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Acaricidal properties of vetiver essential oil from *Chrysopogon zizanioides* (Poaceae) against the tick species *Amblyomma cajennense* and *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)

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ABSTRACT

Ticks are arthropods widely distributed in tropical and subtropical regions, which can transmit infectious agents also responsible for zoonoses. Excessive use of conventional acaricides has resulted in the onset of drug resistance by these parasites, thus the need to use alternative methods for their control. This study evaluated the acaricidal activities of *Chrysopogon zizanioides* (vetiver) essential oils containing different zizanoic and khuzimol (high and low acidity) acid concentrations on *Amblyomma cajennense* and *Rhipicephalus microplus* (Acari: Ixodidae). To this aims, toxicity tests of different concentrations of examined essential oils were conducted on adult females and larval stages. Results showed that the essential oils of *C. zizanioides* with high and low acidity reduced oviposition of females, eggs hatch and larval survival, being more effective than some commercial products widely used to control these ectoparasites. These results indicate that the *C. zizanoides* essential oils are promising candidates as acaricidal agents and represent also an add value to vetiver oil with high acidity, which is commercially undervalued in the cosmetic industry.

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1. Introduction

Especially in tropical and subtropical regions, major economic losses are recorded annually due to tick infections and their blood-sucking habit, which confer to these arthropods medical and veterinary importance (Grisi et al., 2002). Worldwide, the economic losses caused by ticks are on the order of tens of billions dollars per year (Parizi et al., 2011). In Brazil, *Rhipicephalus microplus* and *Amblyomma cajennense* are vectors of etiological agents that cause severe diseases, like tick fever in cattle and spotted fever in humans (Olivo et al., 2008).

The main method of tick control is the use of synthetic pesticides, including synthetic pyrethroids, organophosphates and amitraz (Furlong et al., 2004; Furlong and Martins, 2005; Gazim et al., 2011). However, the indiscriminated and intensive use of acaricides has caused the onset of drug-resistance phenomena by these ectoparasites (Labruna et al., 2004), environmental pollution and harm to human and animal health (Chagas et al., 2002; Chagas, 2004; Nerio et al., 2010). Thus, the search for alternative methods is of great relevance for these economic, health and ecological issues (Bacci et al., 2007).

Biologically active botanical compounds represent a viable strategy for tick control, for having generally lower cost and lower toxicity to animals, humans and non-target organisms (Moreira et al., 2007; Bagavan et al., 2009). The use of plant extracts with potential acaricidal activity has been the focus of a larger number of studies, which have demonstrated repellency, oviposition inhi-

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bition, feed activity reduction and alteration of larval development against these arthropods (Roel, 2001).

Chrysopogon zizanioides (Poaceae) (vetiver) essential oil showed bactericidal, fungicidal, insecticidal and anti-inflammatory properties (Mao et al., 2006; Bizzo et al., 2009; Danh et al., 2010). This essential oil is also widely used in the cosmetic industry as a natural fixative of volatile essences (Monteiro et al., 2011; Santos et al., 2002). There are two variations of the *C. zizanioides* essential oil available in the market: one with high acid value (HAV) (low quality) and the other one with low acid value (LAV) (high quality). For the cosmetic industry, its quality is related to the concentration of fatty acids and sesquiterpene zizanoic acid and khusimol (alcoholic portion) (Dantas et al., 2007; ISO 1242, 1999). The acid value of the essential oil is directly proportional to the fatty acids and zizanoic acid concentration and the reverse occurs with khusimol (Martinez et al., 2004). So far there are no data in literature demonstrating the acaricidal activity of this plant and, despite *C. zizanioides* HAV essential oil has lower acceptability in the perfumery industry, it is not known if it has the desired properties for the control of arthropods. Therefore, this study aimed to evaluate the acaricidal activity of *C. zizanioides* essential oils on *A. cajennense* and *R. microplus*. Tick reproductive parameters and larval mortality were compared with the activity of commercial products largely used in the control of these arthropod species.

2. Material and methods

2.1. Essential oils

Essential oils of *C. zizanioides* HAV and LAV were obtained by hydrodistillation of *C. zizanioides* roots and acquired from the company Raros Naturals, located in Macaíba, Rio Grande do Norte, Brazil. The acid value of the *C. zizanioides* essential oil was measured by the amount (mg) of potassium hydroxide required to neutralize the free fatty acids contained in 1 g of essential oil (ISO 1242, 1999). 45 mg/g potassium hydroxide were used for the essential oils of *C. zizanioides* with high acid value (HAV) and 7.5 mg/g for oil with low acid value (LAV).

2.2. Chemical analysis of essential oils

Samples of the *C. zizanioides* essential oils (HAV and LAV) were analyzed by chromatography using a flame ionization detector (CG–FID) and mass spectrometry (CG–EM). The chromatographic conditions were: fused silica capillary column (30 m × 0.25 mm) with a stationary phase Zebron ZB–5MS (0.25 μm in film thickness); helium as a gas carrier at a flow rate of 1.8 ml/min keeping the temperature programmed in 50 °C for 2 min, followed by an increase of 4 °C/min until 200 °C, then at 15 °C up to 250 °C, keeping this temperature constant for 15 min; detector temperature (or interface) 250 °C; injection volume of 0.5 μl in dichloromethane; partition rate of injected volume 1:100 and column pressure of 166 kPa. Conditions of the mass spectrometer: ion capture detector operating in electronic impact and energy impact of 70 eV; sweep speed 1.000; sweep range of 0.50 fragments and fragments detected on 40–500 Da.

Each component of the essential oils was identified by comparing its mass spectrum, spectra and retention indexes with data reported in database (NIST 21 and NIST 107) and in the literature (Adams et al., 2008). The relative retention ratios (RRR) were determined using a calibration curve of a series of *n*-alkanes (C_8–C_{18}) injected in the same samples chromatographic conditions. The concentration of the constituents was calculated from the integral area of the respective peaks related to the total area of all the sample constituents.

2.3. Ticks

A total of 1000 engorged females of *A. cajennense* and *R. microplus* species were collected with forceps directly on horses and cattle, respectively. Animal hosts were kept isolated without application of acaricidal treatments for a minimum of 45 days. The species *A. cajennense* was collected in the municipality of Umbaúba (11°22′27″S, 37°39′27″W) and *R. microplus* in São Miguel do Aleixo (10°23′26″S, 37°22′42″W), both localities in the state of Sergipe, Brazil. The identification of the ticks was made by using specific taxonomic keys (Fletchmann, 1990).

A total of 580 engorged females were used in immersion bioassays, while the remaining females were kept in biological oxygen demand (BOD) for the production of larvae, which were used in other bioassays.

2.4. Bioassay with adults

A total of 580 engorged female ticks of both species (290 females for each species) kept in the laboratory were washed with distilled water and dried on paper towel for exposure to treatment. The bioassay used was adapted from Drummond et al. (1973) in which the engorged *A. cajennense* (198.2 ± 9.2 mg/female) and *R. microplus* (200.2 ± 4.0 mg/female) females were immersed for 2 min in 3 ml final volume of the solutions tested.

For each bioassay, five engorged females were placed in Petri dishes (4 cm diameter, 2 cm height) lined with filter paper. The experimental design was completely randomized. All products applied were emulsified in distilled water and Triton X-100 at 2%, as surfactant agent. For the negative control females were immersed only in distilled water and Triton X-100 (2%). Ten repetitions (5 females/plate) were performed for each tick species, totalizing 100 females. Positive controls were performed using commercial products Natuneem® (*Azadirachta indica* oil) at the concentration of 10 μl ml^{-1} and Butox® P CE25 (*deltamethrin*) at recommended doses 2 and 1 μl ml^{-1} for *A. cajennense* and *R. microplus*, respectively. In these cases four repetitions (5 females/plate) were performed considering the two acaricides by each tick species, totalizing 80 females. For treatments containing the essential oils of *C. zizanioides* HAV and LAV concentrations of 1, 10, 20, 50 and 100 μl ml^{-1} were used in four replicates (5 females/plate) for both essential oils at five concentrations by each tick species, totalizing 400 females.

The plates containing treated females (=580) were covered with transparent plastic (PVC film) and kept in BOD incubator at 27 ± 1 °C temperature and 75 ± 5% humidity. 14 days after oviposition the eggs were weighed to determine the yield of eggs per female (PO) (g).

For each treatment, a sample (~30 mg) was withdrawn for the evaluation of the egg hatch percentage. The eggs were placed in test tubes previously identified, sealed with PVC film and kept for 60 days under the same conditions of temperature and humidity above mentioned. After this period, the number of larvae counted in each egg mass was recorded.

For the two species of ticks relations corresponding the number of eggs to its mass were laid. For this, 10 groups of about 70 mg of eggs from untreated ticks had their masses and their amount of eggs determined. Thus, it was possible to estimate the number of eggs (or estimated number of larvae) in a mass of eggs for each tick species.

With these data it was possible to determine the following reproductive parameters based on the formulae according to Stendel (1980):

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