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In vitro evaluation of the effects of some acaricides on life stages of *Rhipicephalus sanguineus* (Acari: Ixodidae)

[*Avaliação* in vitro *do efeito de alguns acaricidas nos estádios de vida do* Rhipicephalus sanguineus (*Acari: Ixodidae*)]

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ABSTRACT

The action of five acaricides was evaluated on eggs, larvae, nymphs and adults of the ixodid tick *Rhipicephalus sanguineus*. The acaricides tested were: high cis-cypermethrin (0.01%) + DDVP (0.11%), coumaphos (0.05%), deltamethrin (0.0025%), amitraz (0.025%) and cypermethrin (0.015%). Eggs treated with high cis-cypermethrin and cypermethrin showed eclosion inhibitions of 72.1% and 67.3%, respectively, whereas coumaphos was much less effective (only 11.7%). Except for coumaphos, which resulted in 63.3% and 80.0% mortality of nymphs and unfed females, respectively, all compounds tested killed 100% of all tick stages to which they were exposed.

Keywords: Tick, *Rhipicephalus sanguineus*, acaricide, dog

RESUMO

Avaliou-se a ação de cinco acaricidas em ovos, larvas, ninfas e adultos do carrapato Rhipicephalus sanguineus. *Os produtos testados foram: cipermetrina high cis (0.01%) + DDVP (0.11%), coumaphos (0.05%), deltametrina (0.0025%), amitraz (0.025%) e cipermetrina (0.015%). Os ovos tratados com cipermetrina high cis e cipermetrina mostraram inibição de eclosão de 72,1% e 67,3%, respectivamente, enquanto coumaphos resultou em baixa eficiência (11,7%). Exceto para ninfas (63,3%) e para fêmeas não alimentadas (80,0%) expostas ao coumaphos, todos os outros estádios de carrapato e fêmeas não alimentadas foram sensíveis a todos os produtos químicos testados.*

Palavras-chave: Carrapato, Rhipicephalus sanguineus, *acaricida*, *cão*

INTRODUCTION

The brown tick *Rhipicephalus sanguineus* (Latreille) is an ectoparasite of dogs (Hoogstraal, 1985), which produces debilitating effects due to blood losses and transmission of various pathogens. These include *Babesia canis* (Piana & Galli-Valério) and *Ehrlichia canis* (Donatien & Lestoquard), the etiological agents of canine babesiosis and ehrlichiosis, respectively (Christofers, 1907; Neitz et al., 1971; Smith et al., 1976; Soulsby, 1982; Gothe et al., 1989).

Control of this ectoparasite is based on the application of chemical compounds developed to control other tick species. In general, owners dip their dogs in acaricide solutions when they encounter engorged females. Eggs, larvae, nymphs and adults of *R. sanguineus* are present all over in the animal's environment, and therefore, all places to which the animals have access, particularly the animal bedding, must be treated with acaricides. Different acaricides are effective for controlling the various developmental stages of *R. sanguineus* (Alcaino et al., 1991; Bandyophadhyay & Sarma, 1993; Castellà et al*.,* 1994; Vidotto et al*.,* 1984; Yathiraj et al., 1992). In order to increase the efficiency of disinfestation measures and to minimize the risk of pesticide resistance, it is essential to understand the effects of acaricides against the different stages of this tick life-cycle (Castellà et al., 1994). Fernandes et al. (1997) reported deltamethrin resistance in Brazilian *R. sanguineus*.

The objective of this study was to evaluate the effects of five acaricides on eggs, larvae, nymphs and adults of *R. sanguineus*.

MATERIALS AND METHODS

Ticks were collected from naturally infested dogs and were reared on the ears of dogs and rabbits (*Oryctolagus cuniculus*) in the laboratory. This involved removing engorged females of *R. sanguineus* by hand from dogs, placing them in Petri dishes, and maintaining them in an incubator at 28±1°C and RH $\geq$ 80%. Following oviposition, the eggs were maintained in the incubator until hatching of larvae. Larval *R. sanguineus*, aging 12-15 days, were placed on the ears of hosts and allowed to engorge fully. The engorged larvae were collected and maintained as previously described. After eclosion, nymphs were again placed on dog or rabbit ears until engorgement. The engorged nymphs were then removed and maintained in the incubator until eclosion of adults. Adult female ticks from the first laboratory generation were then allowed to engorge on the host animal ears.

Five acaricides were evaluated against the eggs, larvae, nymphs, unfed and engorged females of *R. sanguineus*: amitraz (Triatox - Coopers Brasil S/A) (0.025%); coumaphos (Assuntol - Bayer do Brasil) (0.05%); cypermethrin (Barrage - Cyanamid Química do Brasil Ltda) (0.015%); high cis-cypermethrin (0.01%) + DDVP (Ectoplus - Ciba-Geigy Química S/A) (0.11%) and deltamethrin (Butox - Químio Russel S/A) (0.0025%).

All treatments were tested in triplicates. The control ticks were treated with distilled water. When control ticks mortality was equal or greater than 15%, the results of the test were not considered.

For evaluation of acaricide efficacy on eggs, six lots of 0.001g of 12 day-old eggs were weighed and placed in filter paper envelopes. Each envelope was immersed for three seconds in 100ml of each acaricide or in distilled water. The eggs were dried on filter paper, placed in plastic syringes and maintained in the incubator. Results were recorded 60 days after the onset of eclosion. The percentage of eclosion inhibition (EI) for each sample was determined as the number of unhatched eggs divided by the number unhatched eggs + the number of dead or live larvae x 100.

In order to evaluate acaricide efficacy on larvae**,** eggs of engorged females were weighed and divided into lots of 20mg. Six lots were placed in plastic syringes, which were sealed with cotton wool plugs and maintained in the incubator. The acaricides were tested on 14-21 day-old larvae, using a method modified from that proposed by Shaw (1966). The larval lots were divided between two filter papers and immersed in 10ml of the acaricide solution. After 10 minutes, excess humidity was removed with a double layer of filter paper. One hundred to 200 larvae were removed from the paper and placed in filter paper envelopes, which were folded and sealed with sticky tape (Cramer - Ind. Brasileira) and placed in the incubator. The envelopes were opened 24 hours later. Live and dead larvae were counted and the percentage of mortality was determined for each lot.

In order to evaluate acaricide efficacy on nymphs**,** 14 day-old larvae were placed on rabbit ears to feed. Engorged larvae were collected and maintained in the incubator. After ecdysis, 10 day-old nymphs were collected and treated with the acaracides. Lots of 50 unfed nymphs were tested using the same method described for larvae.

To evaluate acaricide efficacy on unfed females, engorged nymphs were collected from rabbits and maintained in the incubator. Ten days after ecdysis, the unfed females were divided into six lots of 10 individuals each and placed in filter paper envelopes. The envelopes were dipped in 100ml of each acaricide solution, for five minutes, and were returned to the incubator after removing the excess humidity. The envelopes were examined 24 hours later and the percentage of mortality was recorded for each envelope.

Acaricide efficacy on engorged females was determined using the test designed by Drummond et al. (1971). Engorged females collected from dogs were washed in running water and dried with a paper towel. Six lots, each consisting of 10 females of equivalent weight, were selected. Females were immersed in 100ml of each acaricide solution, for five minutes, after which they were dried on a paper towel, placed in a Petri dish and fixed dorsally with sticky tape. The proterossome of each tick (the gnathosoma together with the propodosomal region) was left free. Dates, treatments, and weight of each lot were written on the filter paper, which was maintained in the incubator for 15 days, after which the eggs of each group were weighed and the reproductive efficiency (RE) was calculated.

The eggs were maintained in the incubator and monitored until the end of the eclosion period. The percentage of eclosion was calculated for each group. The efficacy of each acaricide was calculated according to Drummond et al. (1971).

The effect of acaricides on eggs, larvae, nymphs and adults of *R. sanguineus* was evaluated based on anlysis of variance, and arc sine transformed for proportions (Snedecor & Cochran, 1989)**.**

RESULTS

The highest inhibitions of egg hatching were obtained with high cis-cypermethrin (72.1%) and cypermethrin (67.3%) ($P<0.05$). Coumaphos was the least efficient, producing inhibition of only 11.7%. (Table 1). All compounds tested resulted in 100% of larval and nymphal mortality, except coumaphos ($P<0.05$), which killed only 63.3% of the nymphs (Table 2).

Table 1. Hatching inhibition (%) of *R. sanguineus* treated with different acaricides

Acaricide	Number of Larvae	Number of unhatched eggs	% of inhibition
Control	1080	67	5.8 e
Amitraz	474	600	55.9 b
Coumaphos	1039	138	11.7 d
Cypermethrin	366	752	67.3 a
Deltamethrin	558	404	42.0 c
Cypermethrin high cis	316	818	72.1 a

Different letters within the same column indicate a significant difference (P<0.05).

Table 2. Mortality (%) of larvae and nymphs of *R. sanguineus* treated with different acaricides

Acaricide	Larvae (number)			Nymphs (number)		
	Alive	Dead	Mortality	Alive	Dead	Mortality
Control	181	30	14.2b	32	2	5.9c
Amitraz	-	170	100a	-	33	100a
Coumaphos	1	159	99.4a	11	19	63.3b
Cypermethrin	-	129	100a	-	38	100a
Deltamethrin	-	135	100a	-	46	100a
Cypermethrin high cis	-	128	100a	-	43	100a

Different letters within the same column indicate a significant difference (P<0.05).

Unfed female *R. sanguineus* had 100% mortality, except those treated with coumaphos (80.0%) (Table 3). Engorged females were susceptible to all the compounds tested; however, in one treatment (test 3) the ticks showed a low sensitivity to deltamethrin and cypermethrin (Table 4).

Table 3. Mortality (%) of unfed female of *R. sanguineus* treated with different acaricides

Acaricide	Test 1	Test 2	Test 3	Mean (%)
Control	0	0	10.0	3.3c
Amitraz	100	100	100	100a
Coumaphos	80.0	70.0	90.0	80.0b
Cypermethrin	90.0	100	100	96.7a
Deltamethrin	100	100	100	100a
Cypermethrin high cis	100	100	100	100a

Different letters within the same column indicate a significant difference (P<0.05)

Table 4. Percentage of acaricide efficacy for *R. sanguineus* engorged females

Acaricide	Test 1	Test 2	Test 3	Mean%
Amitraz	100	100	100	100a
Coumaphos	100	100	100	100a
Cypermethrin	100	100	77.0	92.3a
Deltamethrin	100	99.9	77.0	92.3a
Cypermethrin high cis	97.9	89.0	98.7	95.2a

Different letters within the same column indicate a significant difference (P<0.05)

DISCUSSION

According to the Brazilian Ministry of Agriculture (Brasil..., 1990), an acaricide is considered to be adequate when its efficacy is 95% or higher. The results of this study show that all the compounds tested are efficient acaricides for *R. sanguineus* engorged female. One of the three assays involving exposure of engorged females to deltamethrin and cypermethrin, however, revealed a low sensitivity to these compounds in this group. These results are in contrast to those reported by Vidotto et al. (1984) and Alcaino et al. (1991). The authors

recorded a high efficacy for these compounds and suggest that *R. sanguineus* has developed some degree of tolerance to deltamethrin and cypermethrin, at least in the region of Belo Horizonte, Minas Gerais State.

All treatments effectively killed *R. sanguineus* larvae, leading to mortality rates higher than 99.4%. These results are similar to those reported by Maske et al. (1994) and Alcaino et al. (1994). Fernandes et al. (1997) tested different concentrations of deltamethrin on *R. sanguineus* larvae in Goiânia, Goiás State, and obtained mean of mortality of 34.4% and 57.6%, at concentrations of 0.0025% and 0.015%, respectively. Therefore, strains of *R. sanguineus* from Goiânia may be more resistant to deltamethrin than those from Belo Horizonte.

Nymphs and unfed females of *R. sanguineus* are sensitive to pyrethroids, as shown by Yathiraj et al. (1992) and Bandyopadhyay & Sarma (1993). However, our results indicate that coumaphos is not effective against these stages of the life cycle (Tables 2 and 3). This ineffectiveness may be related to organophosphate resistance, first observed in the 1960s and 1970s. This happened shortly after the introduction of these compounds for intensive tick control.

In the present study, all treatments showed a low efficacy against *R. sanguineus* eggs, which showed particularly low levels of susceptibility to coumaphos (Table 1). This agrees with the findings reported by Castellà et al. (1994), although eggs in our study were more sensitive to cypermethrin and high cis-cypermethrin.

An engorged female *R. sanguineus* produces 2,000 to 4,000 eggs, which are deposited in humid sites protected from direct sunlight (Freitas et al., 1978). Oviposition is a dynamic process and eggs can be found in various stages of development in the same infestation sites. One-day old eggs are more sensitive to acaricides than those which are 10-20 day old (Davey et al., 1989). We tested eggs that were 12 day-old, which may explain the low efficacy of the treatments. Cypermethrin was the most effective treatment, producing the highest indices of hatching inhibition. Thus, it may be considered that the best choice for acaricide treatments to control *R. sanguineus* are the egg-laying sites.

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