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Repellent activity of plant-derived compounds against *Amblyomma cajennense* (Acari: Ixodidae) nymphs

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ABSTRACT

Repellence responses of *Amblyomma cajennense* nymphs to callicarpenal, intermedeol, *Hyptis suaveolens* essential oil, extract of *Melia azedarach*, *Cymbopogon nardus*, *Spiranthera odoratissima*, *Chenopodium ambrosioides*, *Ageratum conyzoides*, *Mentha pulegium*, *Ruta graveolens*, and *Memora nodosa* were studied. Among these the extract of *C. nardus* stood out because of the long-lasting repellence, maintaining, in the highest concentration, 35 h of protection against 90% of the nymphs. The essential oil of *H. suaveolens* and the extracts of *C. ambrosioides* and *A. conyzoides* showed good repellence index (66%) when applied in high concentrations. However, greater protection could be obtained at higher concentrations but with a shorter repellence time. Callicarpenal, intermedeol, extract of *M. Pulegium*, and *M. nodosa* leaves showed moderate repellence in high concentrations. Extracts from *M. azedarach*, *R. graveolens*, *S. odoratissima*, and *M. nodosa* roots showed little or no repellent effect. These results show that some plant extracts may represent a promising alternative in the control of infestations by *A. cajennense*.

1. Introduction

Amblyomma cajennense (Fabricius), the Cayenne tick, can be found all over the American continent, from the southern USA to northern Argentina (Guglielmone et al., 2006). It is a three-host tick, whose adults prefer to feed on large mammals such as equines, bovines, tapirs and capybaras. Other domestic and wild animals, as well as human beings, are also infested by this species. It is the highest prevalent species in humans in the geographic region where it occurs (Guglielmone et al., 2006). Due to its low parasite specificity it has a major role in the transmission of pathogens between animals and humans, such as *Rickettsia rickettsii* (Wolbach) Brumpt, causal agent of Rocky Mountain spotted fever, in Central and South America (Bustamante et al., 1946; De Rodaniche, 1953; Dias and Martins, 1939). It is also able to experimentally transmit *Rickettsia parkeri* Lackman et al. (Sangioni et al., 2005), an agent related to clinical symptoms of a rickettsial fever (Paddock et al., 2004) and can be involved in the transmission of a rickettsia closely related to *Rickettsia honei* Stenos et al. (Billings et al., 1998), responsible for clinical symptoms of the spotted group rickettsiosis in Thailand (Stenos et al., 1998).

The World Health Organization (WHO) recommends the use of personal protection mechanisms to arthropod-borne

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diseases in endemic regions (Barnard, 2000). As far as we know the only repellents evaluated against *A. cajennense* were the reference compound DEET (Soares, 2008), formic acid and abdominal secretion of carpenter ants (Falotico et al., 2007). DEET (N,N-diethyl-3-methilbenzamida) is a well-characterized compound that has been extensively evaluated against ticks (Carroll et al., 2005; Pretorius et al., 2003; Schreck et al., 1995; Staub et al., 2002). The use of synthetic compounds such as DEET has elicited concerns about the dangerous effects on humans and the environment. Thus the interest in plants as natural sources of repellent compounds for personal protection against hematophagic arthropods has been renewed. Some plants have traditionally been used for this purpose, with efficiency proven against ticks; such plants include citronella (*Cymbopogon nardus* (L.) Rendle), cloves (*Allium sativum* L.), lily of the valley (*Convallaria majalis* L.), and horehound (*Hyptis suaveolens* L.) against the castor-bean tick *Ixodes ricinus*, and terpenoids extracted from American beautyberry, *Callicarpa americana* L., against the black-legged tick *Ixodes scapularis* and the lone star tick *Amblyomma americanum* (Thorsell et al., 2006; Carroll et al., 2007; Garboui, 2008). This study was developed with the aim to investigate the repellence of extracts of chinaberry tree *Melia azedarach* L., citronella *C. nardus*, "erva-de-santa-maria" *Chenopodium ambrosioides* L., mexican tea *Ageratum conyzoides* L., pennyroyal *Mentha pulegium* L., rue *Ruta graveolens* L., "manacá" *Spiranthera odoratissima* A. St.-Hill, and "carobinha do campo" *Memora nodosa* (Manso) Miers against *A. cajennense* nymphs. The essential oil of *H. suaveolens* and the terpenoids callicarpenal and intermedeol, extracted from the *C. americana* plant, were also evaluated.

2. Materials and methods

2.1. Ticks and volunteers

A. cajennense-engorged females were obtained in naturally infested equines and incubated in a chamber (27 °C, 80% RH) during the oviposition period. Five-day-old larvae were placed to feed on rabbits (*Oryctolagus cuniculus*) using a feeding chamber (Sonenshine, 1991). Engorged larvae were collected from the rabbits and incubated in the same conditions mentioned above. In the repellency bioassays, unfed nymphs with ages varying from 2 weeks to 2 months were tested. According to the protocol established by Sanavria and Prata (1996) for the maintenance of colonies of *A. cajennense* in the laboratory, nymphs with that age are already able to attach and feed. The bioassays were carried out on three female volunteers. The use of the animals as well as the use of human beings were approved by the Research Ethics Committee from UFG (protocol #11 05/03/2007).

2.2. Test compounds

Two terpenoids were used: callicarpenal (13,14,15,16-tetranor-3-cleroden-12-al, C16H27O, MM 235,20) and intermedeol (C15H26O, MM 222,37), extracted from *C. americana*. They were donated by Dr. Charles L. Cantrell, Natural Products Utilization research Unit, USDA, ARS. Hexanic extract from green fruits of *M. azedarach* was obtained by continuous hot Soxhlet extraction. The essential oil of *H. suaveolens* was obtained by hydrolyzation using a Clevenger-type apparatus (Guenther, 1972) and sodium sulphate anhydrous for drying the product. Leaves and aerial parts of *A. conyzoides*, *C. ambrosioides*, *C. nardus*, *M. pulegium*, *R. graveolens*, and *S. odoratissima* and leaves and roots of *M. nodosa* were used to produce ethanolic extracts. These organic materials were dried at room temperature for 3–10 days and then triturated. In approximately 200 g of the powder, 1000 mL of ethanol 95% was added and then mixed for 4 h. The mixture was filtered and the residue was submitted to two more extractions, in the same conditions. The filtered ones were mixed and concentrated in rotavapor at $\leq$40 °C temperature.

Characteristics of the plants, such as family, scientific and common names, collection place, part of the plant used, type of product obtained and concentrations used are shown in Table 1. The callicarpenal and intermedeol were used at similar initial dilutions verified as repellent for the tick *I. scapularis* by Carroll et al. (2007); first at 0.288 mg/cm^2 and 0.272 mg/cm^2 consecutively and then in three more concentrations consecutively diluted by half. The other compounds were initially diluted at 10% and then tested. Those that had percentages of repellence lower than 70% were tested in three more concentrations consecutively increased doubly. The products that showed percentages of repellence higher than 70% were tested in two more consecutive increased concentrations doubly and one diluted by half. The extract of *M. nodosa* leaves was only tested in a concentration of 10% and two more consecutive doubly increased concentrations because of the small amount of extract obtained. Ethanol was used as the solvent, except for the extract of *M. azedarach* that was diluted in water.

2.3. Fingertip bioassay

This bioassay was developed according to Schreck et al. (1995). The proximal phalange of the left index finger of one volunteer was treated with DEET and the right one with ethanol 95% as the negative control. A volume of 2.75 μl/cm^2 per treated area was used, and the test was performed 10 min after the solution had been applied to allow the solvent evaporation. A nymph was released, individually, on the distal phalange; then the finger was vertically positioned tip downward, allowing the tick to climb up the finger due to its negative geotropism. The ticks that dropped off the finger, inverted their direction after touching the treated area, or remained on the releasing point for 1 min were considered repelled. To evaluate the repellent time, the tests were repeated after 50 min and later at each hour, until repellence was lower than 50%. For each concentration and time after application, 30 ticks were evaluated. All nymphs were previously tested in the negative control and only the active ones were used in the tests. The bioassay was conducted in environmental conditions of temperature and humidity, with temperature averages of 22–27 °C and relative humidity of 45–50%.