PUBLIC HEALTH

Toxicity of Extract of *Magonia pubescens* (Sapindales: Sapindaceae) St. Hil. to Control the Brown Dog Tick, *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae)

Fernando F. Fernandes[1], Walmirton B. D'alessandro[2] and Edméia P.S. Freitas[3]

[1]Lab. Artropodologia Médica e Veterinária (LAMV), Setor de Entomologia, Instituto de Patologia Tropical e Saúde Pública (IPTSP), Univ. Federal de Goiás - UFG, 74.605-050, C. postal 131, Goiânia, GO, fernandesff@pesquisador.cnpq.br; [2]Bolsista da FUNAPE-UFG, LAMV, IPTSP, UFG; [3]Bolsista de Iniciação Tecnológica Industrial do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), LAMV, IPTSP, UFG

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Toxicidade do Extrato de *Magonia pubescens* (Sapindales: Sapindaceae) St. Hil. para o Controle do Carrapato Vermelho do Cão, *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae)

RESUMO - Estudou-se a ação do extrato bruto etanólico da casca do caule da saponácea *Magonia pubescens* St. Hil. sobre larvas do carrapato vermelho do cão *Rhipicephalus sanguineus* (Latreille). Larvas do carrapato foram obtidas a partir de teleóginas mantidas em incubadoras, coletadas em canis naturalmente infestados. As larvas foram colocadas em papel filtro impregnado com diferentes concentrações do extrato, obtidas por diluição em dimetilsulfóxido (DMSO) e água destilada. Quatro repetições foram feitas com cada solução ($n \geq 120$). O controle foi feito com DMSO e água destilada. Os bioensaios foram realizados em uma câmara biológica climatizada a 27±1°C, UR ≥ 80% e fotofase natural de 12h. A mortalidade foi observada após 48h de exposição. As larvas sem capacidade locomotora foram consideradas mortas. O extrato de *M. pubescens* demonstrou potencial larvicida sobre *R. sanguineus*. Obtiveram-se as CL_{50} e CL_{99} de 1503 ppm e 9991 ppm respectivamente. Não houve mortalidade significativa no grupo controle. Com base nos resultados do presente estudo, *M. pubescens* deve ser reconhecida como uma futura alternativa acaricida para controle do carrapato vermelho do cão. Esses resultados reforçam a importância da preservação dessa saponácea em seu bioma natural.

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 ($n \geq 120$). 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 (LC_{50}) and 9991 ppm (LC_{99}) 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 pirethroids 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 characterist

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 municipally 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 LC_{50} and LC_{99}. 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 cm^2), containing micropores 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).

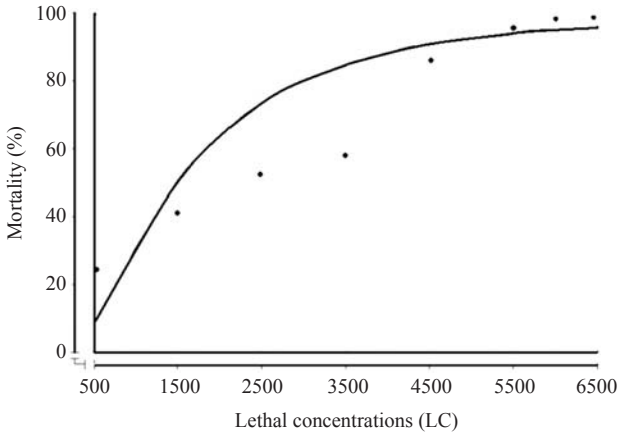

Fig. 2. Larvicidal activity of different concentrations of the crude ethanol extract of *M. pubescens* on *R. sanguineus* larvae, observed after 48 h exposure ($\chi^2 = 38{,}25$; $P < 0{,}05$). The points represent mortality at given concentration and the S-shaped curve is derived of the Probit analysis. There was no mortality in the control group.

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 oleoresinous extract from *Copaifera reticulata* (Caesalpiniaceae) 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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