

Full Length Research Paper

Antitermitic activity of plant essential oils and their major constituents against termite *Heterotermes sulcatus* (Isoptera: Rhinotermitidae)

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Termiticide potential of six plant derived essential oils and their thirteen major active chemical constituents were investigated for their termiticidal activities against the termites, *Heterotermes sulcatus* Mathews 1977 (Isoptera: Rhinotermitidae) using no-choice bioassay method. Responses varied with different plant essential oils and their major chemical constituents at a fixed oil concentration. Among the essential oils tested, strong termiticidal activity was observed with the essential oils of *Pittosporum undulatum*, *Lippia sidoides* and *Lippia gracilis*. Oil was obtained by hydrodistillation for 4 h. Each crude essential oil was subjected to separation over SiO₂ soaked with AgNO₃ (15%) column chromatography eluted with n-pentane, n-pentane: CH₂Cl₂ and CH₂Cl₂. The obtained fractions were analyzed by gas chromatography/flame ionization detector (GC/FID). The major constituent's plant essential oils were identified by GC/MS. The constituents thus identified were tested individually for their termiticidal activities against *H. sulcatus*. Limonene, carvacrol and thymol resulted in 100% termite mortality, after relatively short-time of exposure. However, for the practical use of these components in the field, while agent termiticide, the safety of these compounds in humans and non-target organisms must still be evaluated.

Key words: Subterranean termites, terpenes, alternative control.

INTRODUCTION

By reason of their feeding habits and preferences for relatively undecayed living and dead plant material

(Wood 1996), about 10% (Rouland-Lefèvre, 2011) of the 2,882 described species of termites (Constantino, 2014)

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have been reported as pests. They cause significant losses to annual and perennial crops including, palms coconuts, sugar cane, maize, sorghum rice, wheat, groundnuts and cotton (Rouland-Lefèvre, 2011). Among termite species, the most widespread genera in South America is the subterranean *Heterotermes*, being found both in natural and urban environments (Constantino, 2001). Of the six described species of *Heterotermes* in South America, all are pests (Constantino, 2002), because they have been found attacking various crops such as sugar cane (Pizano et al., 1986; Arrigoni et al., 1989), soybeans, groundnut, cotton, maize (Mill, 1992) and rice (Czepack et al., 1998). *H. tenuis* (Hagen) and *H. longiceps* (Snyder) are the most important due to their wide distribution (Constantino, 2002) and financial losses. *H. sulcatus* has been found inside buildings at several urban areas of João Pessoa municipality (Paraíba State), causing an estimated economic loss of US\$ 2,101,330.00 (Vasconcellos et al., 2002). Recently, this species was found infesting small crops of cashew trees in Northeastern Brazil (personal observation) and probably causing production losses.

The control of the termite based in overuse of pesticides has caused several environmental problems and economic losses because of their high toxicity and low biodegradability (including contamination of soil, water, crops and humans) (Koul et al., 2008). Verma et al. (2009) listed several active ingredients that are used globally as termiticides, as follows: bifenthrin, chlorfenapyr, cypermethrin, fipronil, imidacloprid and permethrin. However, the cryptic lifestyle and the social organization of termites make it difficult to control these species. Thus, it is important to search for new alternative methods for controlling termite pests that are less harmful to the environment.

Essential oils of medicinal and aromatic plants have a wide variety of mixtures of natural organic compounds that are the mainline of defense of plants against herbivores and pathogens, among others functions (Bakkali et al., 2008; Koul et al., 2008). Because of the low molecular weight of their compound, essential oils are highly volatile and so are characterized by a low persistence in the environment (Isman, 2006; Isman et al., 2011). Therefore, products are expected to be environmentally safe, thus providing a good alternative to the use of conventional insecticides for the pests' control. The bioactive compounds of essential oils have demonstrated toxic, repellent and deterrent activities to insects (Aslan et al., 2004; Pavela, 2011), inclusive to some species of termites (Chauhan and Raina, 2006; Zhu et al., 2003). Among their many components, the monoterpenes have

attracted the attention of researchers, due to their potential insecticide action (Mondele et al., 2010). Thus, the objective of our study was to evaluate the termiticidal activity of selected plant derived essential oils and their major active chemical components against subterranean termite *H. sulcatus* (Isoptera: Rhinotermitidae).

MATERIALS AND METHODS

Plant and extraction of essential oils

In the present study, essential oils were extracted from the following plants: *Schinus terebinthifolius*, *Pittosporum undulatum*, *Lippia sidoides*, *Lippia gracilis*, *Mentha arvensis* and *Croton cajucara*. All plants were collected in the Northeastern Region Cerrado Biome in Pernambuco State, Brazil, between 1th January and 19th July 2014. Fresh leaves (approximately 350 g) of each plant species were individually subjected to hydrodistillation in a Clevenger type apparatus for 4 h. After extraction using CH_2Cl_2 , the essential oils were dried over anhydrous Na_2SO_4 , filtered and the solvent was evaporated under reduced pressure. Crude oils were maintained under -40°C until the chemical analysis.

Analysis of essential oils by Gas chromatography (GC)

Each crude essential oil was subjected to separation over SiO_2 soaked with AgNO_3 (15%) column chromatography eluted with n-pentane, n-pentane: CH_2Cl_2 and CH_2Cl_2 . The obtained fractions were analyzed by gas chromatography/flame ionization detector (GC/FID) and that which displayed purity higher than >95% was analyzed by gas chromatography-mass spectrometry (GC-MS) aiming at the chemical identification. Identification of the each compound was performed by comparison of recorded mass spectra with those available in the system library. GC chromatograms were obtained on a Shimadzu GC-2010 gas chromatograph equipped with an FID-detector and an automatic injector (Shimadzu AOC-20i) using a RtX-5 (5% phenyl, 95% polydimethylsiloxane (Restek, Bellefonte, PA, USA, 30 m $\times$ 0.32 mm $\times$ 0.25 m film thickness) capillary column. These analyses were performed by injecting 1.0 μl of a 1.0 mg/ml solution of sample material in CH_2Cl_2 in a split mode (1:10) employing helium as the carrier gas (1 ml/min) under the following conditions: injector and detector temperatures of 220 and 250°C , respectively; oven programmed temperature from 40 to 240°C at $3^\circ\text{C}/\text{min}$, holding 5 min at 240°C . GC/MS analysis (70 V and an ion source temperature of 230°C) was conducted on a Shimadzu GC-17A chromatograph interfaced with a MS-QP-5050A mass spectrometer using helium as the carrier gas.

Termites

Subterranean termites, *H. sulcatus* were collected from corrugated cardboard traps of colonies localized in the city of Cristino Castro (PI, Brazil, S08°49'04", 44°13'27"W). Traps were brought to the laboratory and the termites were transferred to plastic boxes containing moist soil. Only large workers (third instar) were used in

Table 1. Plant species and relative percentages and their principal volatile constituents identified in essential oils.

Plant	Family	Major compound(s) in percentage
<i>Pittosporum undulatum</i>	Pittosporaceae	(±)Limonene 80.8; bicyclogermacrene 5.3; β -pinene 2.8
<i>Lippia gracilis</i>	Verbenaceae	Carvacrol 45.6; o-Cymene 9.5; Y-Terpinene 8.9
<i>Lippia sidoides</i>	Verbenaceae	Thymol 78.5; p- cymene 8.6; (E)- caryophyllene 5.6
<i>Mentha arvensis</i>	Lamiaceae	(-)-Menthol 40.53; (-)-menthone 14.63; (+)-menthofuran 4.32
<i>Croton cajucara</i>	Euphorbiaceae	Linalool 12.79; α -guaiene 11.50; <i>epi</i> - β -santalene 8.70.
<i>Schinus terebinthifolius</i>	Anacardiaceae	β -pinene 30.59; α -Pinene, 28.9; β -phellandrene 6.59

the experiments. Termites were used within four days after field collection.

Termiticidal activity

The no-choice bioassay method of Kang et al. (1990) was employed to evaluate the anti-termite activity of the plant essential oils and their major chemical constituents. Sample of 3.5 mg of the essential oils dissolved in 600 μ l of ethanol were applied to 1 g filter paper samples (Whatman No. 3, 8.5 cm in diameter). A piece of filter paper treated with solvent only was used as a control. After the solvent was removed from the treated filter papers by air-drying at ambient temperature, 100 active termites (90 workers and 10 soldiers) above the third instar were put on each piece of filter paper in a Petri dish (9 cm in diameter $\times$ 1.5 cm in height). Drops of water were periodically sprinkled onto the sterilized sand in the dishes to maintain sufficient moisture for the termites. The experimental delineation used was randomized, with six repetitions for each sample. The mortality of termites was evaluated daily for 14 days.

Statistical analyses

Mortality data in termiticidal tests were analyzed using ANOVA. The means were compared by Student-Newman-Keuls test (SNK) with the help of the software SISVAR 4.6. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Chemical composition of essential oils

Essential oils are very complex natural mixtures which can contain about 20 to 60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20 to 70%) compared to others components present in trace amounts. On the basis of comparisons to GC peak area patterns of authentic chemicals, 18 active plant chemical

constituents were identified from the plant essential oils. These active major plant chemical constituents are represented in Table 1. The most abundant compound of *P. undulatum* oil was (+)limonene (80.8%). *L. gracilis* had carvacrol as the major component (45.6%) which was followed by o-Cymene (9.5%) and Y-Terpinene (8.9%). *L. sidoides* had thymol as the major component (78.5%). Menthol (40.53%) and menthone were detected as the main components in *Mentha piperita*. The major compounds of *C. cajucara* were linalool (12.79%) and α -guaiene (11.50%). β -pinene was the major active constituent identified from *S. terebinthifolius* (30.59%).

Anti-termite activity of plant essential oils

All the tested essential oils by using the “no-choice” bioassay method at 3.5 mg/g concentration caused high mortality of *H. sulcatus* in the feeding tests, after relatively short-time exposure (14 days). As shown in Table 2, the most efficient essential oils were *P. undulatum*, *L. sidoides* and *L. gracilis*, and resulted in 100% of mortality in 10 days, indicating strongest toxicity against *H. sulcatus*. The essential oils of *M. piperita* and *S. terebinthifolius* resulted in 100% mortality in 14 days. Among the six essential oils, *C. cajucara* was unable to kill all termites at a dosage of 3.5 mg/g after 14 days and had the lowest toxic effect to termites.

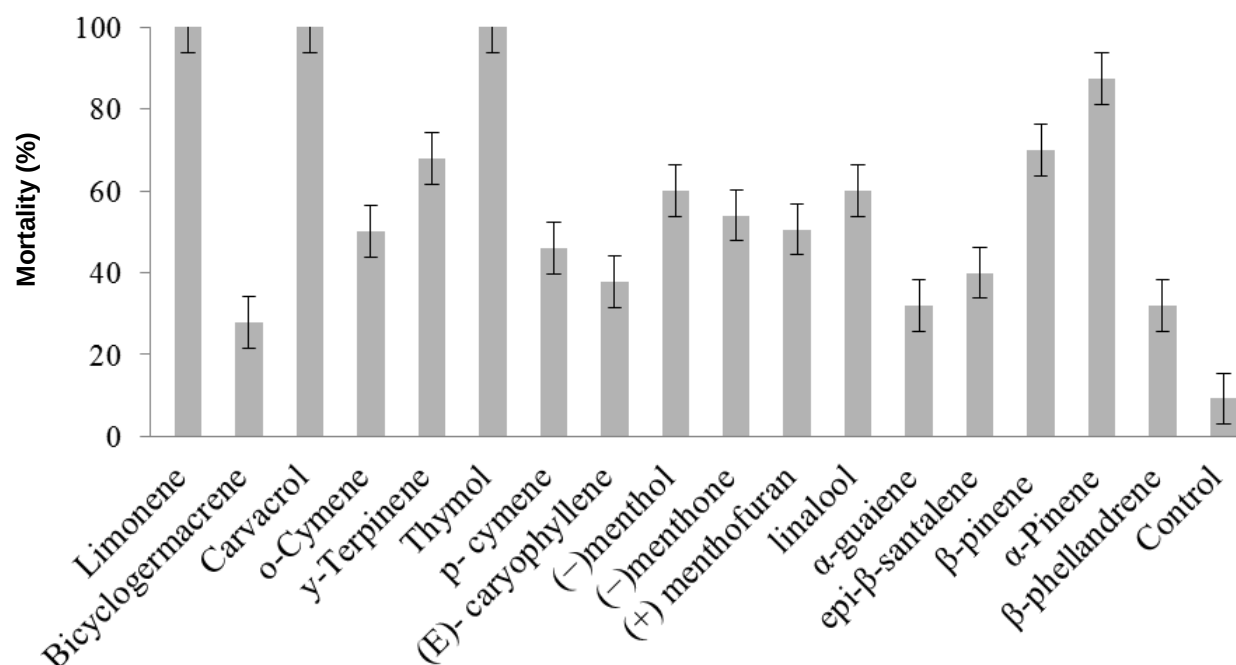
Termiticidal activity of the essential oil constituents

In order to understand the relationship between the constituents of plant essential oils and antitermitic activity, 17 constituents of plant essential oils were tested for antitermitic activity against *H. sulcatus*. Limonene, Bicyclogermacrene, Carvacrol, o-Cymene, γ -Terpinene, Thymol, p- cymene, (E)-Caryophyllene, (-) Menthol, (-)

Table 2. Termiticide-activity of six active plant essential oils against *Heterotermes sulcatus* at a 3.5 mg/g dosage for 14 days after treatment.

Essential oil	Termite mortality (%)			
	1 day	5 days	10 days	14 days
<i>Pittosporum undulatum</i>	5.3 ± 2.3 ^c	88.0 ± 5.0 ^d	100 ± 0.0 ^d	-
<i>Origanum vulgare</i>	4.7 ± 5.0 ^c	82.0 ± 5.2 ^d	100 ± 0.0 ^d	-
<i>Lippia gracilis</i>	4.0 ± 0.0 ^c	78 ± 6.1 ^d	100 ± 0.0 ^d	-
<i>Mentha piperita</i>	2.7 ± 2.3 ^{bc}	40.0 ± 4.0 ^c	73 ± 5.2 ^c	100 ± 0.0 ^b
<i>Croton cajucara</i>	2.7 ± 2.1 ^b	11.0 ± 2.3 ^b	50.3 ± 5.0 ^b	90.0 ± 5.0 ^b
<i>Schinus terebinthifolius</i>	3.3 ± 3.1 ^b	24.0 ± 5.0 ^b	59.5 ± 3.0 ^b	100 ± 0.0 ^b
Control	0.0 ± 0.0 ^a	1.3 ± 1.2 ^a	2.7 ± 1.2 ^a	4.7 ± 1.4 ^a

The means followed by the same letter in the same column do not differ by Student-Newman-Keuls (SNK test) at $P < 0.05$.

**Figure 1.** Termiticide-activity of seventeen active essential oil constituents against *Heterotermes sulcatus* at a 3.5 mg/g dosage for 14 days after treatment.

Menthone, (+)Menthofuran, Linalool, α-Guaiene, epi-β-santalene, β-Pinene, α-Pinene, and β-Phellandrene at a concentration of 3.5 mg/g showed different percentage of mortality after 14 days. The results are summarized in Figure 1. Among these constituents, limonene, carvacrol and thymol resulted in 100% termite mortality. (-)α-pinene

and β-pinene showed 84.5 and 70% termite mortality. The constituents bicyclogermacrene, (E)-caryophyllene, α-guaiene and β-phellandrene showed low termiticidal activity as compared to others tested at the same concentration, with antitermitic activity not higher than 40%.

DISCUSSION

Many phytochemical components are known by their antitermitic, antifeedant (Cárdenas-Marei et al., 2012), or repellent activities against a number of insect pests (Abdelgaleil, 2010; Zahran and Abdelgaleil, 2011). In the present study, all 6 essential oils tested showed antitermitic activity against *H. sulcatus*. *P. undulatum*, *L. sidoides* and *L. gracilis* exhibited higher toxicities to *H. sulcatus*. The major compounds of these oils (limonene, carvacrol, and thymol) also exhibited higher toxicities to *H. sulcatus* resulted in 100% of termite mortality. This can be explained by structure or mechanism of action of the compounds.

Limonene, carvacrol, and thymol showed symptoms of seizures as a way of toxicity for termites, indicating the presence of bioactive molecules present in plant secondary metabolites. Thus, probably the high mortality that both products caused termites is related to their mechanism of action in the nervous system of insects. Although little is known regarding the physiological actions of essential oils on insects, various oils or their components cause symptoms that suggest a neurotoxic mode of action (Regnault-Roger et al., 1995; Choi et al., 2007; Ebadollahi et al., 2013). These symptoms include hyperactivity, seizures, and tremors followed by knock down. Such symptoms are very similar to those produced by the insecticides pyrethroids (Isman, 2006).

Among the essential oils components, limonene is known to have insecticidal, antibiotic and antifungal activity (Ibrahim et al., 2001; Regnault-Roger et al., 2012). Several studies have shown that monoterpenes of relatively simple structure, such as ($\pm$)-limonene causes insect mortality, by inhibiting the enzyme acetylcholinesterase (AChE) (Bruno et al., 1999; Viegas-Jr. et al., 2003). Limonene is a registered active ingredient in 15 pesticide products used as insecticides and insect repellent. Toxicological studies, in recent years, illustrated that carvacrol have very good acute toxicities on various invertebrate pest species including insects, acari, and nematodes (Lei et al., 2010). This compound acts as a positive allosteric modulator for insect binds to γ -aminobutyric acid (GABA) receptors, and can cause inhibitory effect on the insect nervous system (Tong and Coats, 2010). Pandey et al. (2012) also tested the antitermitic activity of seven essential oils and their main components on *Odontotermes assamensis* Holmgren (Isoptera: Termitidae), and found that carvacrol caused 100% mortality of termites in concentration of 2.5 mg/g after 8 days. Carvacrol also proved antitermitic activity to nymphs of the termite

Reticuliter messperatus Kolbe (Isoptera: Rhinotermitidae) in concentration of 1.5 mg/g after 48 h (Ahn et al., 1998; Ebadollahi et al., 2010). Xie et al. (2014) tested the toxicities of forty-two monoterpenes, including eleven monoterpene hydrocarbons and thirty-one oxygenated monoterpenes against *Reticulitermes chinensis* Snyder (Isoptera: Rhinotermitidae) found that (+)-pulegone and carvacrol (ketones and phenols) had the highest toxicity.

Thymol also resulted in 100% termite mortality. This component has shown insecticidal activity against many organisms, such as *Culex quinquefasciatus* Say (Diptera: Culicidae) and *Musca domestica* L. (Diptera: Muscidae) (Pavela, 2011). According to Priestley et al. (2003), thymol binds to GABA receptors associated with chloride channels located on the membrane of postsynaptic neurons and disrupts the functioning of GABA synapses. Lima et al. (2013) also evaluated the biotoxicity of some plant essential oils against the termite *Nasutitermes corniger* (Isoptera: Termitidae) and found that the essential oils of *L. sidoides* containing 44.55% of thymol, proved to be one of the most potent over *N. corniger*, to kill all the termites with 48 h exposure dose 0.27 μ g/mg. According to Hu and Coats (2008), thymol presents few risks to the ecosystem because of its rapid dissipation and low level of residues left in the environment.

In the present study, the compounds of monoterpenes hydrocarbons (limonene, α -pinene, β -pinene) and phenols (carvacrol and thymol) were more effective than alcohols (linalool and menthol) to *H. sulcatus*. This may be explained in a structural as well as pharmacological manner. The termiticidal activity of phenols (thymol and carvacrol) was stronger than that of the monoterpenes alcohol group (linalool). These results are in agreement with those of Seo et al. (2009) who reported that the antitermitic activity of phenols was stronger than that of the alcohol group. Linalool compound was tested against subterranean termite *C. formosanus* in contact toxicity tests in a Petri dish, and caused 59.4% mortality of termites in 7 days in the concentration of 0.5% (Raina et al., 2012). The compound of sesquiterpenes hydrocarbon group ($-$)- α -pinene was more effective than ($-$)- β -pinene. These results agree with those of Cheng et al. (2004) who also reported the antitermitic activity of β -pinene was less stronger than α -pinene. This result indicated that the position of the double bond inside or outside of the six-member ring is also very important in the antitermitic activity (Xie et al., 2014).

In conclusion, the results of this study indicate that the essential oils of *P. undulatum*, *L. sidoides* and *L. gracilis* were the most toxic to *H. sulcatus*. Among the compounds limonene, carvacrol and thymol constitute a

promising alternative to harmful chemicals and persistence in the environment, which are commonly used in termite control. However, for the practical use of these components in the field, while agent termiticide, the safety *L. gracilis* of these compounds in humans and non-target organisms must still be evaluated.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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