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A N I N T E R N A T I O N A L J O U R N A L

Evaluation of Activity of the Crude Ethanolic Extract of *Magonia pubescens* St. Hil (Sapindaceae) Against Larvae of the Cattle Tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887) (Acari: Ixodidae).

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ABSTRACT

The acaricidal potential of the crude ethanolic extract (c.e.e.) of the stem peel of Magonia pubescens *was evaluated against larvae of* Rhipicephalus (Boophilus) microplus. *The larvae were placed in filter paper envelopes impregnated with different concentrations of c.e.e., dissolved in dimethylsulfoxide (DMSO) and distilled water for determination of lethal concentrations (LC). The following treatments were used: 1. Envelopes of dry filter paper; 2. Envelopes of filter paper moistened with distilled water; 3. Envelopes of filter paper moistened with a solution of DMSO in distilled water; and 4. Envelopes moistened with 2 mL of each concentration of the c.e.e. to be tested. The bioassays were carried out in quadruplicate at 27°±1° C and RH ≥80% and 12h light. Mortality was observed after 48 h, LC_{50} and LC_{99} values of 365 and 4,000 ppm being obtained. There was no significant mortality in larvae exposed to the first three treatments ($p < 0.05$).*

Key words: Botanical acaricide, Sapindaceae, *Magonia pubescens*, tinguí-do-cerrado, *Boophilus microplus*, cattle tick

INTRODUCTION

The active principles of plants with acaricidal properties are a promising alternative for the control of ticks and others arthropods (Prates et al., 1993; Ventura and Ito, 2000; Chagas et. al., 2002; Fernandes et al., 2005, 2007, 2008; Fernandes and Freitas, 2007).

Recent analyses of molecular phylogeny showed that the five species of *Boophilus* belong to the genus *Rhipicephalus,* so that the former should be relegated to the status of a subgenus (Beati and Keirans, 2001; Murrel and Barker, 2003; Barros-Battesti et al., 2006).

The search of new control alternatives, more efficient and more environmentally-friendly compounds (Fernandes et al., 2005; Fernandes and Freitas, 2007; Freitas-Ribeiro et al., 2005), to *Rhipicephalus* (*Boophilus*) *microplus* cattle tick (Canestrini, 1887) (Acari: Ixodidae) (Barros-Battesti et al., 2006), has been driven by the growing number of reports of resistance of this genus to synthetic acaricides throughout the Neotropics (Mangold et al., 2000; Fernandes 2000, 2001; Fernandes and Freitas, 2001; Santamaría

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Vargas et al., 2003), as well as detrimental environmental effects resulting from accumulation of chemical residues in the food chain. The principal advantages of botanical acaricides are their low toxicity to mammals, rapid degradation in the environment and slow development of resistance to them in ticks (Chungsamarnyart et al., 1991). The plants from which they are derived thus constitute a valuable natural resource (Cascon and Gilbert, 2000).

Several plants have already been evaluated for *R.* (*B.*) *microplus* control. Chemical components of the essential oils of *Melinis minutiflora* Beauv, especially α-pinene, have demonstrated larvicidal activity to this tick (Prates et al., 1993). Favorable results were also obtained in evaluations of the larvicidal activity to *R.* (*B.*) *microplus* of oils from three species of *Eucalyptus* (Myrtaceae) (Chagas et al., 2002), as well as the oleoresinous extract from *Copaifera reticulata* (Leguminosae: Caesalpinioideae) (Fernandes and Freitas, 2007).

The soapberry *Magonia pubescens*, commonly in Brazil as timboeiro", "tinguí-do-cerrado", "urucurana" or capixingui", is a plant of the Sapindaceae family, characteristic of Brazilian cerrado (Fig. 1). Although able to adapt to many different types of soil, its natural habitat is infertile areas of Brazil (GO, DF, MT, MS, MG and SP), Bolivia and Paraguay (Guarin Neto, et al., 2000). It is a tree of middle to large size, whose wood is used in construction as well as to provide fuel for smelting in Minas Gerais State. It has characteristic fruit, large and brown. Its flowers are considered important to apiculture and its seeds are used in decorative arrangements and for soap manufacture (Pott and Pott, 1994).

Other Previous study with crude ethanolic extract (c.e.e.) of the stem peel of *M. pubescens* also demonstrated larvicidal activity against larvae of the brown dog tick, *R. sanguineus* (Fernandes et al. 2008). Extracts of another soapberry, *Sapindus saponaria* L. also presented larvicidal activity to both ticks *R.* (*B.*) *microplus* (Fernandes et al., 2005), and *R. sanguineus* (Fernandes et al., 2007). However, to date the effect of any type of extract of *M. pubescens* on *R.* (*B.*) *microplus* is unknown.

The proposal of this study was to evaluate the potential of *M. pubescens* as an acaricide, against *R.* (*B.*) *microplus*.

MATERIALS AND METHODS

Engorged females of *R.* (*B.*) *microplus* were collected from naturally infested cattle on farms of the Goiânia municipal district; which had suspended use of acaricidal treatment for at least 45 days prior to the study. Gravid females were conditioned in Biological Oxygen Demand incubator at 27±1ºC RH ≥ 80% and L12:D12h. In order to obtain larvae of uniform age, eggs were collected daily in separate screw-cap polyethylene vials (Fernandes, 2000, 2001).

Samples of *M. pubescens* stem peel for use in bioassays were from areas of Cerrado in the municipally of Francisco Sá, in the Brazilian State of Minas Gerais (Fig. 1). The samples were dried and ground down to a fine powder in a forced ventilation greenhouse at 40°C. Subsequently 800g of this powder were extracted by percolating with 1L of ethanol for 72h at room temperature, repeating this process and then filtering and concentrating the c.e.e. in a rotating evaporator.

A 5,000 ppm stock solution of c.e.e. was prepared 24h before each bioassay, using 1.6 mL of DMSO to 148.4 mL of distilled water. One hour in rest, after dissolution, c.e.e was homogenized for about 15 minutes in magnetic agitator, being adjusted the final volume with distilled water. After allowing the c.e.e to settle for 1h, it was homogenized for 15 min with a magnetic stirrer and adjusted to the final volume with distilled water.

Smaller concentrations (4,000, 3,000, 2,000, 1,800, 1,500, 1,000, 500 and 300 ppm) were obtained by sequential dilution of the stock solution in distilled water, to permit determination of lethal concentrations, particularly LC_{50} and LC_{99}. These were calculated using Probit analysis (Fernandes et al., 2005, 2007), and the $\chi 2$ test (Fernandes 2000, 2001, Fernandes and Freitas, 2007), using Sistema Para Análises Estatísticas® (SAEG) version 9.0© software

The methodology used in the present study for evaluation of larval sensitivity was based on the larval packet test (FAO, 2004; Fernandes and Freitas, 2007), with modifications to improve practicality and decrease cost, without reducing efficiency.

Larvae were exposed to tested solutions in filter paper envelopes (≈327m^2) containing micropores to allow better ventilation (Fernandes et al., 2005, 2007; Fernandes and Freitas, 2007).

Figure 1 - *Magonia pubescens*: tree in its the natural Cerrado biome (A); branches, leaves and fruit (B); and stem peel (C).

The following treatments were used: 1. Envelopes of dry filter paper; 2. Envelopes of filter paper moistened with distilled water; 3. Envelopes of filter paper moistened with a solution of DMSO in distilled water; and 4. Envelopes moistened with 2 mL of each concentration of the c.e.e. to be tested.

The bioassays were carried out in a specially constructed biological chamber for testing botanical acaricides at LAMV, climatizate at 27°±1° C, RH≥80% (Fernandes et al., 2007), and approximately 12 h natural photophase. Hatchings tubes with the highest larval eclosion rate (90-100%) were selected 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 (Fernandes 2000, 2001). 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 and thirty or more specimens with good mobility caught with a n°. 4 paintbrush moistened in test solution, then gently transferred to each envelope. The remaining larvae on the paper were killed by squashing then under adhesive tape (Fernandes and Freitas, 2007).

All larvae used in bioassays were 14-21 days old (FAO, 2004). The envelopes were sealed by folding over the 1-2cm twice and fixing the folds in place with metallic clips. They were then ventilated by suspended them on hooks to prevent contact with any surface and avoid leakage of the solutions or contamination. Bioassays were carried out in quadruplicate. Fresh stock solution and four new envelopes/treatment were prepared for each replicate (Fernandes and Freitas, 2007).

Larval mortality was noted after 48h exposure, when envelopes were opened and inspected under the stereomicroscope.

To allow comparison with data of other authors, immobilized larvae were considered to be dead (FAO, 2004). However, in the present work, replicates of control treatments that produced mortality of 5% or more were discounted and repeated, without using the Abbott´s formula. This had been made to check larger rigidity in the evaluation of the larvicidal activity of the extract, eliminating possible minimal increments in the mortality, promoted by the addition of water, paper or solvents.

RESULTS AND DISCUSSION

The results of larval mortality after 48h exposure to c.e.e are presented in Fig. 2 ($\chi^2 = 3.74 < \chi^2_{.05\ (7)} = 14.06$). LC_{50} and LC_{99} values of 365 ppm (0.365 mg/ml) and 4,000 ppm (4.0 mg/ml) respectively were obtained (Table 1).

Mortality was insignificant (< 5%) in the first three treatments (p <0.005). The absence of mortality of larvae in dry envelopes demonstrates the absence of toxicity of filter paper, which can be recommended for use in larval sensitivity bioassays.

The mortality absence in the 3rd treatment (envelopes of paper filter moistened with the DMSO solution and distilled water) was as expected, based on previous assays of *R.* (*B.*) *microplus* larval sensibility to organic solvents. However, Chagas et al. (2003) observed 22.5% mortality in larvae of *R.* (*B.*) *microplus* after 24h exposure to a higher concentration (25%) of this solvent.

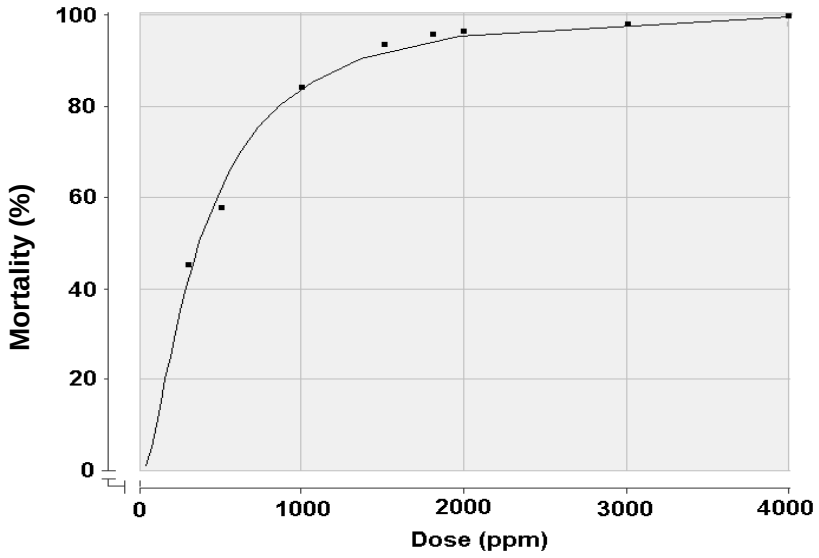

Figure 2 - Susceptibility of *Rhipicephalus* (*Boophilus*) *microplus* larvae to different concentrations of crude ethanolic extract of stem peel of *Magonia pubescens*, observed after 48h exposure.

Table 1 - Susceptibility of *Rhipicephalus* (*Boophilus*) *microplus* larvae to different concentrations of crude ethanolic extract of the stem peel of *Magonia pubescens*, observed after 48h exposure.

Lethal Concentration (LC)	Level of 95% of Significance			
	LC Values (ppm)	Minimum	-	Maximum
LC_{45}	319	(257.09	-	379.68)
LC_{50}	365	(299.34	-	428.60)
LC_{55}	417	(348.13	-	484.36)
LC_{60}	472	(399.69	-	542.96)
LC_{65}	545	(468.64	-	621.57)
LC_{70}	623	(541.97	-	706.42)
LC_{75}	727	(638.70	-	821.69)
LC_{80}	865	(765.33	-	980.36)
LC_{85}	1,063	(939.80	-	1,215.69)
LC_{90}	1,360	(1,191.24	-	1,588.57)
LC_{95}	1,969	(1,677.64	-	2,404.53)
LC_{99}	4,000	(3,165.78	-	5,436.24)

The present work implemented a higher criterion for evaluation of the mortality, with a “control group " composed of three treatments (1, 2 and 3), and without applying the Abbott´s formula. This had been made to check larger rigidity in the evaluation of the larvicidal activity of the extract, eliminating possible increments or addictive effect in the mortality promoted by the addition of the solvente, water ou paper. Chagas et al. (2003) they observed in larvae of *R.* (*B.*) *microplus* a mortality of 22.5% after 24 hours of exhibition to this solvent.

M. pubescens in the present work caused mortality of 99% of the larvae (LC_{99}) of *R.* (*B.*) *microplus*,

in the concentration 4,000 ppm (4.0 mg/ml) (≈ 0.4 %). Chagas et al. (2002) they verified 100% of mortality of larvae of *R.* (*B.*) *microplus* submitted to the concentration of 100,000 ppm (100.0 mg/ml) (≈ 10%) of essential oil of *Eucalyptus citriodora* and *E. staigeriana*, and of 200,000 ppm (200.0 mg/ml) (≈ 20%) of *E. globulus*. Fernandes et al. (2005) they verified mortality of 99% of larvae of *R.* (*B.*) *microplus* submitted to the concentration of 6,360 ppm (6.36 mg/ml) (≈ 0.6 %) of c.e.e of the peel of the stem of *S. saponaria*. In accordance with Fernandes et al. (2008), the larvicidal action on *R.* (*B.*) *microplus* of *M. pubescens*, could be related to the presence of tannins, previously isolated of that species (Oliveira et al., 2001).

The results obtained in this study demonstrate the larvicidal toxicity of this plant to *R.* (*B.*) *microplus*, as well as its potential to provide new compounds for larvicidal control of this species.

The larvicidal activity of *M. pubescens* demonstrated in this study was produced by the crude extract of just one part of the plant, *i.e.*, the stem peel. Further studies should evaluate extracts of the seeds and fruit, as well as bioactive fractions of the stem peel c.e.e. The results obtained in the present study reinforce the importance of this plant as a natural resource, providing a further impetus for measures to preserve the Cerrado.

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RESUMO

Este trabalho objetivou avaliar o potencial acaricida do extrato-bruto etanólico (e.b.e.) da casca do caule de *Magonia pubescens* sobre larvas do carrapato bovino *Rhipicephalus* (*Boophilus*) *microplus.* As larvas foram acondicionadas em B.O.D. climatizada, em envelopes de papel filtro impregnados com diferentes concentrações do e.b.e., solubilizado com dimetilsulfóxido (DMSO) e água destilada, para determinação das Concentrações Letais (CL). Foram realizados os seguintes tratamentos: 1. envelopes de papel filtro seco; 2. envelopes de papel filtro umedecidos com água destilada; 3. envelopes de papel filtro umedecidos com solução de DMSO e água destilada; e 4. envelopes umedecidos com 2 mL de cada concentração do e.b.e. testada. Os bioensaios foram feitos em quadruplicata, em uma câmara climatizada a 27°±1° C, UR≥80% e fotofase natural de 12 horas. A mortalidade foi observada após 48h. Obtiveram-se as CL_{50} de 365 e CL_{99} de 4.000 ppm. Não houve mortalidade significativa para os três primeiros tratamentos ($p<0,05$).

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