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Short communication

Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus (Boophilus) microplus*

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ABSTRACT

The present study aimed to evaluate the activity of *Lippia gracilis* Schauer essential oil obtained from different *L. gracilis* genotypes and their major components, carvacrol and thymol against *Rhipicephalus (Boophilus) microplus* (cattle tick) larvae and engorged females. The larval test was performed parallel to the adult immersion test for engorged females for four *L. gracilis* genotypes. Similar tests were further performed for their major compounds carvacrol and thymol. Carvacrol (LC_{50} of 0.22 and 4.46 mg/mL, to larvae and engorged females, respectively) was more efficient than thymol (LC_{50} of 3.86 and 5.50 mg/mL, to larvae and engorged females, respectively). The lethal concentrations obtained for the isolated essential oil from genotypes LGRA-201 against larvae (1.31 mg/mL) and LGRA-106 against engorged females (4.66 mg/mL) confirmed the acaricidal activity of *L. gracilis* essential oil and its effectiveness in controlling the southern cattle tick.

1. Introduction

Lippia gracilis Schauer (Verbenaceae) is a deciduous branched shrub able to grow to approximately 2 m. This plant is a species of the vegetation typical of a well-drained, semi-arid region in northeastern Brazil. Its aromatic leaves and flowers constitute the plant medicinal components, composed of tissues from which the essential oil is extracted. The plant oil exhibits antimicrobial activity due to its high content of carvacrol and/or thymol (Pessoa et al., 2005). An Active Germplasm Bank of *L. gracilis* is maintained at the Federal University of Sergipe and plant material is available to supply cuttings for production of plantlets needed for large scale cultivation and essential oil production.

Generally, members of the *Lippia* genus have similar chemical composition, with some compounds present in several species. These compounds include thymol, carvacrol, 1,8-cineol, humulene, and *p*-cymene, which exhibit anti-malarial, anti-viral, and cytostatic pharmacological activities (Cavalcanti et al., 2010) and may be used for the control of insects and ticks.

The southern cattle tick, *Rhipicephalus (Boophilus) microplus*, has been associated with losses in milk and beef production as well as damage to animal hides, which in turn have resulted in economic losses throughout the tropical and subtropical region where this tick is distributed (Graf et al., 2004).

The use of synthetic acaricidal products is the most common method for controlling southern cattle tick. However,

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the frequent use of these products in cattle herds may lead to contamination of milk and meat and fostering the selection of resistant ticks. In many countries, acaricide-resistant tick populations have increased to the point that few synthetic acaricides continue to have efficiency greater than 75% (Graf et al., 2004). Castrejón (2003) have previously proposed the use of non-chemical control methods, including the use of plants with acaricide compounds, to reduce the environmental and financial impact of synthetic acaricides. In this context, phytochemicals may represent a useful tool for the control of ectoparasites and can also potentially be combined with other control strategies. Furthermore, phytochemicals may contribute to the production of milk and animal meat free of unsafe chemicals that are harmful to humans, animals, and the environment (Agnolin et al., 2010). Additionally, thymol and carvacrol are Generally Regarded As Safe (GRAS) food flavoring, which is an indication of low toxicity materials (United States Office of the Federal Register, 2009).

The aim of the present study was to evaluate the activity of *L. gracilis* genotypes essential oil and their major components, carvacrol and thymol, to *R. (B.) microplus* larvae and engorged females.

2. Methods

2.1. Plant and biological materials

Leaves were collected from four *L. gracilis* genotypes (Table 1) from the Active Germplasm Bank (BAG) of Medicinal Plants at the Experimental Farm Rural Campus of the Federal University of Sergipe (UFS). Defoliation was performed manually, and leaves were dried in a forced air circulation oven at 40 °C for 5 days. *R. (B.) microplus* ticks used in the bioassays were bred and maintained at the Animal Science Department of the Federal University of Maranhão (UFMA).

2.2. Extraction and analysis of essential oil

The essential oil was extracted by hydrodistillation in a Clevenger apparatus. Each sample consisted of 75 g of dried leaves harvested from three cloned plants, and samples were distilled for 140 min.

The analysis of the essential oil chemical composition was performed using a gas chromatograph coupled to a mass spectrometer (GC–MS) (Shimadzu, model QP 5050A) equipped with an AOC-20i auto injector (Shimadzu) and a fused-silica capillary column (5% phenyl–95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 μm film, J&W Scientific). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The temperature program was as follows: 50 °C for 1.5 min, temperature increased at 4 °C/min until reaching 200 °C, temperature increase at 15 °C/min until reaching 250 °C, and stayed at 250 °C for 5 min. The injector temperature was 250 °C, and the detector (or interface) temperature was 280 °C. The injection volume of ethyl acetate was 0.5 μL, the partition rate of the injected volume was 1:87, and the column pressure was 64.20 kPa. The mass spectrometer conditions were as follows: ionic capture detector impact energy of 70 eV, scanning speed 0.85 scan/s from 40 to 550 Da.

Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (FID), using a Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) instrument, under the following operational conditions: capillary ZB-5MS column (5% phenyl-arylene–95% dimethylpolysiloxane) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) from Phenomenex (Torrance, CA, USA), under same conditions as reported for the GC–MS. Quantification of each constituent was estimated by area normalization (%). Compound concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

The essential oil components were identified by comparing their mass spectra with the available spectra in the equipment database (NIST05 and WILEY8). Additionally, the measured retention indices were compared with those in the literature (Adams, 2007). The relative retention indices (RRI) were determined using the Vandendool and Kratz (1963) equation and a homologous series of *n*-alkanes (C_8–C_{18}) injected under the chromatography conditions described above.

The means of the chemical constituents and essential content were subjected to the analysis of variance *F* test and were compared using the Scott–Knott test at 5% probability.

2.3. Larval sensitivity test

A sensitivity test was performed on *R. (B.) microplus* larvae at the Animal Parasitology Laboratory at the UFMA Chapadinha Campus. The methodology was developed by Stone and Haydock in 1962, and adapted by the Food and Agriculture Organization (FAO, 1984) and Leite (1988). Two sheets of filter paper (4 cm^2) (Whatman, 80g) were treated with 0.4 mL of solution containing 3% dimethyl sulfoxide (DMSO) and essential oil or one of the major components. Ten concentrations ranging from 0.0612 to 25.00 mg/mL of thymol (Merck) or carvacrol (Sigma–Aldrich) or essential oil isolated from each of the four *L. gracilis* genotypes were used for the test.

Approximately 100 tick larvae were placed on one of the sheets and then covered with the other sheet, forming a sandwich. The sandwiched filter papers and larvae were

Table 1
Lippia gracilis genotypes maintained at the Active Germplasm Bank of Medicinal Plants (UFS) and used in the present study.

Code	Source	Geographic data for the source	Herbarium voucher no.
LGRA-106	Tomar do Geru – SE	11 19′ 16.7″ S; 37 55′ 09.2″ W	14733
LGRA-108	Tomar do Geru – SE	11 19′ 22.4″ S; 37 55′ 12.6″ W	14734
LGRA-109	Tomar do Geru – SE	11 19′ 20.7″ S; 37 55′ 16.9″ W	14735
LGRA-201	Rio Real – BA	11 23′ 38.7″ S; 38 00′ 54.1″ W	14736