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Efficacy of 11 Brazilian essential oils on lethality of the cattle tick *Rhipicephalus (Boophilus) microplus*

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ABSTRACT

Herbal extracts have been investigated as an alternative for parasite control, aiming to slow the development of resistance and to obtain low-cost biodegradable parasiticides. The goal of this study was to evaluate the efficacy, *in vitro*, of 11 essential oils from Brazil on reproductive efficiency and lethality of the cattle tick *Rhipicephalus (Boophilus) microplus*. The effects of oils extracted from *Curcuma longa*, *Zingiber officinale*, *Lippia alba*, *Lippia gracilis*, *Lippia origanoides*, *Lippia sidoides*, *Mentha arvensis*, *Mentha piperita*, *Croton cajucara* (white and red), and *Croton sacaquinha* on ticks were investigated by the Immersion Test with Engorged Females (ITEF) and the modified Larval Packet Test (LPT). Distilled water and 2% Tween 80 were used as control treatments. Chemical analysis of the oils was done with gas chromatography coupled with mass spectrometry. Analysis of the *in vitro* tests using Probit (SAS program) allowed the calculation of lethal concentrations (LCs). Lower reproductive efficiency indexes and higher efficacy percentages in the ITEF were obtained with the oils extracted from *C. longa* (24 and 71%, respectively) and *M. arvensis* oils (27 and 73%, respectively). Lower LC_{50} was reached with *C. longa* (10.24 mg/mL), *L. alba* (10.78 mg/mL), *M. arvensis* (22.31 mg/mL), *L. sidoides* (27.67 mg/mL), and *C. sacaquinha* (29.88 mg/mL) oils. In the LPT, species from Zingiberaceae and Verbenaceae families caused 100% lethality at 25 mg/mL, except for *L. sidoides*. The most effective oils were from *C. longa*, *L. gracilis*, *L. origanoides*, *L. alba*, and *Z. officinale*. The LC_{50} and LC_{90} were, respectively: 0.54 and 1.80 mg/mL, 3.21 and 7.03 mg/mL, 3.10 and 8.44 mg/mL, 5.85 and 11.14 mg/mL, and 7.75 and 13.62 mg/mL. The efficacy was directly related to the major components in each essential oil, and the oils derived from *Croton* genus presented the worst performance, suggesting the absence of synergistic effect among its compounds. Since *C. longa*, containing 62% turmerone, was the one most efficient against ticks, this compound may be potentially used for tick control, but further research is needed, especially to assess toxicity of these compounds to the host. These new studies, together with the results presented here, may provide a strong rationale for designing pre-clinical and clinical studies with these agents.

1. Introduction

Brazil is the world's largest beef producer and exporter. In 2013, Brazil had approximately 212 million bovine heads and from July to September of 2014, almost 8.5 million bovines were slaughtered (IBGE, 2014). Due to its territorial size, the country still has enormous potential to expand its animal production, but parasites represent a substantial constraint in tropical areas. Economic losses related to *Rhipicephalus (Boophilus) microplus* are due to a decrease in the production of meat and milk, hide damage, costs for tick control (labor, equipment, facilities, acaricides) and loss or treatment of animals with tick-borne diseases caused by *Babesia bigemina*, *Babesia bovis*, and *Anaplasma marginale* (Leal et al., 2003). Recent estimates of annual losses caused by the cattle tick, also known as southern cattle tick, reach around $3.2 billion. The annual potential economic losses due to the five major ectoparasites (*R. (B.) microplus*, *Haematobia irritans*, *Dermatobia hominis*, *Cochliomyia*

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hominivorax, and *Stomoxys calcitrans*) and gastrointestinal nematodes of cattle in Brazil reach $13.9 billion (Grisi et al., 2014).

Chemical treatment with acaricides and anthelmintics has been the main mode of control of these parasites, but because of the increasing development of parasite resistance and the high cost of chemical products in developing countries, there is an emerging interest in alternative approaches to manage parasite populations (Lem et al., 2014). From the 1960s to the 1990s, resistance of the southern cattle tick to commercial acaricides such as organochlorates, organophosphates, synthetic pyrethroids, amitraz, and macrocyclic lactones was observed (Castro-Janer et al., 2015). Although fipronil and fluazuron were not commonly adopted for tick control, reduction in their costs and tick resistance to other molecules increased their use at the beginning of this century and currently, fluazuron is the only molecule for which no reports have described the presence of resistance in Uruguay. Recently, however, resistance to fluazuron, and cross-resistance between fipronil and lindane in *R. (B.) microplus* have been reported in Brazil (Reck et al., 2014; Castro-Janer et al., 2015). The emergence of highly resistant organisms has created a worrisome scenario that has influenced the course of scientific research in the field of healthcare. Therefore, the search for alternatives to minimize this problem has been proposed, such as the use of botanical insecticides based on plant extracts.

Essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal, and cosmetic applications, especially in the pharmaceutical, sanitary, cosmetic, agricultural, and food industries (Bakkali et al., 2008). Some of these oils constitute effective alternatives with reduced secondary effects, which can be used as single agents or together with synthetic chemical compounds (Carson and Riley, 2003). Extracts from some botanical families have been recognized as presenting biological activities against different organisms. In addition, there are an increasing number of studies concerning the use of essential oils in the veterinary field, particularly as control agents against ectoparasites like lice, mites, and ticks. A possible advantage of essential oils over conventional ectoparasite treatments may be associated with their reported ovicidal efficacy, repellent action due to their volatile components, and effect on tick fecundity when females are exposed to sub-lethal doses of essential oils (Ellse and Wall, 2014).

In the present study, essential oils extracted from plant species of the following families were evaluated: Zingiberaceae (*Curcuma longa* and *Zingiber officinale*), Verbenaceae (*Lippia alba*, *Lippia gracilis*, *Lippia origanoides*, and *Lippia sidoides*), Lamiaceae (*Mentha arvensis* and *Mentha piperita*), and Euphorbiaceae (*Croton cajucara* (red and white morphotypes) and *Croton sacaquinha*). The aim of this study was to investigate the efficacy of these 11 essential oils on the reproductive efficiency of engorged females, as well as whether any of them can be lethal to engorged females and larvae of *R. (B.) microplus*.

2. Materials and methods

2.1. Plant material

The plant species used in the present study (Fig. 1; Table 1) were grown in 2014 at Embrapa Amazônia Ocidental, located in the city of Manaus, Amazonas State (AM), Brazil. Botanical samples of each species were deposited at the EAFM Herbarium of the Instituto Federal do Amazonas, located in Manaus. The cultivation occurred in field plots with soil classified as Dystrophic Yellow Latosol and which were composted using chicken manure (1.0 kg m^2) incorporated before cultivation. The field plots were located at the geographic coordinates 03°06′23.04″S and 60°01′35.14″W, having average altitude of 50 m, average temperature of 25.6 °C, and average annual rainfall of 2200 mm. The equatorial climate is characterized as Af, according to the Köppen classification (Köppen and Geiger, 1928).

2.2. Oil extraction

After harvest in July 2014, the parts of the plants (Table 1) were separated and put to dry in a covered shed for a week at an average temperature of 27 °C. The essential oils from each species and plant part were extracted by hydrodistillation in a modified Clevenger apparatus for 4 h. After separation was complete, oils were dried over anhydrous sodium sulfate and kept refrigerated (8 °C) in closed vials until used.

2.3. Chemical analysis

Chemical analysis of the oils was done with gas chromatography coupled with mass spectrometry (GC–MS) in an Agilent 5973 N system (Agilent Technologies, DE, USA) equipped with an HP-5MS capillary column (5%-diphenyl, 95%-dimethylsilicone, 30 m × 0.25 mm; film thickness 0.25 μm). Helium was used as the carrier gas (1.0 mL min^{-1}). A total volume of 1.0 μL of a 1% solution of the oil in dichloromethane was injected into an injector heated to 250 °C, operating in split mode (split ratio 1:100). Oven temperature was raised from 60 to 240 °C, at 3 °C min^{-1}. The mass detector was operated in electronic ionization mode (70 eV) with the mass analyzer maintained at 150 °C, the ionization source at 220 °C, and the transfer line at 260 °C. Linear retention indices were calculated by injection of a series of n-alkanes (C_7–C_{26}) in the same column and conditions as above. Identification of the essential oil components was done by comparison of both mass spectra and retention indices with the Wiley sixth edition spectral database and other data from the literature.

For quantification, the essential oils were analyzed in an Agilent 7890A chromatograph equipped with a flame ionization detector (FID) kept at 280 °C and fitted with an HP-5MS capillary column

Fig. 1. Plant species used to obtain the essential oils: *Croton cajucara* (morphotype white (A) and red (B)), *C. sacaquinha* (C), *Curcuma longa* (D), *Lippia alba* (E), *L. gracilis* (F), *L. origanoides* (G), *L. sidoides* (H), *Mentha arvensis* (I), *M. piperita* (J), *Zingiber officinale* (L).