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Efficiency of the latex from *Euphorbia splendens* var. *hislopii*, in the control of *Rhipicephalus* (*Boophilus*) *sanguineus* (Latreille, 1806) (Acari: Ixodidae)

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Abstract: The aim of this study was to evaluate the effect of the latex from *Euphorbia splendens* var. *hislopii* (N.E. Br.) Ursch & Leandri, Euphorbiaceae, on the eggs and engorged larvae of *Rhipicephalus (B.) sanguineus* (Latreille, 1806) (Acari: Ixodidae). Six aqueous concentrations: 25, 50, 100, 125, 250 and 500 µL/L of the latex of this plant were tested. The control group was tested only with distilled water. The latex of *E. splendens* var. *hislopii* presented an LD50 of 18.031 µL/L and LD90 of 84.610 µL/L against the eggs of *R. (B.) sanguineus*. The larvae of all the groups treated with the latex presented a low survival rate of 0% at 25 µL/L, 1% at 50 µL/L, 2% at 100 µL/L, 3% at 125 µL/L, 9% at 250 µL/L, 5% at 500 µL/L when compared with the control group (91%). On day 7 and 14 after the application the latex killed more efficiently the treated groups (25, 50, 100, 125, 250 and 500 µL/L). As from day 21 the latex became less effective for all treatments. Our results clearly show that the aqueous concentration of the latex have a strong effect on tick eggs and larvae suggesting that it could become an important acaricide.

Introduction

Several synthetic acaricides have been used for killing ticks, but resistance of *Rhipicephalus (Boophilus) sanguineu*s (Latreille, 1806) to these products has been noted in some countries (Gladney & Dawkins, 1976; Belot & Mishra, 1979). The indiscriminate use on infested dogs and the environment could cause the inefficiency of the acaricide (Furlong, 1993), in addition to economic injury and poisoning other animals and humans (Fernandes, 1997; 2000).

Plants or crude extracts could become an alternative. Kaaya et al. (1995) and Abdel-Shafy & Zayed (2002) demonstrated that extracts of several plants had a strong acaricide effect, being able to reduce food ingestion and alter the fertility and viability of eggs of *Hyalomma anatolicum excavatum, Amblyomma variegatum, Rhipicephalus appendiculatus.*

Euphorbia splendens var. *hisloppi* (N.E. Br.) Ursch & Leandri, Euphorbiaceae, originating from Madagascar, is popularly known in Brazil as "coroa-de-cristo" ("crown-of-christ"), where it is nowadays widespread and cultivated as an ornamental plant and as a hedge (Schall et al., 1992). It has been used as molluscicide since 1930, with the prospect of becoming the most appropriate method to control the snail that is the intermediate host of *Schistosoma mansoni*. However, in literature there is no mention of the effects of this product on *R. (B.) sanguineus.* Gomes et al. (2003) tested latex from *E. splendens* var *hislopii*, to control *Peckya (Peckia) chrysostoma* (Wiedemann, 1830) (Diptera: Sarcophagidae) and observed a significant reduction in the body mass of specimens and viability of their whole cycle. Leles & Fernandes (2005) tested the substances extracted from *Copaifera langsdorffii* (Meliaceae) and *Carapa guianensis* (Caesalpiniaceae) on larvae of *R. (B.) sanguineus,* showing a promising activity against these ticks.

The study of the tick *R. (B.) sanguineus* is important for veterinary medicine, because this arthropod is a transmitter of pathogens to dogs (Simpson et al., 1991), such as *Babesia canis, Ehrlichia canis* and *Hepatozoon canis* (Christophers, 1907; Smith et al., 1976).

Some authors highlight the implications of this tick in public health, since parasitizing other domestic animals, *R. (B.) sanguineus* can also parasitize

man (Feldman-Muhsan, 1956; Dipeolu et al., 1982), transmitting pathogens, such as *Rickettsia rickettsii, Rickettsia conorii* and *Coxiella burnetti* (Mantovani & Benazzi 1953; Racioppi et al., 1981). Harrison et al. (1997) recently confirmed that the immature forms of *R. (B.) sanguineus* fed more frequently on humans than reported in the literature, thereby increasing transmission of some anthropozoonoses (Hoogstraal, 1967), as these develop with high tick density and are prevalent in synanthropic environments in various cities of Brazil.

Therefore, this study aims to evaluate the effectiveness of the latex from *E. splenden*s var. *hislopii* to control the eggs and larvae infestations of *R. (B.) sanguineus*.

Material and Methods

Latex

For the assays performed in the present study, latex samples were always collected at the same site, to avoid possible variations due to factors such as soil, climate etc, of the plant metabolism and active substance concentration as demonstrated by Lugt (1987). The latex from *Euphorbia splendens* var. *hisloppi* (N.E. Br.) Ursch & Leandri, Euphorbiaceae, was extracted by cutting through the stem of a plant in the winter of 2005 on the Fundação Oswaldo Cruz campus, Rio de Janeiro, Brazil. The latex was drained into test tubes. The tubes were hermetically sealed and carried to the laboratory for the preparation of mother solutions. The latex (1 mL) was collected in 9 mL of distilled water, the solution from which the required concentrations were obtained. The concentrations tested were 25, 50, 100, 125, 250 and 500 µL/L. The control group was exposed to distilled water only.

Ticks

A colony of *R. (B.) sanguineus* was maintained in the Laboratório de Educação em Ambiente e Saúde, Instituto Oswaldo Cruz, Fiocruz, RJ. Gravid females were colected from naturally infested dogs, in Rio de Janeiro-RJ, and free of acaricidal residues for at least thirty days prior to the bioassays. The ticks were reared on the ears of rabbits (*Oryctolagus cuniculus*) in the laboratory. The females were placed in BOD incubators acclimatized to 27±1 °C, 80% RH and a 12:12 h light/dark photoperiod. In order to obtain eggs of the same age cohort, separated plastic syringes, which were sealed with cotton wool plugs.

The experiments were conducted with 12 day old eggs and engorged larvae (14-21 day-old larvae). After exposure to latex, the samples were kept in BOD incubators acclimatized to 27±1 °C, 80% RH and a 12:12 h light/dark photoperiod until the end of the experiment.

Eggs exposure

For evaluation of latex efficacy on eggs, seven lots of 110 eggs of *R. (B.) sanguineus* were used for each concentration of latex. Eggs were exposed to 1 mL of each concentration inside the syringe (experimental groups) and the control group received only distilled water, since no other solvent was required to solubilize extracts of *E. splendens*. The concentration distributed uniformly on the internal surface of the syringes. After 5 min of exposure, the excess humidity was absorbed by the cotton, it was replaced by a dry one, and was returned to the incubator. After the treatment, eggs were observed daily, to check eclosion inhibition and possible changes resulting from the exposure to latex.

Engorged (fed) larvae

Larvae of *R. (B.) sanguineu*s were placed on rabbit ears until engorgement. In order to evaluate the larvicidal efficacy of the latex, seven lots of fifty larvae of *R. (B.) sanguineus* were placed in plastic syringes and were tested as described above for the eggs. The tests were made in triplicate.

Evaluation of biological parameters

With the aid of an optical microscope the parameters evaluated were the eclosion of the eggs and the number of live and dead larvae (engorged) of *R. (B.) sanguineus*. The results were confirmed with analysis of variance (ANOVA). The difference between the percentage of hatching eggs and engorged larvae (live and dead) was evaluated through the Chi-Square and Tukey test (significance level: $p<0.05$) (Zar, 1999). The lethal dose (LD90 and LD50) against egg hatching was computed by Probit analysis (Finney, 1971).

Results and Discussion

The biocontrol activity of the latex from *E. splendens* var. *hislopii*, on the eggs of the tick *R. (B.) sanguineu*s is shown in Figure 1. The egg hatching was observed in all groups and there was a significative difference in the eclosion percentage among treatments (Chi-Squared value=389.312, df=6, $p<0.001$) (Table 2). The egg hatching rate was inversely proportional to the concentration tested, *i.e.* the lower number of eclosions was observed for the higher concentrations of 125, 250 and 500 µL/L (1% for these concentrations). For the other concentrations tested the egg hatching rates were: 45% with 25 µL/L, 30% with 50 µL/L and 10% with 100 µL/L (Figure 1).

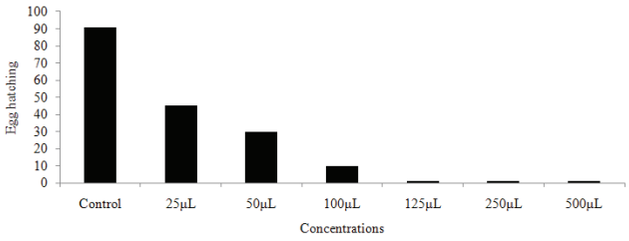

Figure 1. Percentage of eggs hatching of *Rhipicephalus (B.) sanguineus* exposed to different concentration of aqueous extracts of *Euphorbia splendens* var. *hislopii*.

For the control group, the egg hatching rate was approximately 90%. Kaaya et al. (1995) using a different methodology, showed the efficiency of water-soluble extracts of *Margaritaria discoidea* an Euphorbiacea. The extracts of this plant induced a high mortality in nymphs and adults of *R. appendiculatus* and *A. variegatum* but the same was not observed for adults of *A. variegatum.*

Although eclosion occurred in all groups, for control and eggs treated with 25 µL/L of latex it began 24 h after treatment, while for the groups treated with the other concentrations the eclosions began only 72 h after exposure, thus demonstrating a possible embryogenic action.

The extract of *E. splendens* var. *hislopii* presented activity against eggs of *R. (B.) sanguineus*. The LD50 was 18.031 µL/L and LD90 was 84.610 µL/L, showing significant differences among the concentrations tested (X^2=14.15; df=5 p<0.05). Gomes et al. (2003), tested latex from *E. splendens* var. *hislopii* as a candidate for as a biological control agent of *P. chrysostoma* (Wiedemann, 1830) (Sarcophagidae) with concentrations of 0.1, 0.2 and 0.3%, that equaled are equivalent to 100, 200 and 300 mg, respectively, of latex in 100 g of meat and observed interference on the post-embryonic development, promoting a reduction in the number of adults. Due to morphologically alterations these adults were unable to procreate. Mello et al. (2010) also evaluated the effect of crude lyophilized latex of *E. splendens* var. *hislopii* on the post-embryonic *Megaselia scalaris* (Loew, 1866) (Diptera: Phoridae) with concentrations of 5, 10 and 20 µg/mL, inoculated placed directly onto the bodies of the newlyhatched larvae with an automatic pipette (1 µL/larva), *i.e.*, not applied the latex concentrations on the larvae differently as performed from the methodology used in the present study. These authors observed that the larval, pupal and newlyhatched larvae to adult development times were faster in the presence of this substance when compared with control group. This result was the inverse opposite of the obtained in the present study, in other words, in the present study there was a direct relationship in this study, between the increasing the latex concentration (50, 100, 125, 250 and 500 µL/L) there was an increase and the time of development (72 h). It is important detach to point out that the animal insect and the methodology above employed was were different of from the present study, and also that the latex was used in its crude lyophilized form, dissolved in distilled water and the solution was placed topically on the larvae, and whereas in the present study the eggs were exposed to 1mL of each concentration (crude latex dissolved in distilled water) inside the syringe. So this may have caused the observed differences between these studies.

Several studies with plant extracts of *Ocimum suave, Gynandropsis gynandra, Azadirachta indica* have reported the effectiveness of such plants as repellents, feeding inhibitors and other actions on the biology of these ticks (Malonza et al., 1992; Mwangi et al., 1995).

Based on the conditions related in the present study, the latex of *E. splendens* var. *hislopii* demonstrated an anti-embryogenic activity, since the unhatched eggs were pale and showed rough surfaces and the embryos were morphologically altered. Also the extracts influenced the hatching period of the eggs.

The efficacy of the treatments is demonstrated by the results in Table 1. On the first 1 day after treatment the effect of product proved to be was very small or negligible in the treated groups treated with 25 µL/L (4), 125 µL/L (3.33) and 250 µL/L (1.66). Pereira & Famadas (2004), observed mortality of engorged females of *Boophhilus microplus* (Canestrini, 1887) subjected to treatment with

Table 1. Average count of dead engorged larvae of *Rhipicephalus (B.) sanguineus* per days exposed to different concentrations of aqueous extracts of *Euphorbia splendens* var. *hislopii* and control group (without extract), under laboratory conditions.

Days post-treatment	NNNumber of Tick						
	Control	25 µL/L	50 µL/L	100 µL/L	125 µL/L	250 µL/L	500 µL/L
1	0	4	12	9.66	3.33	1.66	5.66
3	0	2.66	7	8.66	6	10	9.33
7	1	11.33	15	14.66	17.67	11.66	11
14	1	17.66	15	15.33	18.33	17.33	16
21	1	11	0,67	0.66	3	5	5.33
28	1.66	3.33	0	0	0	0	0

Data are from triplicate experiments with groups of fifty *R. (B.) sanguineus* each.

root extract from *Dahlstedtia pentaphylla* (Leguminosa, Papilionoidae, Millettiedae), which only becomes evident as from second day of exposure to the product. In the present study the best result obtained was seen 1 and 3 days after the application of the extract at a concentration of 100 µL/L (9.66 and 8.66) and 500 µL/L (5.66 and 9.33) respectively when compare to the control group.

On day 7 and 14 after application, the latex show is more efficient in killing at in the treated group (25, 50, 100, 125, 250 and 500 µL/L). As from day 21 the latex became less effective for all treatments, and by day 28 the product already had no effect whatsoever. These results suggest a better effect of the latex against engorged larvae, this because according to Pereira & Famadas (2004), scientific studies have proved that the larval stage of *Boophilus microplus* (Canestrini, 1887) is about eight times as sensitive to the product as the adult stage. In another study, Pereira & Famadas (2006) observed that the efficiencacy of *Dahlstedtia pentaphylla* (Leguminosae, Papilionoidae, Millettiedae) at in the concentrations of (1:10 and 1:20 mL) persisted up to day 7, and as from day 14, the product became less effective.

The engorged larvae were observed, displaying a low survival rate with all treatments showing a significant difference (Chi-Squared value=693.78, df=6, $p<0.001$): 0% at 25 µL/L, 1% at 50 µL/L, 2% at 100 µL/L, 4% at 125 µL/L, 9% at 250 µL/L and 5% at 500 µL/L, respectively (Figure 2 and Table 2).

The larvae that emerged had difficulties to move and were paralyzed. The survival rate of the control group was 91%. Although the results do not provide linearity, the latex presented an activity on eggs and larvae originated from engorged adults of *R. (B.) sanguineus*, acting directly on the biology of the ticks.

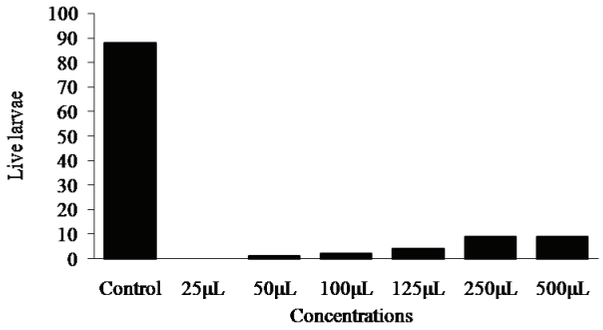

Figure 2. Percentage of live larvae engorged of *Rhipicephalus (B.) sanguineus* exposed to different concentration of aqueous extracts of *Euphorbia splendens* var. *hislopii.*

According to Chagas (2004), tests with plant extracts usually give a non-linear relationship between concentrations and action. Still according to the author, this may occur depending on the process of extraction and production of the active compound of the extract.

The results from the present study suggest that latex from *E. splendens* var. *hislopii* interfere with the development (eggs and engorged larvae) of *R. (B.) sanguineus,* thus indicating a potential candidate for biological control.

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Table 2. Mean±SD per day of eggs hatched and dead engorged larvae of *Rhipicephalus (B.) sanguineus* exposed to different concentrations of aqueous extracts of *Euphorbia splendens* var. *hislopii* and control group (without extract), under laboratory conditions.

Concentrations	Eggs Hatched	Dead Engorged Larvae
	(Mean±S.D.)	(Mean± S.D.)
Control	2.3±2.7^{a}	1.7±6.0^{a}
25µL/L	3.2±5.3^{a}	13±7.3^{b***}
50µL/L	3.4±6.1^{a}	4.9±7.4^{c***}
100µL/L	0.94±3.1^{b*}	6.8±6.1^{c***}
125µL/L	0.027±0.09^{b***}	9.2±5.3^{d***}
250µL/L	0.027±0.28^{b***}	9.2±6.3^{d***}
500µL/L	0.027±0.28^{b***}	8.9±6.5^{cd***}

Values within a column followed by the same letter are not significantly different at the 5% level according to Tukey's. ***$p<0.001$ when compared to control group. Data with groups of 110 (eggs) and 150 (engorged larvae).

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