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Review Article

Efficacy of Anti-termite Extracts from Four Saharan Plants against the Harvester Termite, *Anacanthotermes ochraceus*

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ABSTRACT

This study aimed to examine the anti-termite potential of four Saharan plant extracts, namely, the apple of Sodom or rubber bush, C*alotropis procera*; pergularia, *Pergularia tomentosa*; jimsonweed, *Datura stramonium*, and Egyptian henbane, *Hyoscyamus muticus* from Bechar (southwest of Algeria) on workers of the harvester termite, *Anacanthotermes ochraceus* (Isoptera: Hodotermitidae). A direct contact application test was conducted with five fractions from aqueous extracts of each part of plant species (leaves, stems) using hexane, dichloromethane, ethyl acetate, butanol, and exhausted fraction. A repellent test was realized with aqueous extracts (10%) of plant species leaves and stems. According to the direct contact application test, all tested plants fractions showed termiticidal activities with different degrees. Butanolic fractions presented the best effects from leaves of *C. procera* and *P. tomentosa* with median lethal time (LT_{50}) = 231.03 and 244.96 min, respectively. In the second test, wood samples were exposed to termite attack for four weeks, and the weight loss percentage was determined. The weight loss ranged from 0.034 to 16.90% at concentrations of 10% of plant extracts. The best repellent effect was obtained from leaves of *C. procera* (weight loss = 0.034%) and leaves

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of *D. stramonium* (weight loss = 1.29%). It was concluded that some Saharan plants are a good source of anti-termite compounds, especially *C. procera*.

Keywords: *Anacanthotermes ochraceus*, direct contact application, repellent test, Saharan plants

INTRODUCTION

Termites are one of the most agriculturally important insects (Sahay et al., 2014). Nevertheless, despite some advantages of termites in soil reclamation, they are often called "silent destroyer" (Kamble & Thakor, 2017).

About 2,500 termite species worldwide, and about 10% are economically important as pests and consumption of cellulose-based plant materials (Ranjith et al., 2015). The termites are among the most problematic pests in agriculture and urban area. They cause significant annual crop loss and damage to buildings, especially in semi-arid tropics and sub-humid (Mokeddem et al., 2017). In addition, the harvester termite, *Anacanthotermes ochraceus* (Isoptera: Hodotermitidae), found in North Africa and Western Asia, is highly destructive on wood.

Previously, the control of termites is completely based on chemical products, particularly synthetic insecticides, such as persistent organochloride and organophosphate insecticides (Ranjith et al., 2015).

Indiscriminate use of chemical pesticides to control insect pests has contributed to a number of biological and environmental hazards. These man-created problems have further resulted in phytotoxicity, mammalian toxicity, biomagnification of pesticide residue, insect resistance, insect resurgence, and increased cost of production (Dipendra et al., 2012). Therefore, replacing synthetic insecticides with bio-rational control is universally acceptable and practical (Ranjith et al., 2015).

With the restriction of synthetic chemical use, termite control is currently focusing on various safer strategies. A wide range of toxic plants, repellent, or have some anti-feeding properties are considered as being insecticidal (Dodji et al., 2016).

There has been a growing interest in termite control by using plants that are known to have chemicals that protect them from different insect pests and microorganisms. Insecticides of plant origin have received significant attention due to their effectiveness on many economically important harmful insects and environmental compatibility. In addition, plant products are considered safer and an alternative to toxic synthetic chemicals (Dipendra et al., 2012). Therefore, various plant extracts have been studied for their toxicity, repellency, and attractancy on termites and insect species (Nazeer et al., 2016).

This study is the continuation of work carried out, done in our laboratory. It was demonstrated that the aqueous extracts of four Saharan plant species, namely, the apple of Sodom or rubber bush, *C. procera*; pergularia, *P. tomentosa*; jimsonweed, *D. stramonium*, and Egyptian henbane, *H. muticus*, were effective against *A. ochraceus* using direct contact application (Bourmita et al., 2013). This investigation aims to evaluate the efficacy of fractions from aqueous extracts from these plants on *A. ochraceus*. In addition, a repellent test was also conducted with these aqueous extracts (10%) to evaluate their full insecticidal effect.

MATERIALS AND METHODS

Plant Materials

The plants collected from the Bechar region, Southwestern Algeria and used in this study were: *Calotropis procera* (Asclepiadaceae) CA04/02, *Pergularia tomentosa* (Asclepiadaceae) CA00/44, *Datura stramonium* (Solanaceae) CA00/50, and *Hyoscyamus muticus* (Solanaceae) CA00/43 (Cheriti, 2002). The plants were identified, and voucher specimens were deposited at the herbarium of Phytochemical and Organic Synthesis Laboratory (POSL), Bechar University, Bechar, Algeria.

Plant Extract Preparations

The leaves of each plant species were dried for seven to fifteen days in the shade at an ambient temperature of 25 to 35 °C. The dried leaves (150 g) were powdered and extracted with 1.5 L of distilled water for 3 hr, and the obtained filtrates were evaporated to remove one-third of water and extracted with four solvents. In a separating funnel, we performed an extraction was performed with solvents. After stirring, each portion of solvent (100 mL) remained in contact with the aqueous phase for 20 min. The operation was repeated four times for each solvent. Five fractions were obtained (hexane, dichloromethane, ethyl acetate, butanol, and exhausted fraction). The extracts were concentrated under reduced pressure at 45 °C, and the obtained residues were stored at 5 °C until use for testing against *A. ochraceus*.

Collection of Termite

The termite, A. ochraceus, was collected on Bechar University (UTMB), Bechar, Algeria, and acclimatized under laboratory conditions 28 to 30 °C and 70 to 80% relative humidity in total darkness. The captured termites were immediately separated from the deadwood and placed inside a room containing wooden sticks as a food source.

Biological Tests

Direct Contact Application Method. Whatman No.1 filter papers were soaked with 400 μL of each extract (dilution: 10%) and put with ten workers of *A. ochraceus* in Petri dishes (90 mm diameter). Filter papers were given as a sole source of food for insects. Filter papers soaked with 400 μL of distilled water were used as control. The mortality was calculated every 30 min for 24 hr.

Determination of Survival Duration

The average percent mortality of termite was corrected by using Schneider-Orelli's formula (Schneider-Orelli, 1947) before subjected subjecting it to regression analysis as in Probit analysis by Finney (1971) for determination of the median lethal time, LT_{50} values, as in Bourmita et al. (2013). Schneider-Orelli's corrected mortality formula was as follows:

$$M_c = \frac{M_2 - M_1}{100 - M_1} X100$$

where,

M_C = Corrected mortality (%)
M_2 = Mortality in the treated population (%)
M_1 = Mortality in the control population (%)

Repellent Test (Weight Loss)

The test was performed to check the repellent effect of each aqueous extract from leaves and stems of four plant species. The test was carried out by using wood pieces treated with different extracts. A sufficient number of white wood pieces were cut and well dried, and weighed. All samples have constant dimensions (20 x 5 x 2 cm).

The wood samples were treated with 10% aqueous extracts dilution for 1 hr in plastic bottles. The treated wood samples were air-dried and implanted in termite-infested areas (Lahmar region, southwest of Algeria). They were exposed to termite attacks for four weeks (López-Naranjo et al., 2014), then collected, cleaned, dried, and weighed to determine the weight loss (Figures 1 and 2).

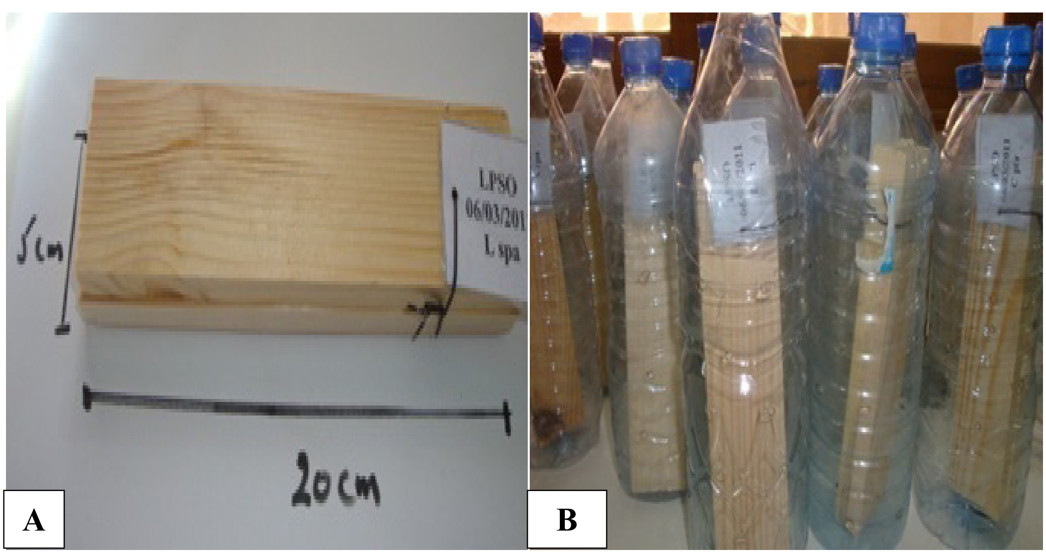

Figure 1. (A) Dimension of wood sample; (B) Samples treated with 10% aqueous extracts for 1 hr in plastic bottles

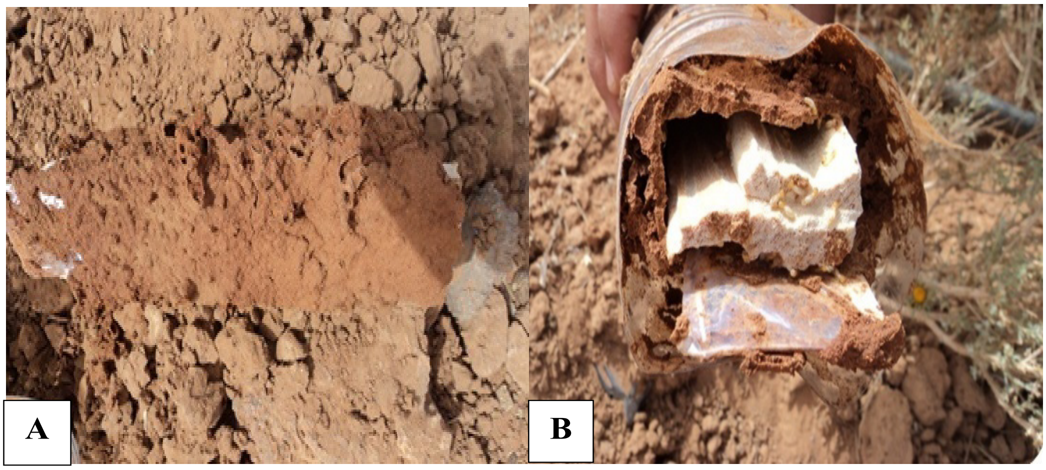

Figure 2. (A) Samples exposed to termite attack for four weeks; (B) Positive trapping of wood attacked by termite after four weeks.

Weight Loss Analysis

After four weeks of the exposure period, the wood samples were removed, cleaned, dried during the day, and weighed to determine the weight loss.

The difference between damaged and intact wood samples was measured, and the weight loss percentage was calculated as follows:

$$\% \text{ weight loss} = (W_3\text{-}W_4 / W_3) \times 100$$

where,

W_3 = weight of test block after treatment

W_4 = weight of test block after exposure to termite attack (Funke et al., 2015)

The weight loss in wood sample was recorded as percentage

RESULTS

The aqueous plant extracts from *C. procera*, *P. tomentosa*, *D. stramonium*, and *H. muticus* were evaluated for their anti-termite efficiency against *A. ochraceus* by direct contact application and repellent test. The results are illustrated in Table 1 and Figure 3 below: as for the termite mortality after 8 hr, it was 100% with 10% of the leaves extract of the plant after 24 hr of treatment compared with the control using distilled water.

The test result showed that the LT_{50} of the butanolic extracts of the leaves of *C. procera*, *P. tomentosa*, and *H. muticus* were 231.030, 244.960, and 257.520 min, respectively; and that of the ethyl acetate extracts of the leaves of *D. stramonium* was 265.089 min.

Repellent Test (Weight Loss)

The weight loss in a wood sample due to termite attack was recorded in percentage, as shown in Figure 3. The results corresponded with the extent of termite attack on the wood samples treated with aqueous

Table 1

Effect of different plant extracts on median lethal time (LT_{50}) of Anacanthotermes ochraceus *population*

Plant species	Extract	Regression equation	Coefficient of regression (R^2)	Median lethal time (LT_{50}) (in min)
Pergularia tomentosa	*n*-hexane	y = 2.728x - 2.295	R^2 = 0.645	472.193
	Dichloromethane	y = 2.794x - 2.352	R^2 = 0.646	427.910
	Ethyl acetate	y = 2.747x - 2.260	R^2 = 0.644	439.423
	n-butanol	y = 2.737x - 1.539	R^2 = 0.842	244.960
	Exhausted fraction	y = 2.781x - 2.608	R^2 =0.575	544.134
Hyoscyamus muticus	*n*-hexane	y = 2.937x - 2.538	R^2 = 0,687	368.607
	Dichloromethane	y = 2.850x - 2.493	R^2 = 0.687	425.718
	Ethyl acetate	y = 2.838x - 2.496	R^2 = 0.689	437.821
	n-butanol	y = 3.050x - 2.353	R^2 = 0.721	257.520
	Exhausted fraction	y = 2.950x - 2.893	R^2 = 0.623	473.797
Calotropis procera	*n*-hexane	y = 2.686x - 1.965	R^2 = 0.760	391.800
	Dichloromethane	y = 2.676x - 1.485	R^2 = 0.840	265,089
	Ethyl acetate	y = 2.633x - 1.541	R^2 = 0.814	304.950
	n-butanol	y = 2.835x - 1.701	R^2 = 0.805	231.030
	Exhausted fraction	y = 2.868x - 2.817	R^2 = 0.626	531.600
Datura stramonium	*n*-hexane	y = 2.889x - 2.600	R^2 = 0.684	430.026
	Dichloromethane	y = 3.061x - 2.756	R^2 = 0.666	341.830
	Ethyl acetate	y = 2.655x - 1.435	R^2 = 0.841	265.290
	n-butanol	y = 3.236x - 3.129	R^2 = 0.564	325.126
	Exhausted fraction	y = 2.847x - 2.509	R^2 = 0.689	434.023

extracts of *C. procera*, *D. stramonium*, *H. muticus*, and *P. tomentosa*.

The changes in weight loss of wood samples treated with different aqueous extracts of plants (natural resistance) compared with the wood treated with distilled water as controls are shown in Figure 3. Regarding extracts as a preservative substance, 10% of the extract concentrations strongly affect the *A. ochraceus* termite species tested. Figure 3 showed the percent weight loss of wood samples treated by aqueous extracts, initial and final weight of each sample after planting in the experimental sites for 30 days; some differences were noted. Regarding the use of aqueous extract as a preservative substance, the concentrations of 10% showed the weight loss of the leaves of *C. procera* (0.034%), the stems of *H. muticus* (1.71%), stems and

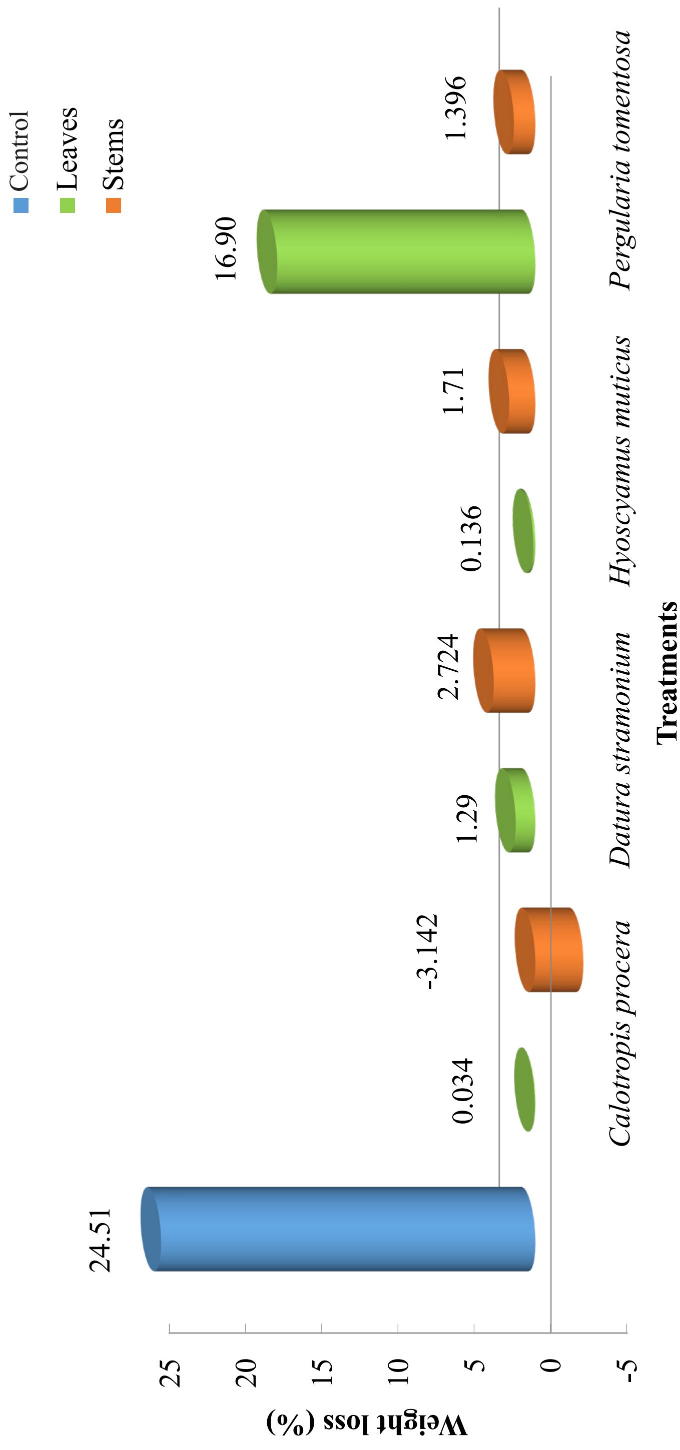

Figure 3. Weight loss of wood samples treated with 10% aqueous plant extracts after four weeks

leaves of *D. stramonium* (2.724% and 1.29%), respectively, and the leaves of *P. tomentosa* with 16.90%. Such increases indicated that the wood without preservatives (lower control) lost more than 24.51% weight compared to 10% of the plant extracts treatment. The negative weight loss of the stems of *C. procera* (-3.142%) and the stems of *P. tomentosa* (-4.396%) was due to the presence of humidity during this planting period.

DISCUSSION

The four Saharan plants, namely, *C. procera*, *P. tomentosa*, *D. stramonium*, and *H. muticus*, were selected to determine their biological activities and examined their anti-termite potential and efficacy to develop an alternative control against the termite, *A. ochraceus*. In addition, the assessment of mortality using the LT_{50} and the repellent test (weight loss) were carried out with their leaf aqueous extracts.

Vinothkumar et al. (2018) reported that among various control measures, chemical control stood first in termite management in sugarcane in Tamil Nadu, southern India. The continuous use of synthetic insecticides in termite control is known to cause aquatic and environmental pollution, lethal effects on non-target organisms and has resulted in the need to search for plant-derived compounds as an alternative (Hridayesh et al., 2016). However, many plant species can be used as botanical insecticides (Abbasipour et al., 2011a). These compounds act as fumigants, contact insecticides, repellents, and antifeedants (Zoubiri & Baaliouamer, 2014).

The result of the bioassay reported in Table 1 and Figure 3 showed the toxic action of the extracts from the leaves of *C. procera*, *P. tomentosa*, *D. stramonium*, and *H. muticus* to workers of *A. ochraceus* after 24 hr of treatment. In addition, the changes in weight loss of wood samples treated with different aqueous plant extracts compared with those treated with distilled water as control were observed (Figure 3). This change is due to their repellent effects against termite attacks. Conceivably, the plant extracts may contain bioactive substances that are toxic and deter feeding to termites. In contrast, the direct contact application method revealed that the percent mortality recorded at 10% concentration of the leaf extract of *C. procera* caused the highest mortality with LT_{50} = 231.03 min (Table 1).

The perusal of literature revealed a finding supporting results of the present work as in the study conducted in our laboratory by Bourmita et al. (2013). It demonstrated that the aqueous leaf extracts of these plants significantly affected the mortality of *A. ochraceus* workers. The study also revealed the LT_{50} of the 5% concentration of the leaf and stem extracts of *C. procera* were at LT_{50} = 54.24 min and LT_{50} = 58.54 min, respectively, and the 4% concentration of the leaf extract of *P. tomentosa* was at LT_{50} = 94.97 min.

Mueen et al. (2005) reported that C. *procera* is possessed acaricidal, schizonticidal, antimicrobial, anthelmintic, insecticidal, anti-inflammatory, antidiarrheal, anticancerous, and larvicidal activities. In medicine, it was used to treat common ailments such as fever, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, and diarrhea (Rashmi et al., 2011). Elimam et al. (2009) examined the insecticidal activity of the leaf extract of *C. procera* and reported that the aqueous leaf extract of *C. procera* showed a high level of toxicity against the larvae of mosquitoes, *Anopheles arabiensis* and *Culex quinquefasciatus*. Sharief et al. (2019) also examined the insecticidal activity of ethanolic, butane, and distilled water extracts of the leaves and flower of *C. procera* and found that they were effective against the house fly *Musca domestica*.

Butanolic extract of the leaves of *P. tomentosa* with LT_{50} = 244.960 min showed significant toxicity against *A. ochraceus*. A 100% mortality was obtained by 10% concentration of the extracts after 24 hr of treatment. *Pergularia tomentosa* has been exploited in traditional medicine as having antioxidant, antibacterial, antifungal, insecticidal, and cytotoxic activities documented by Al-Mekhlafi and Masoud (2017). The insecticidal activity of *P. tomentosa* was also reported that its crude methanol extract and its isolated cardenolides had potent antifeedant activity against the cotton leafworm, *Spodoptera littoralis* (Green et al., 2011). Furthermore, Acheuk and Doumandji-Mitiche (2013) reported that the alkaloids extract from the aerial part of *P. tomentosa* showed significant toxic and growth inhibitory effects against the fifth instar nymph of the migratory locust, *Locusta migratoria*. Miladi et al. (2018) also reported that the flowers of *P. tomentosa* exhibited insecticidal activity against *L. migratoria.* However, a comparative study of ethanolic leaf extracts from the four selected plants against the larval stages of the dengue fever mosquito vector, *Aedes aegypti*, *P. tomentosa*, showed some larvicidal properties but with less efficacy (Asiry et al., 2017).

The ethyl acetate extracts of the leaves of *D. stramonium* with LT_{50} = 265.089 min showed significant toxicity against *A. ochraceus*. Mortality of 100% was obtained by 10% concentration of the extract after 24 hr of treatment. The various parts of the plant (leaves, seeds, roots, and fruits) are used in medicine for different purposes (Tayoub et al., 2016). The plant extracts are acaricidal, insect repellent, antimicrobial, larvicidal, pesticide toxicity, antifungal, and vibriocidal (Aderonke et al., 2017). In addition, the plant comprises bioactive constituents like scopolamine, hyoscamine, tropane alkaloids, tannins, proteins, carbohydrates, sapronins, steroids, flavonoids, phenols, and glycosides (Al-snafi. 2017; Debnath & Chakraverty, 2017). Abbasipour et al. (2011b) reported *D. stramonium* aerial part extract as and insect mortality factor. The concentration at LC_{50} of acetone extract is active as a toxicant against the cowpea weevil, *Callosobrchus maculatus*, and an oviposition deterrent. Swathi et al. (2012) reported that the ethanolic leaf extract *of D. stramonium* also has significant larvicidal and repellent activities against the mosquitoes, *Aedes aegypti*, *Anopheles* *stephensi*, and *Culex quinquefasciatus*. A study by Jawalkar et al. (2016) revealed that the ethanol, chloroform, and acetone extracts of *D. stramonium* seeds were effective against the rice weevil, *Sitophilus oryzae*.

The results showed that butanolic leaf extract of *H. muticus* was significantly toxic against *A. ochraceus* with LT_{50} = 257.520 min. The mortality of 100% was obtained by 10% concentration of the extracts after 24 hr of treatment. *Hyoscyamus muticus* was also reported for its tropane alkaloid content, and the main alkaloids present are scopolamine and hyoscyamine (Abed Elmaksood et al., 2016). Elsharkawy et al. (2018) reported that the methanolic extract of *H. muticus* exhibited an antifeedant effect on the fourth instar larvae of the cotton leafworm, *Spodoptera littoralis*. Abdel-Aziz et al. (2009) demonstrated that the alkaloidal chloroform fraction of the leaf and root extracts of the leaves and roots of *H. muticus* exhibited an acaricidal activity against the two-spotted spider mite, *Tetranychus urticae*.

The results obtained from our investigation showed that the plant extracts of *C. procera*, *P. tomentosa*, *D. stramonium*, and *H. muticus* used in this study have repellent and toxic effects on *A. ochraceus*. Therefore, they can be used as an effective anti-termite agent and can be suggested and used as a natural insecticide for termite control because they constitute a rich source of bioactive chemicals.

In addition to preliminary tests, developing new efficient anti-termite treatment using natural products needs bio-guided fractionation. Therefore, less efficient fractions or extracts could be neglected for more advanced tests.

CONCLUSION

The harvester termite, *Anacanthotermes ochraceus* (Isoptera: Hodotermitidae), is one of the most destructive termite pests. Therefore, using natural products of some Saharan plant species is a principal goal developed in our laboratory to discover effective and environmentally friendly termite control agents. The purpose of this study was to investigate the anti-termite effect of fractions against *A. ochraceus* from the aqueous leaf and stem extracts of four Saharan plant species, namely, the apple of Sodom or rubber bush, *Calotropis procera*, pergularia, *Pergularia tomentosa* (Asclepiadaceae), and jimsonweed, *Datura stramonium*, and Egyptian henbane, *Hyoscyamus muticus* (Solanaceae).

Of these plant species, our results have provided evidence on the efficacy of some Saharan plant species to be important sources of anti-termite treatment, especially *C. procera* and *P. tomentosa*. Therefore, our upcoming investigation will isolate and evaluate the anti-termite effect of compounds isolated from fractions and extracts from these plant species.

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