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Biotoxicity of some plant essential oils against the termite *Nasutitermes corniger* (Isoptera: Termitidae)

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ABSTRACT

The termite *Nasutitermes corniger* is an important structural pest in the Neotropics. Here we investigated the insecticidal activity of plant essential oils from *Corymbia citriodora, Croton sonderianus*, *Cymbopogon martini*, *Lippia alba, L. gracilis*, *L. sidoides* and *Pogostemon cablin* on *N. corniger*. The chemical composition of the essential oils was analyzed with gas chromatography coupled to mass spectrometry (GC–MS). Toxicity bioassays were performed by a topical application of the essential oils on the termites. Mortality was evaluated at 4, 24 and 48 h after the bioassays were conducted. The essential oils featured the following major compounds: citronellal and citronellol (*C. citriodora*); geraniol (*C. martini*); bicyclogermacrene and α-pinene (*C. sonderianus*); carvone and limonene (*L. alba*); carvacrol and methyl thymol (*L. gracilis*); thymol, ρ-cymene and methyl thymol ether (*L. sidoides*); and patchoulol, α-bulnesene and α-guaiene (*P. cablin*). The lowest LD_{50} and LD_{90} (μg of oil/mg of *N. corniger*) were observed for *P. cablin* (0.37 and 0.85, respectively) and *L. sidoides* (0.38 and 1.98, respectively), while *C. sonderianus* was least toxic (9.48 and 38.43, respectively). All essential oils were more toxic to the workers than to the soldiers. The oils from *P. cablin* and *L. sidoides* were up to 25 times more toxic than the oil from *C. sonderianus*. These results show that the essential oils from *P. cablin* and *L. sidoides* may be promising for further studies aiming the control of termites in the field.

1. Introduction

Termites (Isoptera) are known for their destructive potential due to their ability to utilize cellulose as food resources. Some species have been considered pests in agricultural and urban environments, where they consume plants, roots, wood and attack buildings and other materials (Constantino, 2002a). According to Korb (2007), the estimated damages resulting from the global activity of termites was $50 billion in 2005.

The genus *Nasutitermes* has the highest diversity among the Isoptera. *Nasutitermes corniger* (Termitidae: Nasutitermitinae) (synonymous to *N. costalis*) to is one of the most abundant and widespread species in the Neotropics, occurring from southern Mexico to northern Argentina (Scheffrahn et al., 2005). Although information on the status of many pest species of termites in South America is still limited, *N. corniger* is recognized as a pest of high importance with a high capacity to adapt to urban environments (Constantino, 2002a). This species causes damage to buildings, mainly due to the consumption of wood, and it is considered to be one of the most important structural pests in some regions of South America (Fontes and Milano, 2002; Torales, 1998).

Pest control 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). Although this, organosynthetic insecticides are still the main method of termite control (Elango et al., 2012; Santana et al., 2010). 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 main line of defense of plants against herbivores and pathogens, among others functions (Bakkali et al., 2008; Hartmann, 2007; Koul et al.,

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2008). Because of the low molecular weight of their compounds, essential oils are highly volatile and so are characterized by a low persistence in the environment (Isman, 2006; Isman et al., 2011). Therefore, these 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, 2011a), inclusive to some species of termites (Chauhan and Raina, 2006; Zhu et al., 2001, 2003).

Euphorbiaceae, Poaceae, Myrtaceae, Verbenaceae and Lamiaceae are important families of medicinal and aromatic plants with a potential insecticidal action. The quince *Croton sonderianus* Müll. Arg. (Euphorbiaceae) is a shrub commonly found in northeastern Brazil, and its essential oil has been shown to be rich in compounds with a potential insecticidal action (Lima et al., 2006). The *Corymbia citriodora* (Hook) K.D. Hill & L.A.S. Johnson (Myrtaceae) stands out in Brazil as one of the most used species for extraction of an essential oil that has insecticidal activity (Ootani et al., 2011). The palmarosa lemon grass *Cymbopogon martini* (Roxb) W. Watson (Poaceae), which is a species native to India, is world renowned for its aroma and repellent activity (Rao, 2001). The genus *Lippia*, which includes species such as *L. alba* (Mill.) N.E. Brown, *L. gracilis* Schauer and *L. sidoides* (Cham) (Verbenaceae), represents a group of great economic importance for medicine and agriculture (Pascual et al., 2001). Additionally, the essential oil from patchouli *Pogostemon cablin* (Blanco) Benth (Lamiaceae) is one of the oils of greatest commercial interest in the world (Bizzo et al., 2009). The essential oil of this species is rich in sesquiterpenes and has demonstrated insecticidal activity against *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) (Zhu et al., 2003).

Thus, the aim of this study was to analyze the chemical composition of the essential oils of these medicinal and aromatic plants and to evaluate their toxicity on the termite *N. corniger*.

2. Materials and methods

2.1. Plant material and extraction of essential oils

In the present study, essential oils were extracted from the following plants: *C. citriodora*, *C. sonderianus*, *C. martini*, *L. sidoides*, *L. gracilis*, *L. alba* and *P. cablin*. All plants were collected in the Active Germplasm Bank located on the Rural Campus of the Federal University of Sergipe (UFS), which is located in the municipality of São Cristóvão, Sergipe State, Brazil (11°00′ S, 37°12′ W). The temperature and mean annual precipitation in the region are 26 °C and 1590 mm, respectively. Voucher specimens were deposited in the Herbarium of the UFS, Department of Biology, São Cristóvão, Sergipe, Brazil. Essential oils were extracted from plant leaves. Leaves were collected and dried at 40 ± 1 °C for 4 days in a forced air oven (Marconi MA 037). The essential oils were obtained by hydrodistillation for a period of approximately 140 min after the onset of boiling (Ehlert et al., 2006). The essential oils were separated from the aqueous phase, deposited in amber glass bottles and kept in a freezer until use.

2.2. Analysis of essential oils by GC–MS

Samples of essential oils were analyzed by gas chromatography coupled to mass spectrometry (GC–MS) (Shimadzu, model QP 5050A). The chromatographic conditions were a fused silica capillary column (30 m × 0.25 mm) with a Zebron ZB-5 MS stationary phase (0.25-μm film). The carrier gas was helium with a flow of 1.8 mL min^{-1}. The initial temperature was programmed to be 50 °C for 2 min, which was followed by an increase of 4 °C/min up to 200 °C and then 15 °C up to 250 °C that was kept constant for a further 15 min. The temperature of the detector (or interface) was maintained at 250 °C, the injection volume was 0.5 μL in dichloromethane, the injected rate partition volume was 1:100 and the pressure in the column was 166 kPa. The mass spectrometer conditions were the following: ion capture detector operating by electron impact with an impact energy of 70 eV; a scan speed 1000; a scan interval of 0.50 fragments/s; and fragments of 40–500 Da were detected.

Each component of the essential oils was identified by comparing its mass spectra with the spectra obtained from the equipment database (NIST 21 and NIST 107), with spectra from the literature and by a comparison of retention rate indices from the literature. The relative retention indices (RRI) were determined using a calibration curve from a homologous n-alkane (C_8–C_{18}) series that was injected under the same chromatographic conditions as the samples. The concentration of the compounds was calculated by the total area of their respective peaks in relation to the total area of all compounds in the sample.

2.3. Termites

The studied *N. corniger* workers and soldiers were obtained from fragments of arboreal nests that were collected at the São Cristóvão Campus, UFS (10°55′37″ S, 37°6′40″ W). The nest fragments containing termites were kept in plastic containers (diameter of 50 cm and height of 20 cm) in the dark under ambient conditions (25–27 °C; relative humidity 60 ± 5%) for 24 h before testing. During this time, only water was supplied to the termites. The identification of the samples was performed based on the literature (Constantino, 2002b) and by a comparison with reference material that was deposited in the Termitology Museum of the University of Brasília (UnB), Federal District, Brazil (15°45′51″ S, 47°14′08″ W).

2.4. Toxicity bioassays

The toxicity bioassays were conducted with *N. corniger* workers and soldiers at the Clinical Plant Health Laboratory of the UFS, São Cristóvão, Sergipe, Brazil. Because *Nasutitermes* workers are known to exhibit polymorphism and age polyethism (McMahan, 1977) only large workers (third instar beyond) were used in the experiments. Recruitment activity during foraging is related with quantitative differences in the sizes of worker (Traniello, 1981), indicating that larger workers have more pheromone concentration (Traniello and Busher, 1985). So larger worker, as used here, could be more participation in foraging.

Preliminary tests were performed to determine the density and the ratio worker/soldier used in the toxicity bioassay. To analysis the effect of the soldiers' proportion on the survival of termites one experiment was performed in Petri dishes (60 mm× 15 mm) containing 100 termites. The treatments consisted in 6 different proportions of workers (w):soldiers (s) (100:0, 90:10, 80:20, 70:30, 60:40, and 50:50 w/s). Eight replicates were made to each treatment. Other experiment was performed in order to verify a possible effect of the density of individuals on the survival of termites group. The percentage of termite mortality was analyzed in Petri dish (60 mm× 15 mm) with 100 and 10 individuals, both cases with 20% of soldier. Ten replicates were made to each group size. All preliminary tests were conducted without applications of essential oils and the mortality of the termites was observed after 48 h.

The treatments of the bioassays of toxicity consisted of the essential oils from *C. citriodora*, *C. sonderianus*, *C. martini*, *L. sidoides*, *L. gracilis*, *L. alba* and *P. cablin* in addition to a control (acetone solvent). The preliminary tests were performed with a topical application of acetone to ensure that the solvent did not affect the survival of the insects. The experimental design was completely