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ACTIVITY OF SOME ACARICIDES AGAINST ENGORGED FEMALES AND EGGS OF *RHIPICEPHALUS SANGUINEUS* (LATREILLE, 1806) (ACARI: IXODIDAE)

M.J. GRACIA-SALINAS, C. SÁNCHEZ-ACEDO, A. ESTRADA-PEÑA & M. GALMES-FEMENIAS
Departamento de Patología Animal, Parasitología y Enfermedades Parasitarias, Facultad de Veterinaria, Universidad de Zaragoza, C/ Miguel Servet nº 177, 50013 Zaragoza, Spain

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ABSTRACT: Engorged females and eggs of the brown dog tick, *Rhipicephalus sanguineus*, were dipped in water dilutions of commercially available formulations of coumaphos, cypermethrin, diazinon, phoxim, ronnel and trichlorfon. Against females, effects on estimated reproduction (ER) (ER = g eggs/g females × % hatch of eggs × 20000) were determined. Percent inhibition of ER was subjected to probit analysis. Concentrations that inhibited 50% of ER (LC50) ranged from LC50 of 0,12 ppm for phoxim to LC50 of 49682 ppm for ronnel. Against eggs, none of the acaricides are ovicides. Effectiveness of the compounds in killing larvae posthatching ranged from LC50 of 7,18 ppm for phoxim to LC50 of 4680,9 ppm for ronnel.

KEY WORDS: Ticks, *Rhipicephalus sanguineus*, engorged females, ovicides, control, acaricides.

INTRODUCTION

Rhipicephalus sanguineus (Latreille, 1806), the brown dog tick, is a 3-host species. It is recognized worldwide as one of the most cosmopolitan ixodids (HOOGSTRAAL, 1956) and is an important ectoparasite of dogs in Spain (OCABO-MELENDEZ, 1988). The pest rarely attacks man, however it is well adapted to living inside buildings, where it can be a nuisance. *R. sanguineus* is also capable of transmitting both canine piroplasmosis, *Babesia canis*, and canine ehrlichiosis, *Ehrlichia canis* (URQUHART *et al.*, 1987; SOULSBY, 1988).

For the foreseeable future, acaricides will continue to be the primary tool for the control of ticks. Most workers preferred to use engorged females for testing acaricides because of their greater resistance to toxicants and their uniform response.

An ideal acaricide should be effective not only against adult ticks but also against their eggs, larvae and nymphs. The ovicidal property of an acaricide is very important for killing or interfering with hatchability of eggs, which would ensure proper tick control.

The purpose of our study was to determine the efficacy of six acaricides, commonly used as chemicals in Spain, against engorged females and egg stages of *R. sanguineus.*

MATERIAL AND METHODS

Engorged females of *R. sanguineus* were collected from dogs in Zaragoza (Spain) in 1989. Previous exposure of these ticks to acaricides is unknown. In the laboratory, in order to obtain samples at the homogeneous physiological stage, the ticks were weighed and only specimens of 200 mg or more were used (see GRAHAM & DRUMMOND, 1964). The ticks used for testing engorged females were sorted and groups of 5 active females, normal in appearance, were used for each dosage. The other females were introduced into test tubes plugged with cotton and placed in an incubator at 27° C and 90 ± 5 % relative humidity (RH). The eggs laid were transferred daily to separate test tubes and stored.

The acaricides used were 5 organophosphate compounds: coumaphos (Asuntol-50, 50% coumaphos, BAYER), diazinon (Neocidol-60, 60% diazinon, CIBA-GEIGY), phoxim (Sebacil, 50% phoxim, BAYER), ronnel (Vaposit, 40% ronnel, SOBRINO) and trichlorfon (Neguvon, 100% trichlorfon, BAYER), and one synthetic pyrethroid, cypermethrin (Barricade, 5% cypermethrin, SHELL).

Stock solutions of the acaricides (12800 ppm) were prepared by transferring the acaricide into a 10 ml volumetric flask and adding water. Subsequent solutions were made by pipetting from the stock solution and making up to 10 ml with water in a volumetric flask. Serial dilutions were made as desired. Dilutions were prepared just before the ticks were treated.

For each acaricide the following concentrations (treatments) with five replicates of each treatment were used: 1,56, 3,12, 6,25, 12,5, 25, 50, 100, 200, 400, 800, 1600, 3200, 6400 and 12800 ppm.

Against engorged females, exposure to each chemical dilution was accomplished by a technique reported by DRUMMOND *et al.* (1971). The ticks were dipped for three minutes in 10 ml of the tested concentration with continuous stirring of the solution and then dried on filter paper to remove excess liquid. The control group received 10 ml of distilled water. After treatment, the engorged females were held at 27° C and 90 ± 5 % RH.

The effect of acaricides was measured in terms of failure to produce viable eggs rather than in terms of mortality, because it is extremely difficult to separate dead and moribund females under *in vitro* conditions. Daily, the egg masses were weighed and the hatch of eggs estimated.

Effectiveness of a concentration was measured by the effect on the estimated reproduction (ER) of the females (DRUMMOND *et al.*, 1971). The ER was calculated as follows:

$$ER = \text{wt eggs (g)}/\text{wt females (g)} \times \%\ \text{hatch} \times 20000$$

In this formula, the constant (20000) is the estimate of the number of larvae which normally hatch from 1 g of eggs.

The percentage control of ER by a given treatment was then determined by comparing the ER of treated ticks with the ER of the control ticks as follows:

$$\%\ \text{Control} = \text{ER (control)} - \text{ER (treated ticks)} / \text{ER (control)} \times 100$$

The ovicidal effect of the acaricide was tested on 5-day-old eggs. Egg exposure to each chemical dilution was accomplished by slightly modifying a technique reported by SHAW (1966). Watman 01 filter paper (9 cm diameter) was used to line the insides of Petri dishes. One cm^3 of each acaricide dilution was added directly to the filter in each dish. The control group received 1 cm^3 of distilled water. One hundred 5-day-old eggs were placed on the moist filter paper and covered with another filter paper treated with 1 cm^3 of the acaricide dilution. This sandwich was held for 24 h in an incubator at 27° C and 90 ± 5% RH. After treatment, egg samples were placed in test tubes in an incubator at 27° C and 90 ± 5% RH and were observed daily for impact on hatchability of eggs, and for post-hatch effect. Ticks were considered dead when they were unable to move when prodded with a brush.

Significant differences in the mean percentage control of the acaricides was determined by analysis of variance and Duncan's New Multiple Range test (DUNCAN, 1951). The LC50 was determined from the test with an average of 5 engorged females and 500 eggs for each concentration used. The data were analyzed by a log-probit programme (DAUM, 1970) designed for a personal computer (RAYMOND, 1985).

RESULTS

Results against engorged females

Results of treatment with coumaphos, diazinon, trichlorfon, and phoxim indicate that there is a relation between dosage and % control, and that generally there was an increase in % control when the dosage was increased. With phoxim and diazinon the % control obtained was higher than 80% with dosages of 6,25 and 12,5 ppm respectively. Coumaphos and trichlorfon required 400 ppm to obtain the same level of control. On the other hand, treatment with cypermethrin and ronnel displays a lack of relation between the increase of dosage and the increase in % control. The % control was lower than 65% even with the highest dosage (Table 1).

ANOVA test shows that with the acaricides diazinon and phoxim a significant difference ($P < 0,001$) occurred between untreated and treated groups, even with the lowest dosage. Coumaphos and trichlorfon differ significantly ($P<0,001$) from the control group with dosages of 100 and 400 ppm respectively. Finally, with the acaricides cypermethrin and ronnel a significant difference ($P<0,05$) occurred between untreated and some treated groups, but this difference was independent of the acaricide concentration (cypermethrin 400 and 3200; ronnel 800 and 3200 ppm).

Based on the mean lethal concentrations of the acaricides in descending order of effectiveness (increasing LC50), phoxim (0,12 ppm) and diazinon (2,42 ppm) were the most effective acaricides, followed by coumaphos (81,1 ppm) and trichlorfon (396,3 ppm). The least effective compounds were cypermethrin (3033,2 ppm) and ronnel (49682,02 ppm).

Results against eggs

The effect of reduction in hatchability (Table 2) was low with the acaricides coumaphos and cypermethrin and ANOVA test indicates that no significant difference was found between untreated and treated groups. However, with the acaricides trichlorfom, diazinon, phoxim and ronnel a significant difference occurred between untreated and some treated groups ($P<0,05$), but the percentage level of prevention of eclosion was independent of the acaricide concentration.

Nevertheless, the acaricides affect the viability of the larvae posthatch (Table 3) and this mortality (with the exception of trichlorfon and ronnel) was directly related to the acaricide concentration. A significant difference ($P<0,001$) occurred between untreated groups from doses of 6,25, 6,25, 50 and 800 ppm onwards with the acaricides phoxim, diazinon, coumaphos, and cypermethrin respectively. With trichlorfon and ronnel the percentage level of inhibition of the larvae posthatch was independent of the acaricide concentration. A mortality rate higher than 80% was achieved with phoxim and diazinon at 25 ppm, a level at which the acaricides coumaphos, cypermethrin,

	COUMAPHOS	CYPERMETHRIN	TRICHLORFON	DIAZINON	PHOXIM	RONNEL
CONTROL	0(a)(1)	0(a)	0(a)	0(a)	0(a)	0(a)
6,25 ppm	14,5(a,b)	1,3(a)	0(a)	42,5(b)	83,2(b)	0(a)
12,5 ppm	19,3(a,b)	6,6(a,b)	0(a)	86,8(c)	92,3(b,c)	0,9(a)
25 ppm	21,8(a,b)	12,4(a,b)	0,1(a)	100(c)	100(c)	1,2(a)
50 ppm	38,7(a)	22,6(a,b)	0,4(a)	86,2(c)	100(c)	1,3(a)
100 ppm	42,2(b)	49,5(a,b)	0,3(a)	100(c)	100(c)	3(a)
200 ppm	44,4(b)	44,5(a,b)	8(a)	100(c)	100(c)	4,6(a,b)
400 ppm	100(c)	64,4(b)	80(b,c)	100(c)	100(c)	15,7(a,b)
800 ppm	100(c)	47,6(a,b)	83,4(b,c)	100(c)	100(c)	48,3(b)
1600 ppm	100(c)	51,8(a,b)	81,5(b,c)	100(c)	100(c)	21,8(a,b)
3200 ppm	100(c)	57,2(b)	100(c)	100(c)	100(c)	38,3(b)
6400 ppm	100(c)	35(a,b)	58,7(b)	100(c)	100(c)	21,9(a,b)
(2)	***	*	***	***	***	*

Table 1.— ANOVA showing the efficacy (% of mortality) *in vitro* of 6 acaricides against engorged females of *Rhipicephalus sanguineus*. (1) Different letters indicate that the treatment was significantly different from the control ($P<0,05$); (2) *=significant at 0,05 level; ***=significant at 0,001 level.

	COUMAPHOS	CYPERMETHRIN	TRICHLORFON	DIAZINON	PHOXIM	RONNEL
CONTROL	2,6	2,6	2,6(a)(1)	2,6(a)	2,6(a)	2,6(a)
1,5 ppm	1,6	1,4	1(a)	1,2(a)	8,4(a,b)	2,8(a)
3,12 ppm	1,6	2	1,8(a)	2,6(a)	6,4(a)	3,2(a,b)
6,25 ppm	3,2	1,6	1,2(a)	4(a,b)	20,4(a,b)	2,8(a)
12,5 ppm	1,8	1,8	2(a)	9,2(a,b)	6(a)	2,8(a)
25 ppm	1,6	1,8	0,8(a)	7,4(a,b)	5,4(a)	3(a,b)
50 ppm	0,8	2,4	2,2(a)	18,8(b)	13,6(a,b)	3,2(a,b)
100 ppm	3,6	2	22(b)	5,2(a,b)	34(b)	3,6(a,b)
200 ppm	2,8	3	23(b)	10,2(a,b)	18,6(a,b)	10,4(a,b)
400 ppm	4,6	2,6	8,2(a,b)	11(a,b)	34(b)	3(a,b)
800 ppm	4	4	11,4(a,b)	16,2(b)	12,2(a,b)	9(a,b)
1600 ppm	2,4	3	9(a,b)	5(a,b)	14(a,b)	11,2(b)
3200 ppm	1,6	2,6	3(a)	5,8(a,b)	37(b)	12,4(b)
6400 ppm	3,4	2,2	2(a)	4,6(a,b)	10(a,b)	9,8(a,b)
12800 ppm	2,8	0,4	0,8(a)	6,4(a,b)	10,8(a,b)	4,8(a,b)
(2)	N.S.	N.S.	*	*	*	*

Table 2.— ANOVA showing the inhibition of hatchability (% of mortality) of 6 acaricides against eggs of *Rhipicephalus sanguineus*. (1) Each value is the average of 500 eggs; different letters indicate that the treatment was significantly different from the control ($P<0,05$); (2) N.S. not significant; * - significant at 0,05 level.

	COUMAPHOS	CYPERMETHRIN	TRICHLORFON	DIAZINON	PHOXIM	RONNEL
CONTROL	2,6(a)(1)	2,6(a)	2,6(a)	2,6(a)	2,6(a)	2,6(a)
1,5 ppm	2(a)	1,4(a)	1(a)	1,6(a)	8,4(a)	2,8(a)
3,12 ppm	2(a)	2(a)	1,8(a)	6,8(a,b)	7,2(a)	3,2(a)
6,25 ppm	4,8(a)	1,6(a)	1,2(a)	24,2(b)	74(c)	2,8(a)
12,5 ppm	9(a)	2(a)	2(a)	65,4(c)	50,2(b)	2,8(a)
25 ppm	30(a)	1,8(a)	0,8(a)	81,6(c,d)	92,2(d)	3(a)
50 ppm	44,6(b)	2,6(a)	2,2(a)	91,6(d)	99,8(d)	3,2(a)
100 ppm	68,4(c)	2,4(a)	22(b)	96,4(d)	99,8(d)	3,8(a)
200 ppm	75,4(c)	3,4(a)	23(b)	94,2(d)	100(d)	10,4(a,b)
400 ppm	97,8(d)	8,4(a,b)	8,2(a,b)	86(d)	100(d)	3,2(a)
800 ppm	99,4(d)	18(b)	11,6(a,b)	99,8(d)	100(d)	9(a,b)
1600 ppm	99,8(d)	40(c)	9,4(a,b)	94,2(d)	100(d)	11,2(a,b)
3200 ppm	100(d)	91,2(d)	89,8(c)	82,4(c,d)	100(d)	25,6(b)
6400 ppm	100(d)	100(d)	100(c)	94,8(d)	100(d)	15,8(a,b)
12800 ppm	100(d)	100(d)	100(c)	100(d)	100(d)	98,4(c)
(2)	***	***	***	***	***	***

Table 3.— ANOVA showing the inhibition of larvae posthatch (% of mortality) of 6 acaricides against eggs of *Rhipicephalus sanguineus*. (1) Each value is the average of 500 eggs; different letters indicate that the treatment was significantly different from the control ($P < 0,05$); (2) *** = significant at 0,001 level.

trichlorfon and ronnel did not differ significantly from that of control. For coumaphos at 400 ppm, cypermethrin and trichlorfom at 3200 ppm and ronnel at 12800 ppm, see Table 2.

Based on the mean lethal concentrations of the acaricides in descending order of effectiveness (increasing LC50), phoxim (7,18 ppm) and diazinon (13,4 ppm) were the most effective acaricides, followed by coumaphos (61,01 ppm). The least effective compounds were cypermethrin (1727,5 ppm) and ronnel (4680,9 ppm). It should be mentioned that with the acaricide trichlorfom, probably due to its irregular response, it was impossible to obtain LC50.

DISCUSSION

With both stages the same effectiveness was observed. Phoxim was the most active acaricide, followed by diazinon, coumaphos, and trichlorfon, whilst cypermethrin and ronnel were the least effective acaricides.

Phoxim has already shown its efficacy against nymphs of the lone star tick, *Amblyomma americanum* (L.) (MOUNT, PIERDCE & LOFGREN, 1971), and of the brown dog tick, *R. sanguineus* (GLADNEY & DAWKINS, 1976), and it was the product recommended by LIEBISH (1981) for the control of *Dermacentor marginatus* (S.) in Germany.

Diazinon was also highly effective and ranked just below phoxim in LC50 value. The concentration of drug used to inhibit 50 % of the population was inferior to those obtained by other authors against another species, where the results were good (DRUMMOND *et al.*, 1972; DRUMMOND, 1988).

The next most effective acaricides were coumaphos and trichlorfon. Our results agreed with DRUMMOND *et al.* (1972) and DRUMMOND (1988) who also observed greater efficacy with diazinon compared to coumaphos and trichlorfon. The greater efficacy of diazinon compared to coumaphos was also shown by DAVEY, AHRENS & GEORGE (1989) in the treatment of *Boophilus microplus* eggs. Nevertheless DRUMMOND *et al.* (1971) and DRUMMOND (1981) observed that diazinon was less effective than coumaphos and trichlorfon. In our study the LC50 of these acaricides were inferior to those obtained by other authors, where the results were good with coumaphos (DRUMMOND, 1988; BELOT & MISHRA, 1979) although not so good with trichlorfon (DRUMMOND, 1981).

The concentration of drug used with phoxim, diazinon, coumaphos and trichlorfon to inhibit the population of *R. sanguineus* females and larvae posthatch (with the excepcion of trichlorfon) was inferior to that recommended for treatment *in vivo* (DRUMMOND, 1986).

Against eggs, the observed lack of connection between the increase of dosage and the increase in prevention of eclosion was also reported when eggs of *Boophilus microplus* (Canestrini) were treated with coumaphos and diazinon (DAVEY, AHRENS & GEORGE, 1989). We therefore believe that, as many of these chemicals are excellent ovicides for other arthropods (CAMPBELL & REW, 1986), another mechanism could be responsible for the lack of observed activity and this could be the poor penetration through the cuticule. However, we therefore believe that although phoxim, diazinon and coumaphos are ineffective as ovicides, they provided high enough larvae posthatch activity to warrant consideration as candidates for premises application in cleaning and disinfesting operations against *R. sanguineus* eggs.

The synthetic pyrethroids (HELLER-HAUPT, VARMA & CROOK, 1979; HELLER-HAUPT & VARMA, 1982; STENDEL, 1980; YOUNG *et al.*, 1985; YOUNG, DE CASTRO & KIZA-AURU, 1985) were extremely effective against other stages and species, but at much lower concentrations than were used in our study. We therefore believe that they should not be considered as serious candidates for control of *R. sanguineus*.

For the same reasons, ronnel also fails to offer acaricidal qualities. It was the worst of the test and our results agreed with those of DRUMMOND (1988).

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