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Acaricidal activity of *Palicourea marcgravii*, a species from the Amazon forest, on cattle tick *Rhipicephalus* (*Boophilus*) *microplus*

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ABSTRACT

Leaves of *Palicourea marcgravii* were extracted successively with hexane, ethyl acetate and ethanol in order to evaluate their acaricidal activity on larvae and adult stages of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. The ethyl acetate extract showed the highest bioactivity of the tested extracts, which contained 0.12% monofluoroacetic acid. On engorged female, the ethyl acetate extract showed a lethal concentration 50% – LC_{50} = 30.08 mg ml^{-1}, inhibitory concentration 50% – IC_{50} = 5.79 mg ml^{-1} and lethal time 50% – LT_{50} = 4.72 days; 100% reproduction was controlled at concentrations of 50 mg ml^{-1} and on larvae the ethyl acetate extract showed a LC_{50} = 2.46 mg ml^{-1}. No alkaloids were detected in any of the extracts. This is the first report on the acaricidal activity of *P. marcgravii* extracts against *R. microplus* as well as the acaricidal properties of a plant species containing monofluoroacetic acid.

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1. Introduction

The cattle tick is known to cause large economic losses to livestock producers worldwide damaging the leather, transmitting diseases and lowering overall productivity (Cordovés, 1997). Tick control is accomplished through the intensive use of synthetic acaricides, which are usually employed in different dosage forms such as concentrates by dipping, spray, injection or pour-on treatment (Vargas et al., 2003; Rodriguez-Vivas et al., 2006; Patarroyo et al., 2009). The repeated application rate of these compounds caused the development of tick resistance to organophosphates and pyretroids, a phenomenon that is a major concern for cattle breeders (Patarroyo et al., 2009).

This fact prompted the search for new alternatives for tick control like biopesticides, either entomopathogenic fungi or biorationals from plants (Ojeda-Chi et al., 2010; Rosado-Aguilar et al., 2010). Pesticides of botanical origin are of interest, because the development of tick resistance is supposed to be slower than chemical pesticides due to the association of several active ingredients and multiple modes of action, making possible a wide range of use while retaining a selective action (Quarles, 1992). However, the speed at which resistance develops depends mostly on the frequency of applications.

The Amazonian forest is an unexplored source of potential bioactive molecules. Previous results of our research on Amazonian biodiversity for new acaricides showed that the essential oil from the spiked pepper, *Piper aduncum*, is highly toxic to *Rhipicephalus* (*Boophilus*) *microplus* larvae (Silva et al., 2009). In the present research we investigated the acaricidal activity on *R. microplus* cattle tick of *Pali-*

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courea marcgravii, a toxic plant with reported insecticidal properties (Silva, 2005; Gonzaga et al., 2008).

2. Materials and methods

2.1. General

Solvents employed were reactive grade purchased from Mallincrodt Baker, Philipsburg, NJ, USA and distilled from glass prior to use. GC–MS analyses were performed using a Shimadzu 5050 mass detector coupled to a Shimadzu GC17 A, Shimadzu corporation, Kyoto, Japan, equipped with a fused silica carbowax capillary column 0.1 mm i.d. × 25 m long, SGE, BP20, SGE Analytical Science Pty, Ltd., Victoria. Australia.

2.2. Collection and processing of botanic material

Leaf samples from *P. marcgravii* were collected at the Federal University of Amazonas (UFMA), experimental farm, km 40 of the BR-174 road (s02° 39.236; w60° 03.432) in the state of Amazonas-Brazil. The leaves were dried in a greenhouse at a temperature of 37 °C for five days and then powdered (Prista et al., 1981).

2.3. Extraction of botanic material

The plant material was extracted in a Soxhlet equipment in three successive steps, using solvents of increasing polarity: hexane, ethyl acetate and ethanol for 24 h each. After extraction, the solutions were decanted, filtered and the solvents removed by evaporation under reduced pressure. The resulting crude extracts were stored at 4 °C.

2.4. Tick collection

Females of *R. microplus* in the final stage of engorgement were collected between january and march of 2007 from cattle belonging to a herd located at the Desidério Finamor Institute of Veterinary Research in Eldorado do Sul-RS-Brazil. Ticks were used in the biological tests within 24h after the collection. Larvae were obtained from eggs produced by engorged females from untreated animals.

2.5. Acaricidal bioassay

Hexane, ethyl acetate and ethanol extracts were solubilized in Tween-80 5% (v/v) at concentrations of 5 mg ml^{-1}, 25 mg ml^{-1}, 50 mg ml^{-1}, 75 mg ml^{-1} and 100 mg ml^{-1}. Three hundred fifty engorged females were separated into groups of ten, weighed, in order to get a sample of uniform weight and immersed separately in each solution for five minutes. Two controls were employed: one was immersed in Tween-80 5% (v/v) and the other in distilled water (Drummond et al., 1973).

After treatment, each group was placed in a Petri plate and maintained at 27 ± 1 °C and 80 ± 5% relative humidity. The mortality rates were evaluated daily over a six days period. Oviposition rates were evaluated over a 15-day period. Reproductive efficiency and reproduction control were evaluated over 15 days, after weighing the eggs, using the following formula (Stendel, 1980).

$$\mathrm{IO} = \frac{\mathrm{EW}}{\mathrm{IWF}}$$

$$\mathrm{IO}(\%) = \frac{\mathrm{IO(control)} - \mathrm{IO(treated)}}{\mathrm{IO(control)}} \times 100$$

$$\mathrm{RE} = \frac{\mathrm{EW}}{\mathrm{IWF}} \times \%\mathrm{E}$$

$$\mathrm{CR} = \frac{\mathrm{RE(control)} - \mathrm{RE(treated)}}{\mathrm{RE(control)}} \times 100$$

where IO is the inhibition of oviposition; IFW is the initial female weight; EW is the egg weight; E is the egg eclosion; RE is the reproductive efficiency; CR is the control of reproduction.

The toxicity of hexane, ethyl acetate and ethanol extracts of *P. marcgravii* against larvae was tested at concentrations of 1 mg ml^{-1}, 5 mg ml^{-1}, 10 mg ml^{-1}, 15 mg ml^{-1}, and 20 mg ml^{-1} using a modification of a previously described methodology by Shaw (1966) and Souza et al. (2008). One hundred larvae around 14 and 21 days old were put into a previously prepared 5 ml syringe. The syringe was cut next to the needle's region leaving an orifice of approximately 0.1 mm diameter in the middle. Before the immersion of the larvae, the part cut was closed with a fine weft fabric fixed with an orthodontic rubber band. Two control groups were employed: one immersed in Tween-80 5% (v/v) and the other in distilled water. The larvae were immersed in the extracts for five minutes and maintained at 27 ± 1 °C and 80 ± 5% relative humidity. This procedure was repeated for each concentration. The same basic procedure was used for the two controls. After 24 h the number of live and dead larvae was counted and the percentage mortality was calculated as %mortality = number of dead larvae/total number of larvae × 100 (FAO Plant Protection Bulletin, 1971).

2.6. GC–MS analysis

2.6.1. Derivatization

10 mg of the ethyl acetate (AcOEt) extract were dissolved in 10 ml of 1% HCl in ethanol (EtOH) and left overnight at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in 5 ml of hexane, the suspension sonicated for 5 min and the residue was filtered off, and the resulting solution was injected into the GC–MS system.

The GC–MS analysis was performed using the following program temperature: 40 °C, 10 min, temperature was increased at 5 °C/min to 140 °C with the MS detector operating in the full scan mode. The monofluoroacetic acid ethyl ester was identified with a >90 SI using the Wiley Registry of Mass Spectral Data (Wiley, 2000).

2.7. Statistical analysis

The experiments were totally randomized with five treatments, five replicates and two control groups for the

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