### **Notas Científicas**

## Larvicidal potential of Sapindus saponaria to control the cattle tick Boophilus microplus

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Abstract – The objective of this study was to evaluate the larvicidal potential of a crude ethanol extract (CEE) of soapberry *Sapindus saponaria* stem peel on the cattle tick *Boophilus microplus*. Tick larvae obtained by incubating engorged females, collected from naturally infested cattle, were placed in envelopes of filter paper impregnated with different concentrations of CEE in the test group, and distilled water in the control group. Four repetitions were made with each solution (n≥120). Mortality was observed after 48 hours. Lethal concentration values of 1,258 ppm (LC<sub>50</sub>) and 6,360 ppm (LC<sub>99</sub>) were obtained.

Index terms: Sapindaceae, Ixodidae, botanical acaricide, soapberry, tick control.

# Potencial larvicida de *Sapindus saponaria* para controle do carrapato bovino *Boophilus microplus*

Resumo – Este trabalho objetivou avaliar o potencial larvicida do extrato bruto etanólico (EBE), da casca do caule de *Sapindus saponaria*, sobre *Boophilus microplus*. Larvas desse carrapato foram obtidas pela incubação de teleóginas, coletadas de bovinos naturalmente infestados. As larvas foram acondicionadas em envelopes de papel-filtro, impregnados com diferentes concentrações do EBE no grupo teste, e com água destilada no grupo controle. Quatro repetições foram feitas com cada solução (n≥120). A mortalidade foi observada após 48 horas. Foram obtidas concentrações letais CL<sub>50</sub> de 1.258 ppm e CL<sub>99</sub> de 6.360 ppm.

Termos para indexação: Sapindaceae, Ixodidae, acaricida botânico, saponácea, controle de carrapatos.

The development of resistance by *Boophilus microplus* (Can., 1887) (Acari: Ixodidae) to chemical acaricides, in several parts of Brazil (Leite, 1988; Furlong & Martins, 2000; Fernandes, 2001), together with the well-documented damage these compounds cause to the environment and food chain have resulted in a worldwide trend towards reducing their use as much as possible. An alternative of lower environmental impact to the control of cattle ticks would be to use active principles of plants with acaricidal properties, based on encouraging results of experiments with some plants (Prates et al., 1993; Chagas et al., 2002).

Soapberry *Sapindus saponaria* L. (Sapindaceae) is found in the USA, Mexico, Argentina and Brazil, where it occurs in the States of Amazonas, Goiás, Mato Grosso and Mato Grosso do Sul. This tree flowers in July and August and is extensively used in urban landscaping. Its wood is used in construction, as well as in the

manufacture of toys, boxes and souvenirs. Its fruits are eaten by bats and are considered to have medicinal properties (Lorenzi, 1992). Fruits and seeds produce a foam due to the presence of saponins. According to Pott & Pott (1994), the triturated fruit can be used as soap and the seeds produce oil which is employed in soap manufacture, as well as being commonly used as an insecticide (Guarin Neto et al., 2000). The crude ethanol extract (CEE) of another member of the same family, the tingui Magonia pubescens St. Hil., has also shown activity against culicine mosquito larvae (Silva et al., 2004). These observations provided the incentive to carry out the present study. The objective of this work was to evaluate the activity of the CEE of S. saponaria stem peel as a potential control measure for larvae of Boophilus microplus.

Samples of *S. saponaria* stem peel were collected in Goiás, especially in the cities of Formosa and Santa

Terezinha. These were transported to the laboratory to obtain CEE using the method of Silva et al. (2004). A 5,000 ppm stock solution was prepared by weighing the CEE on an analytical balance with a precision of 100  $\mu$ g. After dissolving in distilled water, the CEE was left to stand for about 1 hour, and was homogenized using a magnetic stirrer for about 15 min, then adjusted to the final volume with distilled water. Lower concentrations were obtained sequentially by dilution in distilled water, to determine lethal concentration (LC) values, particularly LC<sub>50</sub> and LC<sub>99</sub>. These were calculated by interpolating the mortalities obtained for different concentrations by means of Probit analysis.

Gravid females of *B. microplus* were collected from naturally infested cattle, in the vicinity of Goiânia, Goiás, and free of acaricidal residues for at least 45 days prior to the bioassays (Fernandes, 2001). The females were placed in BOD incubators acclimatized to  $27\pm1^{\circ}$ C, RH>80% and a 12:12h photoperiod. In order to obtain larvae of the same age cohort, egg batches were collected daily in separate polyethylene tubes with screw caps.

An established method was used to determine larval susceptibility to CEE-impregnated papers (FAO, 1995; Leite, 1988; Fernandes, 2000, 2001), incorporating slight modifications to improve practicality and reduce cost, without compromising efficiency. The bioassays were carried out in a BOD incubator under the same conditions as described above. Larvae were exposed to the solutions in filter paper envelopes (327 cm<sup>2</sup>), containing micropores to ensure adequate circulation of air. Each envelope received 2 mL of the solution, distributed uniformly with a pipette on internal surfaces. Envelopes of the control group received only distilled water, since no other solvent was required to solubilize extracts of S. saponaria. Only larvae 14-21 days old were used in the tests, with a minimum of 30 larvae for each envelope. Four replicates were carried out for each solution (n≥120 larvae), and mortality was recorded after 48 hours of exposure, when the envelopes were opened and inspected under the stereomicroscope. To permit comparison with results of previous studies, immobile larvae were considered to be dead.

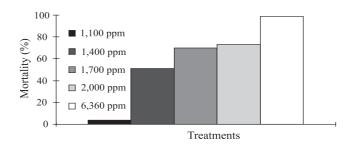
The CEE of *S. saponaria* showed activity against larvae of *B. microplus*, with LC<sub>50</sub> and LC<sub>99</sub> values of 1,258 and 6,360 ppm respectively being obtained (Figure 1). No significant mortality was observed in the control group (p<0.005).

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% for *E. staigeriana* and *E. citriodora*, and 20% for *E. globulus*. The results of the present study show *S. saponaria* to be an even more potent acaricide, since it killed 99% of *B. microplus* at a concentration of only 6,360 ppm.

It is noteworthy that promising results were obtained using only the stem peel 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 subfractions of the CEE tested.

The principal advantages of botanical acaricides over chemical compounds include low mammalian toxicity, rapid degradation in the environment, and delayed development of resistance (Chungsamarnyart et al., 1991). Furthermore, it is known that diverse chemical substances isolated from plants are truly novel (Cascon & Gilbert, 2000).

The larvicidal action of *S. saponaria* could be related to the presence of tannins, similar to those isolated from the CEE of *M. pubescens* by Silva et al. (2004). Three fractions isolated from this plant presented activity against 3<sup>rd</sup> instar larvae of the mosquito *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). This line of research should be continued, including phytochemical fractionation of *S. saponaria*, and evaluation of bioactive fractions against *B. microplus*, as part of the ongoing search for alternatives in the control of ticks and other arthropods of medical and veterinary importance.



**Figure 1.** Larvicidal activity of different concentrations of the crude ethanol extract of *Sapindus saponaria* on larvae of *Boophilus microplus*, observed 48 hours after exposure.

Furthermore, results of the present study reinforce the importance of *S. saponaria* as a potentially autosustainable resource, among the flora of Brazil and the Americas. Steps should be taken to ensure its preservation in its natural environment.

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#### References

CASCON, V.; GILBERT, B. Characterization of the chemical composition of oleoresins of *Copaifera guianensis* Desf., *Copaifera duckei* Dwyer and *Copaifera multijuna* Hayne. **Phytochemistry**, v.55, p.773-778, 2000.

CHAGAS, A.C.S.; PASSOS, M.W.M.; PRATES, H.T.; LEITE, R.C.; FURLONG, J.; FORTES, I.C.P. Efeito acaricida de óleos essenciais e concentrados emulsionáveis de *Eucalyptus* spp. em *Boophilus microplus*. **Brazilian Journal of Veterinary Research and Animal Science**, v.39, p.247-253, 2002.

CHUNGSAMARNYART, N.; JIWAJINDA, S.; RATANAKREETAKUL, C.; JASAWAN, W. Practical extraction of sugar apple seeds against tropical cattle ticks. **Kasetsart Journal (Natural Science)**, v.25, p.101-105, 1991.

FAO. **Acaricide resistance test kit**. Instructions for use. 11.ed. Berlin: World Acaricide Resistance Reference Centre, 1995. 9p.

FERNANDES, F.F. In vitro activity of permethrin, cipermethrin and deltamethrin on larvae of *Rhipicephalus sanguineus*. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.52, p.621-626, 2000.

FERNANDES, F.F. Toxicological effects and resistance to pyretroids in *Boophilus microplus* from Goiás, Brasil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.53, p.548-552, 2001.

FURLONG, J.; MARTINS, J.R.S. **Resistência dos carrapatos aos carrapaticidas**. Juiz de Fora: Embrapa Gado de Leite, 2000. 25p. (Circular técnica, 59).

GUARIN NETO, G.; SANTANA, S.R.; SILVA, J.V.B. Notas etnobotânicas de espécies de Sapindaceae Jusieu. **Acta Botanica Brasilica**, v.14, p.327-334, 2000.

LEITE, R.C. *Boophilus microplus* (Canestrini, 1887): susceptibilidade, uso atual e retrospectivo de carrapaticidas em propriedades das regiões fisiográficas da Baixada do Grande Rio e Rio de Janeiro; uma abordagem epidemiológica. 1988. 144p. Tese (Doutorado) - Universidade Federal Rural do Rio de Janeiro, Seropédica.

LORENZI, H. **Árvores brasileiras**: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Nova Odessa: Plantarum, 1992. 240p.

POTT, A.; POTT, V.J. **Plantas do pantanal**. Brasília: Embrapa-CPAP; Embrapa-SPI, 1994. 320p.

PRATES, H.T.; OLIVEIRA, A.B.; LEITE, R.C.; CRAVEIRO, A.A. Atividade carrapaticida e composição química do óleo essencial do capim-gordura. **Pesquisa Agropecuária Brasileira**, v.28, p.621-625, 1993.

SILVA, H.H.G.; SILVA, I.G.; SANTOS, R.M.G.; RODRIGUES FILHO, E.; ELIAS, C.N. Atividade larvicida de taninos isolados de *Magonia pubescens* St. Hil. (Sapindaceae) sobre *Aedes aegypti* (Diptera, Culicidae). **Revista da Sociedade Brasileira de Medicina Tropical**, v.37, p.396-399, 2004.

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