temperature for development, and pellets of material displaced (Ripa & Luppichini 2004). In Chile, the first detection of a termite of the genus *Reticulitermes* occurred in 1986 in the Metropolitan Region which was identified as *R. flavipes* by

genetic studies. Ripa & Luppichini (2004), and Karsulovic et al. (2008) established prevention and population control methods for this termite.

Synthetic insecticides have been used effectively and widely for decades for reducing pests. However, some of them threaten human health and the environment, and also produce an evolution of insecticide resistance in pest populations. Botanical insecticides have acquired a great importance today as a substitute for synthetic insecticides because they cause lesser side effects to the environment and are an alternative for integrated pest management (Amri et al. 2013, Isman 2020). These products are plant-derived pesticides that are considered the most potential substitutes of the highly harmful synthetic pesticides, derived from plant roots, stems, leaves, flowers, fruits, seeds, and wood. More than 2,000 species of plants belonging to 60 families have pesticide activity, and many are used as insect growth regulators, ecdysone affecting substances, behavior modulators, feeding deterrents, repellents, attractants, and other effects (Verma et al. 2018). Botanical insecticides have been used in agriculture for at least 2000 years, particularly in Asia and the Near East (Thacker 2002). The interest in plant compounds for pest control is based in their efficacy, degradability, and physiological activity (Isman 2020). *Melia azedarach* L. (Meliaceae) is a tree native from Iran, India, China, and Australia (Hong & Ellis, 1998) which has important insecticide properties. In Chile it is frequent and widely used as an ornamental tree in streets and avenues, and their management represents high volumes of materials with possibilities of use. This plant has become the objective of studies to evaluate the properties from different plant structures, in particular insecticide, antiviral, antioxidant, bactericide, and antiparasitic activities (Ahmed et al. 2008, Gende et al. 2008). The insecticide activity of *M. azedarach* is found on leaves, stems, fruits, and seeds, and is due to a group of biologically active triterpenoids that have antifeeding effects. Most of these substances in *M. azedarach* would inhibit the action of oxidases in the midgut, from which the juvenile insect dies or develops in abnormal pupa or adult, from the deficiency of nutrients or interference in physiological processes. This is caused by feeding inhibition, a decreased rate of metabolism, growth, and development. The lesser emergence of deformed adults cause inhibition of oviposition and mortality (Mazzonetto & Vendramin 2003, Pérez-Pacheco et al. 2004). Because of the previous background of the effectiveness of extracts from unripe fruit of *M. azedarach* (Huerta et al. 2008, Chiffelle et al. 2011) on other insects, together with the absence of studies on *R. flavipes* and the need to evaluate less toxic and more friendly products to the environmental, herein we evaluated the repellence effect of extracts from unripe fruit onto this termite.

2. MATERIAL AND METHODS

2.1. Material

Unripe fruits were collected at random as in Huerta et al. (2008) from adult trees of *M. azedarach* in the Faculty of Forestry and Nature Conservation Sciences, University of Chile (33°34'S; 70°38'O) in Santiago, Chile, in December. Individuals of *R. flavipes* (Figure 1) were collected from a pest outbreak of in the Estación Central, La Pintana, and San Ramón municipalities, in Santiago, Chile. These samples of workers were transferred in containers in darkness with soil, wood chips, and stable temperature and humidity to the Forest Entomology Laboratory, Department of Silviculture and Nature Conservation, University of Chile, Santiago, Chile.



Fig. 1. Workers of *R. flavipes* (left) and unripe fruits of *M. azedarach* (right).

2.2. Methods

The extracts from unripe fruits of *M. azedarach*, one in distilled water and the other in 96% v/v ethanol, were prepared in the Laboratory of Chemistry, Department of Agroindustry and Enology, Faculty of Agronomic Sciences, University of Chile, Santiago, Chile. From both extracts, several concentrations were obtained by dilution. The fruits were dried at 45°C in a forced air oven (Thelco, Mod. 18, Colorado, US) until constant weight. Then they were ground in a mechanical mill and sieved to 250 µm. The powder obtained was mixed with distilled water or ethanol to obtain solutions at the maximum concentration possible. These solutions were homogenized for 18 h in a shaker (Heidolph®, MR 3001 K Schwabach, Germany), warming at 37°C the first hour. Then, the supernatants were separated using a centrifuge (Sigma, Mod. 1-14, Osterode am Harz, Germany) at 1,500 rpm for 15 min. After this, the solutions were filtered again to obtain the extracts to be evaluated. Finally, when the concentration of each stock solution was determined, they were diluted to 5.4; 10.1, and 13.5 % w/v for the water extract, and 3.0; 5.6; 8.6, and 7.4 % w/v for the ethanol one.

The repellent effect on worker *R. flavipes* termites was evaluated using the direct test method in Manzoor et al. (2011). Two halves were separated in a Petri dish with moistened filter paper discs, 0.6 cm in diameter, one treated with one of four concentrations of each extract and the other disc untreated. The treated and untreated discs were added pure solvent (ethanol or distilled water) and allowed to dry for 12 h. Then 10 termite workers were set into each Petri dish, with five replicates per treatment. The numbers of termites in contact with each disc of filter paper, treated and untreated, for periods of 5 min were counted during 60 min (12 observations in total). A Sony DCR-SX45 video camera was used to facilitate this process and record the behavior of the termites during this time period. Differences in the mean number of termites in contact with the paper discs were separated using t-tests for paired comparisons.

3. RESULTS

The results of the evaluation experiment of the repellent effect, determined with the direct selection test, indicate that both in the controls with only distilled water ($t_{(11)} = 1.77$; $p \leq 0.05$), as well as with both extracts (water-ethanol $t_{(11)} = 1.82$; $p \leq 0.05$), indicate that the averages of *R. flavipes* in the filter paper discs offered simultaneously were similar (Figure 2).

In all the concentrations evaluated in the tests with ethanolic and water extracts from the green unripe fruits of *M. azedarach*, the termites presented a significant repellence response towards the treated discs. The number of termites in contact with the solvent (water or ethanol) disc during the 60 min period was significantly greater than those in contact with the corresponding extract-treated paper disc, which indicates that the results were due to their repellent effect (Figure 2).

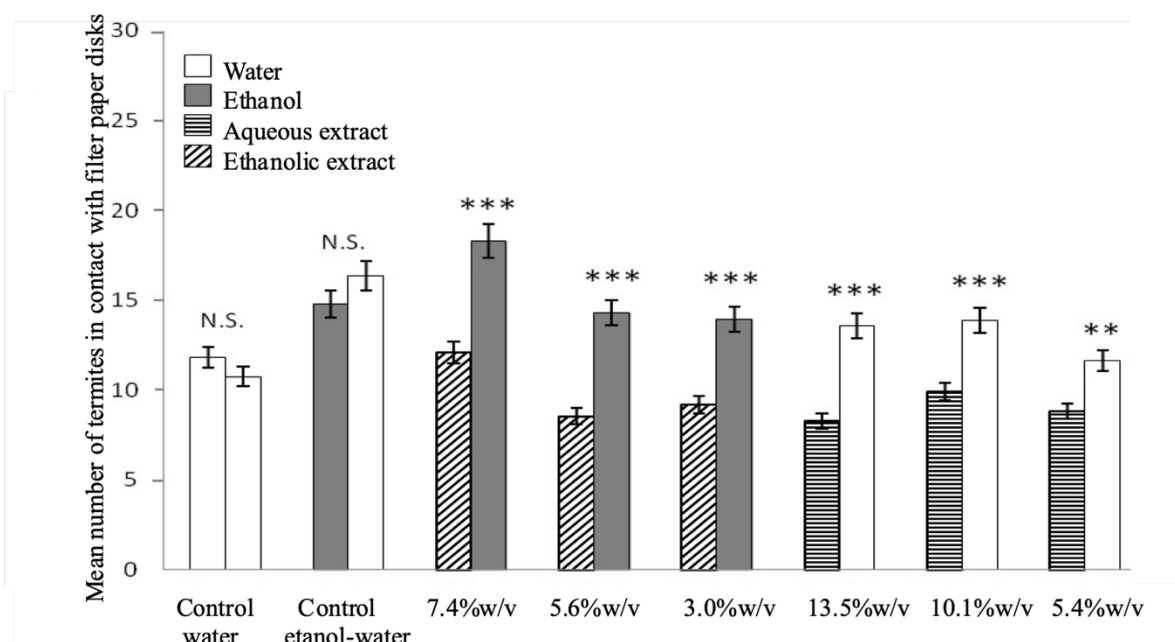


Figure 2. Repellence in a choice test of *M. azedarach* unripe fruit extracts-treated filter paper disks on *R. flavipes* termite workers. Each bar represents the mean number $\pm$ SD of the termites in contact with a filter paper disk in a 60 min period (n=12 counts). Empty bars: water solvent-treated filter paper; gray bars: ethanol solvent-treated filter paper; horizontal hatched bars: aqueous extract-treated filter paper; diagonal hatched bars: ethanol extract-treated filter paper. Each experiment was evaluated by a paired comparison t-test. *** $P < 0.001$; ** $P < 0.01$; N.S.: no significant difference.

The average number of termite workers in contact with the filter paper discs offered simultaneously was greater in those discs with the ethanol extract than with the water one. With the ethanol extract at the greatest concentration (7.4% w/v) the difference in the average number of termites in contact between discs of filter paper treated with the extract and untreated was 6.19 contacts. While with the water extract at the greatest concentration (13.5% w/v), the difference was smaller (5.28 average contacts). With the least concentration of the ethanol extract (3.0% w/v), the difference between the averages was 4.75 contacts, compared to 2.81 with the least concentration of the water extract at 5.4% w/v. Thus, a greater repellence of the ethanol extract from *M. azedarach* unripe fruits was observed, as well as a direct relationship between the concentration of the extract and its repellent effect, as with both extracts minor differences between the termite contact averages were obtained with the least concentrations (Figure 2).

4. DISCUSSION

The repellence action of the ethanol extract from green unripe fruits of *M. azedarach* was more effective than that obtained with the water extract, since the former obtained greater differences between the averages of the individuals of *R. flavipes* in contact with the treated filter paper discs with extracts and those treated only with the respective solvent. This may be due to the fact that ethanol, compared with water, obtains greater yields due its dry weight dissolving capacity and therefore is capable of dissolving a greater amount of secondary metabolites when preparing the extracts (Wu et al. 2006).

The compounds affecting insects in *M. azedarach* are found in leaves, stems, fruits, and seeds, mainly meliartenin, limonoid (triterpene) (Rodríguez 1999). Manzoor et al. (2011) observed with bark extracts from *M. azedarach* significant differences between the repellence produced with ethanol

extracts of the powdered bark at 10, 50 and 100% applied onto workers of *Heterotermes indicola* Wasmann (Isoptera: Rhinotermitidae) and reported a difference of 7 contacts with the 100% ethanol extract between the average number of individuals in contact with the extract-treated and untreated filter paper discs. The results in our study are similar, with extracts from unripe green fruits of *M. azedarach*, since with the maximum concentration of the ethanol extract (7.4% w/v) the difference between the filter paper discs treated with extracts and those only treated with ethanol was 6.19 contacts. Thus, in both studies a direct relationship was observed between the increase in concentration of the extracts and the repellence effect on the respective termite.

In another study, Chiffelle et al. (2009) found that larvae of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) treated with ethanol and water extracts from green fruits of *M. azedarach*, applied to food for ingestion (similarly to that of our study), presented a repellence effect, which was more noticeable in those treatments at the highest concentrations of the extract. Thus, there was a trend in behavior similar to that observed in our study herein, where a direct relationship was observed between the increase in concentration of the extracts and the repellence effect on *R. flavipes*.

Yuan and Hu (2012) assessed the repellence activity using a similar method but with the chloroform extract of dry leaves from *Lantana camara* L. (Lamiales: Verbenaceae) against worker *R. flavipes*. In the choice tests, significantly fewer termites made contact ($\square 1$) with the treated paper discs at test concentrations $\square 0.016 \text{ mg/cm}^2$ (equivalent to 0.0023 w/w), showing a strong repellence. Those results were more satisfactory than those obtained in our study.

Using the same method with extracts from young leaves of *Schinus molle* L. (Anacardiaceae), Huerta et al. (2015) detected the same trends in repellence effectiveness on *R. flavipes* workers, both with the water and ethanol extracts, with a greater degree of repellence in the latter.

5. CONCLUSIONS

The water and ethanol extracts of unripe green fruits of *M. azedarach* assessed in laboratory bioassays were effective in causing repellence to *R. flavipes*, finding a greater level with the ethanol extract. The results are promising and suggest further research on the development of a repellent based on extracts of unripe fruit of *M. azedarach* for *R. flavipes*, a method of application on wood, and its economic feasibility. Field tests are also recommended to confirm the effectiveness of the extracts in real conditions and in the presence of various factors that may affect results, and positive outcomes should include this technology in the integrated management of this urban pest.

REFERENCES

1. Abbott, WA 1925, 'A method for computing the effectiveness of an insecticide', *Journal of Economic Entomology*, vol. 18, pp. 265-267.
2. Ahmed, M, Ahmed, M, Thayyil, H, Zameeruddin, K, Ibrahim, M 2008, 'Antioxidative activity of *Melia azedarach* Linn leaf extract', *Iranian Journal of Pharmacology and Therapeutics*, vol.7, pp. 31-34.
3. Amri, I, Hamrouni, L, Hanana, M, Jamoussi, B 2013, 'Reviews on phytotoxic effects of essential oils and their individual components: news approach for weeds management', *International Journal Applied Biology and Pharmaceutical Technology*, vol.4, pp. 96-114.
4. Blaske, UV, Hertel, H 2001, 'Repellent and toxic effect of plant extracts on subterranean termites (Isoptera: Rhinotermitidae)', *Journal Economic Entomology*, vol.94, no. 3, pp. 1200-1208.
5. Boucias, D, Cai, Y, Sun, Y, Lietze, V, Sen, R, Raychoudhury, R, Scharf, M 2012, 'The hindgut lumen prokaryotic microbiota of the termite *Reticulitermes flavipes* and its responses to dietary lignocellulose composition', *Molecular Ecology*, vol. 22, pp. 1836-1853.

6. Castiglioni, E, Vendramin, J, Tamai, M 2002, 'Evaluación del efecto tóxico de extractos acuosos y derivados de meliáceas sobre *Tetranychus urticae* Koch (Prostigmata: Tetranychidae)', *Agrociencia*, vol.6, no.2, pp. 75-82.
7. Chiffelle, I, Huerta, A, Azúa, F, Puga, K, Araya, JE 2011, 'Antifeeding and insecticide properties of aqueous and ethanolic fruit extracts from *Melia azedarach* L. on the elm leaf beetle *Xanthogaleruca luteola* Müller', *Chilean Journal of Agriculture Research*, vol.71, no.2, pp. 218-225.
8. Edmonds, R, Agee, J, Gara, R 2011, *Forest health and protection*, 2nd ed., Long Grove, Illinois.
9. Funaro, CF, Böröczkyb, K, Vargoc, EL, Schal C 2018, 'Identification of a queen and king recognition pheromone in the subterranean termite *Reticulitermes flavipes*', *Proceeding of the National Academy of Sciences*, vol. 115, no.15, pp. 3888-3893.
10. García, A, Pérez-Urría E 2009, 'Metabolismo secundario de plantas', *Reduca (Biología). Serie Fisiología Vegetal*, vol., no.3, pp. 119-145.
11. Gende, L, Principal, J, Maggi, M, Palacios, S, Fritz, R, Eguaras, M 2008, 'Extracto de *Melia azedarach* y aceites esenciales de *Cinnamomum zeylanicum*, *Mentha piperita* y *Lavandula officinalis* como control de *Paenibacillus larvae*', *Zootecnia Tropical*, vol. 26, pp. 151-156.
12. Hong, T & Ellis, R 1998, 'Contrasting seed storage behaviour among different species of Meliaceae', *Seed Science and Technology*, vol. 26, pp. 77-95.
13. Huerta, A, Chiffelle, I, Lizana, D 2008, 'Actividad insecticida de extractos del fruto de *Melia azedarach* en distintos estados de madurez sobre *Drosophila melanogaster*', *Boletín de Sanidad Vegetal Plagas*, vol. 34, no. 3, pp. 425-432.
14. Huerta, A, Constenla, S, Chiffelle, I 2015, 'Repellency of *Schinus molle* L. leaf extracts onto *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae)', *Proceeding 46th The International Research Group on Wood Protection (IRG) Annual Meeting. Section I Biology*, Viña del Mar, Chile, IRG/WP 15-10848.
15. Isman, M 2020, 'Botanical Insecticides in the Twenty-First Century-Fulfilling Their Promise?', *Annual Review of Entomology*, vol. 65, pp. 233-249.
16. Karsulovic, J, Bozo, A, Araya, JE, Vargas, Y, Gaete, V, Tejer, B 2008, 'Aplicación de ondas sónicas y ultrasónicas como medio de control del ataque de termitas subterráneas *Reticulitermes flavipes* (Kollar) en madera de pino radiata', *Maderas. Ciencia y Tecnología*, vol. 10, no. 2, pp. 99-111.
17. Manzoor, F, Pervez, M, Hassan, M, Malik, S 2011, 'Effects of three plant extracts on the repellency, toxicity and tunneling of subterranean termite *Heterotermes indicola* (Wasmann)', *Journal Applied Environmental Biological Sciences*, vol. 1, no.7, pp. 107-114.
18. Mazzonetto, F, Vendramin, J 2003, 'Effect of powders from vegetal species on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) in stored bean', *Neotropical Entomology*, vol. 32, pp. 145-149.
19. Pearce, M 1997, *Termites: Biology and pest management*, CAB International, Wallingford, UK.
20. Pérez-Pacheco, R, Rodríguez, C, Lara-Reyna, J, Montes, R, Ramírez, G 2004, 'Toxicidad de aceites esenciales y extractos vegetales en larvas de mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae)', *Acta Zoológica Mexicana*, vol. 20, pp. 141-152.
21. Ripa, R, Luppichini, M 2004, 'Termitas de importancia económica en Chile', *Tierra Adentro*, vol. 59, pp. 42-51.
22. Thacker, JRM 2002, *An introduction to arthropod pest control*, Cambridge Univ. Press, Cambridge, UK.

23. Verma, M, Verma, S & Sharma, S 2018, *Eco-friendly termite management in tropical conditions. Termites and Sustainable Management*, Springer, Cham.
24. Yuan, Z, Hu, XP 2012, 'Repellent, antifeedant, and toxic activities of *Lantana camara* leaf extract against *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)', *Journal of Economic Entomology*, vol. 105, pp. 2115–2121.
25. Wu, S, Tsai, J, Chang, S, Lin, D, Wang, S, Huang, S 2006, 'Supercritical carbon dioxide extract exhibits enhanced antioxidant and anti-inflammatory activities of *Physalis peruviana*', *Journal of Ethnopharmacology*, vol. 108, no, 3, pp. 407-413.