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PALAVRAS-CHAVE: Acaricida botânico, saponácea, tingui-do-cerrado, controle de carrapato

ABSTRACT - The action of crude ethanol extract of the stem bark of the soapberry *Magonia pubescens* St. Hil. was studied upon larvae of the Brown Dog tick *Rhipicephalus sanguineus* (Latreille). Tick larvae were obtained by maintaining gravid females in an incubator, after collecting them from naturally infested kennels. The tick larvae were placed in envelopes of filter paper impregnated with different concentrations of the extract dissolved in dimethylsulfoxide (DMSO) and distilled water. Four tests were repeated with each solution (n ≥ 120). The control was carried out in DMSO and distilled water. The bioassays were performed at 27±1°C, RH ≥ 80% and 12:12 light cycle. Mortality was observed after 48h exposure. All motionless larvae were considered to be dead. The extract of *M. pubescens* showed larvicidal potential against *R. sanguineus*. The lethal concentrations of 1503 ppm (LC₅₀) and 9991 ppm (LC₉₉) were obtained. There was no mortality in the control group. Based on the results of the current study, *M. pubescens* should be recognized as an future alternative acaricide for the control of Brown Dog tick. These results reinforce the importance of the preservation of this soapberry in its natural biome.

KEY WORDS: Botanical acaricide, soapberry, tingui, tick control

The resistance related in *Rhipicephalus sanguineus* (Latreille) to some synthetic acaricides in some parts of the world (Fernandes 2000, Fernandes & Freitas, 2001, Miller *et al.* 2001), together with the well-documented damage that these compounds can cause to the environment have resulted in a worldwide trend towards reducing their use as much as possible. In Brazil, acaricide resistance in *R. sanguineus* to some pirethrroids was first recorded by Fernandes (2000), in Goiânia, State of Goiás. Based on encouraging results of experiments with some plant species (Fernandes *et al.* 2005, 2007; Fernandes & Freitas 2007), active principles of plants with acaricidal properties could be used as an alternative with lower environmental impact to control ticks.

The soapberry *Magonia pubescens* St. Hil. is characteristic
of savannah, cerrado, and occurs in the states of Goiás, Distrito Federal, Mato Grosso, Mato Grosso do Sul, Minas Gerais and São Paulo (Fig. 1). It is also present in Bolivia and Paraguay. Despite adapting to any soil type, it is frequently observed at great densities in areas with poor soils. The trees are characterized by medium to great load, which wood is used in construction (Lorenzi 1992), and it is also considered useful for metallurgical coal. The species is distinguished easily by the fruit characteristics, which is big and of brown color. The flowers are used by the bees, although they lead to a slightly toxic honey; seeds are also used in ornamental arrangements and in the soap production (Pott & Pott 1994).

The crude ethanol extract of another member of the same family, the tingui Sapindus saponaria L., has also shown activity against larvae of the cattle tick, Rhipicephalus (Boophilus) microplus (Canestrini) (Fernandes et al. 2005). These observations provided the incentive to carry out the present study. Activity of the ethanol extract of the stem bark M. pubescens was therefore evaluated as a potential acaricide for larvae of R. sanguineus.

Material and Methods

Samples of the stem bark of M. pubescens were collected in July of 2003, in cerrado areas of the municipality of Francisco Sá, in Minas Gerais State, Brazil (Fig. 1). These samples were transported to the laboratory to obtain the ethanol extract using the method of Arruda (2000). A 10,000 ppm stocked solution was prepared weighing the extract on an analytical balance with a precision of 100 μg. After dissolving in dimethylsulfoxide (DMSO) and distilled water (in the proportion of 3.2 ml of DMSO for 146.8 ml of distilled water), the extract was left to stand for about 1h, homogenized using a magnetic stirrer for about 15 min, and then adjusted to the final volume with distilled water. Lower concentrations were obtained sequentially by dilution in distilled water (9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000 and 1000 ppm), to determine the values of lethal concentration (LC), particularly LC50 and LC99. These concentrations were calculated by interpolating the mortalities obtained for different concentrations by means of Probit analysis, using the “Sistema para Análises Estatísticas” (SAEG) software v. 9.0.

Engorged female R. sanguineus were in naturally infested kennels from residences distributed in different districts of Goiânia, State of Goiás, and free of acaricidal residues for at least 45 days prior to the bioassays. The females were placed in BOD incubators acclimatized to 27 ± 1ºC, RH > 80% and a 12:12h photoperiod. In order to obtain larvae of the same age, eggs batches were daily collected in separate polyethylene tubes with screw caps (Fernandes 2000, 2001).

An established method was used to determine larval susceptibility to plant extract impregnated filter paper, “the larval packet test (lpt)” (FAO 2004), incorporating slight modifications to improve practicality and reduce cost without compromising efficiency (Fernandes 2000, Fernandes et al. 2007, Fernandes & Freitas 2007).

The bioassays were carried out in a BOD incubator under the same conditions as described previously (Fernandes 2000, 2001). Larvae were exposed to the solutions in filter papers envelopes (= 327 cm²), containing micro pores to ensure adequate circulation of air. Each envelope received 2 ml of the solution, distributed uniformly with a pipette on its internal surfaces (Fernandes et al. 2005). The control group was subdivided in three types of treatment, conditioning the larvae in: 1 - envelopes of dry filter paper; 2 - filter paper humidified with distilled water; and 3 - filter paper humidified with a solution of DMSO and distilled water, in the same proportion used in the test group.

Only 14-21-day old larvae were used in the tests (FAO 2004), with a minimum of 30 larvae in each envelope. Hatching tubes with the highest larval eclosion rate (90-100%)

Fig. 1. Tree of soapberry M. pubescens in its the natural cerrado environmental (A); fruits, leaves and branches (B); and stem bark (C).
were elected and placed in the base of a bottle, inverted in the centre of a Petri dish that was subsequently filled with water, which prevented their escape. A sample of the larvae from this tube was put in the centre of a sheet of white paper, fixed to the bench with adhesive tape. Thirty or more specimens with good mobility were caught with a no. 4 paintbrush moistened in test solution, then gently transferred to each envelope. The remaining larvae on the paper were killed by squashing them under adhesive tape. The opening of the envelopes (treated and inoculated with larval ticks) was folded (10 mm) and re-sealed with metallic clip, with its identification mark (tested solution and concentration) on the outside (Fernandes & Freitas 2007).

In each bioassay, four replicates of exposures to each concentration tested were carried out, i.e., four envelopes were impregnated with each test concentration. The entire bioassay was repeated on four different days, preparing a new stock solution each day. The mortality was recorded after 48h of exposure, when the envelopes were opened and inspected under the stereomicroscope (Fernandes et al. 2007). To allow comparison with results of previous studies, motionless larvae were considered to be dead (FAO 2004).

Results and Discussion

The ethanol extract of the stem bark of the soapberry M. pubescens showed activity against larvae of R. sanguineus, with LC_{50} and LC_{99} values of 1503 ppm (1350-1642, Confidence Interval (CI) at 95% probability), and 9991 ppm (8033-13270, CI 95%), respectively. No significant larval mortality was observed in the control group after 48h of exposure in the laboratory (Fig. 2).

There are several studies on the activities of Brazilian plants against tick larvae. Fernandes et al. (2005) registered 99% mortality of larvae of R. (B.) microplus submitted to 6,360 ppm (6,36 mg/ml) (= 0,6 %) of crude ethanol extract of the stem bark of ther soapberry, S. saponaria. The same extract also had R. sanguineus larvicidal activity with LC_{99} values of 3,922 ppm (3,92 mg/ml) (= 0,4 %) (Fernandes et al. 2007). The oleoresin extract from Copaifera reticulata (Caesalpinaceae) produced 99% R. (B.) microplus larval mortality at 3,491 ppm (3,49 mg/ml) (=0,3%). Previously, Prates et al. (1993) evaluated larvicidal activity of chemical components of the essential oil of Melinis minutiflora Beauv. on B. microplus, and obtained favorable results, particularly for alpha-pinene. Chagas et al. (2002) evaluated the essential oils of three Eucalyptus species (Myrtaceae) on B. microplus, observing 100% mortality among larvae exposed to concentrations of 10% (= 100000 ppm) for E. staigeriana and E. citriodora and 20% for E. globulus (Chagas et al. 2002). The results of the present study show M. pubescens to be an even more potent acaricide, since it killed 99% of R. (B.) microplus at the concentration of 9991 ppm (< 1%).

It is noteworthy that promising results were obtained using the stem bark of the plant. Even more satisfactory result may be obtained with other parts of this plant, e.g. seeds and fruits, or with fractions and sub-fractions of the tested extract. Furthermore, it is known that diverse chemical substances isolated from plants are truly novel, that is, are only encountered in these plants (Cascon & Gilbert 2000).

The crude ethanol extract of the stem peel of M. pubescens has also shown activity against 3rd instar larvae of the mosquito Aedes aegypti (L.) (Diptera: Culicidae) (Arruda 2000) and against larvae of the brown dog tick, R. sanguineus (Fernandes et al. in press). This line of research should be continued, including phytochemical fractionation of M. pubescens and evaluation of bioactive fractions against R. sanguineus, as part of the ongoing search for alternatives in the control of this tick and other arthropods of medical and veterinary importance. Furthermore, the results of the present study reinforce the importance of M. pubescens as a potentially auto-sustainable resource among the flora of the Brazilian cerrado. This comes to foment efforts for the preservation of this plant species in its natural habitat.

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