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Full Length Article

Repellent and Toxic Effects of some Plant Extracts on Subterranean Termite *Psammotermes hybostoma* (Isoptera: Rhinotermitidae)

Khalid A Asiry[1*], Abir S Al-Nasser[2] and Refaat A Abohassan[3]
[1]*Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University. Jeddah, Saudi Arabia*
[2]*Department of Biology, Faculty of Science, University of Jeddah, Jeddah, Saudi Arabia*
[3]*Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University. Jeddah, Saudi Arabia*
[*]For correspondence: Kasiry@kau.edu.sa; aalnasser@uj.edu.sa; rabuhasan@kau.edu.sa
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Abstract

The current study was conducted to evaluate the toxicity and repellent effects of ethanolic extracts of *Lantana camara*, *Moringa oleifera*, *Rhazya stricta* and *Ruta chalepensis* on subterranean termite, *Psammotermes hybostoma*. All the tested extracts demonstrated noticeable toxicities, however, *L. camara* and *R. stricta* showed relatively more toxic effects, at 24 h and 48 h with LC50s of 177.5, 131.5 $mg.kg^{-1}$ and 199.8, 140.3 $mg.kg^{-1}$, respectively. Toxic effect was observed to be dependent on dose as well as exposure-time. No significant differences were observed between *L. camara*, *R. sricta* and positive control fipronil 2.5EC. Moreover, *L. camara* and *R. stricta* extracts, at 500 $mg.kg^{-1}$, showed potential repellent effects on *P. hybostoma* (Desneux) with repellency of 88.3 and 81.67%, respectively. Our work highlights the importance of screening the plant-based products for anti-termites' activity. We suggest that tested plants could possibly provide an alternative means for termite control strategies in protecting the economically important crops.

Keywords: *Lantana camara; Moringa oleifera*; Plant extracts; Repellent; Acute and Chronic Mortality; Termites; *Psammotermes hybostoma*; *Rhazya stricta*; *Ruta chalepensis*; Termiticide

Introduction

Termites are social insect pests present in a wide-ranging terrestrial environment scattered all over the globe. Termites are the most detrimental pest in the tropics which cause significant challenges in housing and agriculture. Subterranean termites are highly destructive polyphagous insect pests and it is anticipated that billions of dollars are expended annually to manage termites worldwide (Tsunoda 2003; Buczkowski and Bertelsmeier 2017). Colonies of *Psammotermes hybostoma* (Desneux) are common subterranean termites prevalent in rural and suburban areas in Saudi Arabia and are held responsible for largely damaging agricultural crops, forestry and household wooden structures (Adeyemi 2010; Alshehri *et al.* 2014; Buczkowski and Bertelsmeier 2017; Ahmad *et al.* 2021). Termites feed on the decaying organic wastes (Kambhampati and Eggleton 2000). They can also feed on live plants parts of groundnuts, maize, and millets (Ravan *et al.* 2015; Ahmad *et al.* 2021). In general, several chemical insecticides like aldrin, BHC, DDT and dieldrin are being used for long term to provide safety from termite invasion. However, these has now been barred in various countries because their residues have adversely influenced the terrestrial and aquatic environment (Elango *et al.* 2012; Bakaruddin *et al.* 2018). The destructive effects of chemical-based termiticides and the enhanced prevalence of termite-resistance have led to the necessity of discovering alternate bio-pesticides which are safer and more effective termiticides.

The use of chemicals costs higher, and has resulted in phytotoxicity, mammalian toxicity, pesticides residues, effects on non-target organisms, development of insect resistance and outbreaks (Elango *et al.* 2012). Therefore, interest has been developed among researchers to investigate the cheaper botanical insecticides to reduce the damages caused by termites and to be safe for human health (Singh *et al.* 2004; Senthil *et al.* 2005). Plants are eco-friendly and may provide alternative remedy to use of synthetic insecticides. Plants are rich sources of bioactive chemicals that act as natural insecticides against different insects and other organisms as well (Hussain *et al.* 2012). Recently, scientific interest for development of environment friendly plant-based pesticides and insect growth regulators

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has surged out (Arihara *et al.* 2004; Isman 2006; Cheng *et al.* 2007; Erb and Kliebenstein 2020). Manzoor *et al.* (2011) reported *Curcuma longa* extracts to be effective in soil treatments to protect food substrate against termites. Addisu *et al.* (2014) found that *Macrotermes* spp. can be easily managed by plant extracts as bio-termiticides in integrated pest management approach and Elsayed (2011) recorded that two desert plant extracts have a toxic effect on two termite species.

Several researchers have documented the toxic effects of plant extracts of *Lantana camara* (lantana or shrub verbena), *Rhazya stricta* (Harmal), *Ruta chalepensis* (fringed rue) and *Moringa oleifera* (horseradish tree or drumstick tree) in controlling some insect pests from different countries of the world (Ghosh *et al.* 2012; Ojiako *et al.* 2013; Addisu *et al.* 2014). These plants are widely distributed in the Kingdom of Saudi Arabia (Mossa *et al.* 1987) but their efficacy against termites is less explored.

The drawbacks related to the mismanagement and overuse of synthetic pesticides have incited the necessity for alternate pest management possibilities. In this regard, plant extracts, comprised of various bioactive compounds, are considered as promising alternative to synthetic insecticides. Therefore, this study was conducted to assess the concentration and time dependent efficacy of locally available plants. *Lantana camara, Rhazya stricta, Ruta chalepensis* and *Moringa oleifera* to contact toxicity and behavior of termite *P. hybostoma* (Desneux).

Materials and Methods

Termite

Termite species of *Psammotermes hybostoma* (Desneux) was obtained from the Research Station of King Abdulaziz University at Hada Elsham, Saudi Arabia. Termites were kept in plastic enamel trays and were retained and nurtured in the laboratory according to Upadhyay *et al.* (2010). Water and carton papers were used as a food material. Termites were kept in glass jars in dark conditions at 25°C and 75 ± 5 RH.

Plant materials

Plant materials *viz*., *L. camara, R. stricta, R. chalepnsis* and *M. oleifera* were collected across many parts in Saudi Arabia.

Preparation of plant extracts

Extracts of test plants were prepared by using a modified method. Leaves of the test plants were air dried for a week and then ground with micro plant grinding machine and subsequently, sieved through a 0.25 mm pore size mesh sieve to acquire uniform fine dust particles (Selase and Getu 2009). The powders obtained were kept separately in glass containers and stored at room temperature (25 ± 3ºC) in the dark. Next, 10 g of powder was mixed with 100 mL of absolute ethanol (99.9%) at room temperature (25 ± 3ºC). The mixture was stirred for 30 min with magnetic stirrer and left for 24 h. Further, it was concentrated in a rotary evaporator in a water bath at 55°C, and the residue obtained was stored at 4°C until use.

Mortality test

Stock solutions of the four plant extracts were designed by soaking 0.5 g of crude extract in 100 mL warm distill water and a range of concentrations of 100, 200, 300, 400 and 500 mg.kg^{-1} were obtained from stock solutions. Further, filter papers (Whatman No.1) of 9 cm diameter treated with 1 mL of different concentrations of ethanolic extracts of test plants were put in Petri dishes and allowed to dry at room temperature for 30 min. Next, twenty worker termites were arbitrarily chosen from stock population and kept in the treated Petri dishes. In all experiments, Fipronil 2.5EC (a synthetic insecticide) and water served as a positive and negative controls, respectively. All the treated Petri dishes were wrapped with a double layer of black plastic sheet to imitate the darkness for termites. Five different concentrations of each plant extract were replicated three times and placed in an incubator at 28 ± 3°C, 75 ± 2 RH. Mortality percentage of termite was recorded at 24 h and 48 h after treatment and values % for the natural mortality in the control treatment corrected by using Abbott (1925) equation.

Termite repellency test

For repellency assay, concentrations ranging from 100, 200, 300, 400 and 500 mg.kg^{-1} of each plant extracts were prepared. Petri dishes were spotted with Whatman No.1 filter paper (9 cm) cut into two equal parts with a distance of 2 cm. One part was treated with different concentrations of plant extracts and the other was left untreated by only distill water (Addisu *et al.* 2014). Twenty termites were established at the center of both treated and untreated filter papers and set in dark to reduce the effect of light on the termites. Three replications were used for each concentration of plant extracts. Number of termites on both treated and untreated filter paper in each Petri dish was recorded 30 min post-treatment. Based on the number of termites stayed on the extract-treated filter paper, repellency was determined.

Statistical analysis

The percentage mortality of *P. hybostoma* was calculated and were separately subject to a repeated measure ANOVA to assess the effect of the following factors: time (repeated factor with 3 observations), concentrations of four plants (17 experimental unites including control) and interactions between these factors. Before applying the repeated measure

ANOVA, data were transformed by applying Log 10 (Max+1-X) to meet the normality and improve variances. Where significant treatment differences ($P \leq 0.05$) were detected, the Fisher's Least Significant Difference (LSD) tests were performed to identify differences in treatment means. In addition**,** LC_{50} was calculated according to Finney (1971). Data was corrected for control mortality using Abbott's formula (1952). As for Repellency test, percentages of repellency rate (PR) were calculated using the method of Jilani *et al.* (1988). Then, obtained data (PR) were analyzed by one-way ANOVA to determine what is the best of the four used plants with tested concentrations (16 experimental unites) as a repellent for emerged adults of *P. hybostoma*. The mean differences were compared using Fisher's LSD test. All data analyses were performed within a SPSS ver.22 (IBM Corporation 2013).

Results

Time dependent toxicity

As presented in Table 1 and Fig. 1, a 100% mortality of the worker termites was recorded in the positive control (Fipronil 2.5EC) and ethanolic extract of *L. camara*, at 500 mg.kg^{-1} after 48 h. *L. camara* extract was the most effective among all the plant ethanolic extracts tested on *P. hybostoma*. At 500 mg.kg^{-1}, the percentage of mortality after 24 h and 48 h exposure was 95.0 and 100% with *L. camara* extract, respectively, but it was 92.3 and 97.3% *with R stricta* extract, whereas *it was* 81.3 and 93.3% *with R. Chalepensis* extract and *M. oleifera* extract at the same concentration showed 73.3 and 77.3% mortality after 24 h and 48 h respectively.

The LC_{50} values for *L. camara* extract and *R. stricta* extract at 24 h and 48 h were 177.5, 131.3 and 199.8, 140.3, respectively (Table 2). whereas the LC_{50} values at 24 h and 48 h with *R. chalepensis* and *M. Oleifera*, were 227.9, 178.2 and 388.1, 240.2, respectively.

Repellency assay

As shown in Table 3, the maximum and significant insect repellence (91.67%) was exhibited by fipronil 2.5EC, followed by non-significant difference shown by the extract of *L. camara* (88.33%, at 500 mg.kg^{-1}). *R. stricta* and *R. chalepensis* exhibited concentration dependent % repellents in the range of 48.33 to 81.67 with significantly different values of 81.67 and 70.0, respectively, at 500 mg.kg^{-1} each. On the contrary, *M. oleifera*, could show 60.0% repellents at 500 mg.kg^{-1}.

Discussion

Economically, the termites are the most critical pest which produce substantial destruction of agricultural crops and domestic materials. The continued usage of chemical termiticides has made us to look for safety of environments and has ensued the necessity to quest for plant-based products as replacements in controlling termites. Our study

Table 1: Percentage mortality of *P. hybostoma* in media containing ethanolic plant extracts

Plant materials	Concentration (mg. kg^{-1})	% Mortality after 24 h	48 h
Lantana camara	100	57.2	68.2
	200	67.5	83.3
	300	79.9	88.3
	400	90.1	98.8
	500	95.0 a	100.0 a*
Rhazya stricta	100	53.1	63.5
	200	63.3	77.3
	300	74.8	84.3
	400	86.9	93.3
	500	92.3a	97.3 a
Ruta chalepensis	100	48.3	56.8
	200	55.3	70.3
	300	63.5	75.9
	400	74.3	87.3
	500	81.3b	93.3 b
Moringa oleifera	100	42.3	48.3
	200	51.3	62.3
	300	54.5	64.2
	400	64.3	70.3
	500	73.3 c	77.3 c
Fipronil (positive control)	2.5 EC	97.5 a	100.0 a
Water (negative control)		1.3 d	2.5 d

*Means not sharing the same letter with columns are significantly different ($P < 0.05$)

Table 2: LC_{50} values and 95% confidence limits of *P. hybostoma* in media containing ethanolic extracts of tested plants

Plant extracts	Assay time (h)	Slope	LC50 (95% CL)
Lantana camara	24	1.96	177.5 (140.22-253.33)
	48	2.11	131.5 (94.35-201.44)
Rhazya stricta	24	1.75	199.8 (161.64-297.21)
	48	1.87	140.3 (101.64-220.55)
Ruta chalepensis	24	1.24	227.9 (203.77-401.33)
	48	1.60	178.2 (128.32-277.11)
Moringa oleifera	24	1.11	388.1 (280.44-530.22)
	48	1.51	240.2 (15.1.43-383.01)

Table 3: Mean Repellency percentage of *P. hybostoma* treated with different plant extracts

Plant extracts	Concentration (mg.kg^{-1})	Mean repellency (%)
Lantana camara	100	55.00 cd*
	200	61.67 c
	300	70.00 b
	400	80.00 b
	500	88.33 a
Rhazya stricta	100	50.00 cd
	200	53.33 cd
	300	63.33 c
	400	75.00 b
	500	81.67 a
Ruta chalepensis	100	48.33 d
	200	50.00 cd
	300	58.33 c
	400	63.33 c
	500	70.00 b
Moringa oleifera	100	43.33 d
	200	48.33 d
	300	51.67 cd
	400	55.00 c
	500	60.00 c
Fipronil (positive control)	2.5 EC	91.67 a
Water (negative control)		1.67

*Means with the same letter are not significantly different, $P < 0.001$

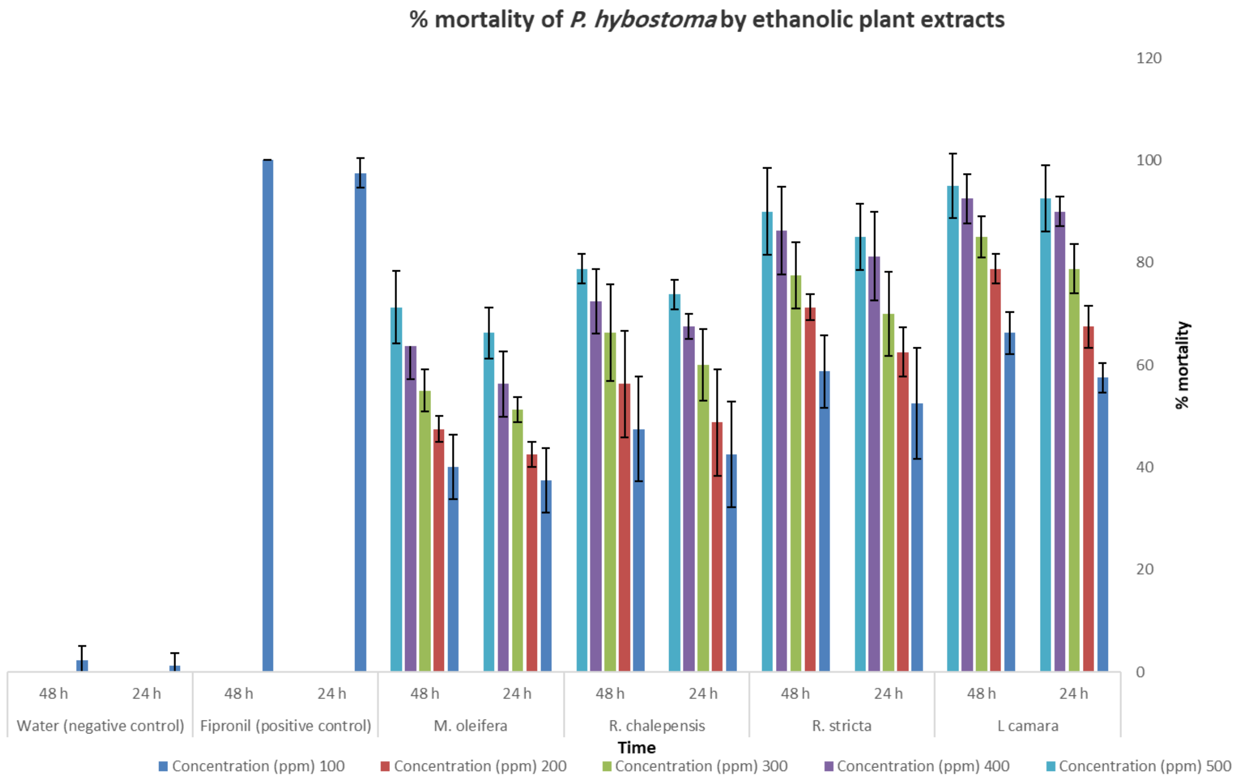

Fig. 1: Concentration and time dependent effects of ethanolic extracts of plants *L. camara*, *R. stricta*, *R. chalepensis* and *M. oleifera* on mortality of *P. hybostoma*

has demonstrated the biocontrol potential (termiticidal effect) of ethanolic extracts of four different plants against subterranean termites *P. hybostoma* worker in Saudi Arabia. All plant extracts were toxic to *P. hybostoma* workers in a dose dependent manner, and their efficiency varied depending on exposure-time. Although the toxic effect of *L. camara* was at par with positive control fipronil, toxicity was relatively low for *M. oleifera*. Significant differences were shown between *L. camara, R. Stricta* and other plant extracts, while *M. oleifera* exhibited less significant mortalities than other ethanolic plant extracts. The ethanolic extracts from *L. camara* and *R. stricta* showed lesser significant differences between them and showed small value of LC_{50s} (24 h and 48 h) compared to the ethanolic extracts from *R. chalipensis* and *M. oleifera.* However, no mortality of *P. hybostoma* was detected for negative control over the total exposure period (48 h). In general, the ethanolic extracts of *L. camara* and *R. stricta* were more toxic than of other plants tested.

The ethanolic extracts of test plants were expected to have higher phenolic, alkaloids and flavonoid contents (Khan *et al.* 2016; Najem *et al.* 2020; Al-Solami 2021; Kumari and Sidhu 2021). These phytochemicals interfere with the behaviour, feeding, growth, moulting and reproduction in insects (Musayimana *et al.* 2001; Simmonds 2001).

The toxic effect of *L. camara* was previously reported on tobacco caterpillar *Spodoptera litura* (Deshmukhe *et al.* 2011), stored grain pests (Rajashekar *et al.* 2014) cabbage white butterfly, *Pieris brassicae* (Sharma and Gupta 2009), and the rice moth, *Corcyra cephalonica*. Ayalew (2020) reported that stored maize could be protected from the infestation of *S. zeamais* by using extracts and oils of *L. camara* leaf and the author suggested that this repellent and mortality effect for insects could be because of the presence of bioactive compounds like 1-Eicosano, Paromomycin, Phytol, Pyrroline and Pyrrolizin. Alvi *et al.* (2018) reported the toxic effect of *R. stricta* extract in controlling insect pests like *Rhyzopertha dominica* and *Trogoderma granarium*. The toxic effect of *R. stricta* extract is credited to the presence of high-level alkaloids (Ali *et al.* 2000).

Repellent effects of testing plant extracts on the worker *P. hybostoma* were significantly different; the repellent percentage dependent extracts concentrations; at 500 $mg.kg^{-1}$, repellent action of *L. camara* was at par with fipronil, followed by *R. stricta*, *R. chalepensis* and *M. oleifera*. Yuan and Hu (2012) showed strong repellent and modest toxic and antifeedant activities of chloroform leaf extract of *L. camara* against subterranean termite, *Reticulitermes flavipes*. Tampe *et al.* (2016) have reported the repellent effect of *R. chalepensis* oils against the weevil *Aegorhinus superciliosus*. This is in agreement with our result as *R. chalepensis* extract showed 70% repellents to *P. hybostoma*. Najem *et al.* (2020) showed the effectiveness of *R. chalepensis* L. essential oil against *Tribolium castaneum*.

Plants based repellents are expected to impart least adverse effects on the environment because they make pests away by arousing their sensory organs ahead of attacking the plants and are also easily degraded in a short time (Addisu *et al.* 2014; Cespedes *et al*. 2014). The products of these tested plants specially, *L. camara* and *R. stricta* can be well utilized to prepare phytochemicals from which all non-target organisms can be rescued from insecticides. It has been anticipated that complex mixtures of secondary metabolites are regulator of plant defense delivering multiple mechanisms of action, as a result its use lower the predisposition of the development of insect resistance (Kortbeek *et al.* 2019; Erb and Kliebenstein 2020).

Various research using plant extracts in agriculture and household pest management has provided promising results towards human and animal health safety (Pascual-Villalobos and Robledo 1999; Scott *et al.* 2004; Pino *et al.* 2013; Najem *et al.* 2020; Al-Solami 2021). Therefore, replacing the synthetic-insecticides by bio-pesticides have become a universally accepted and suitable tactic, and is encouraged.

Conclusion

The immense crop losses experienced in Saudi Arabia are due to deterioration of crops by termites. The present study showed that all the tested plant extracts against subterranean termite, *P. hybostoma* possess termiticidal potential that can be exploited in the management of *P. hybostoma* pests. Depending on the results of our study that showed more efficacy of most plant extracts specially *L. camara* plant with 500 mg.kg^{-1} concentrate. Moreover, the common availability of these tested plants in many parts of Saudi Arabia makes them a significant natural termiticide to be exploited in integrated management of termite *P. hybostoma*. These findings, however, require in-depth future studies on actual assessment as biocides on pests without damaging the environment.

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Author Contributions

KAA designed the experiment, collected and prepared the materials, analyzes the data and drafted the manuscript. ASN prepared the plant extracts, collected data and reviewed the manuscript. RAA collected data and revised the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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