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Effect of a spray formulation on the reproductive parameters of a susceptible population of *Rhipicephalus* (*Boophilus*) *microplus*

Efeitos de uma formulação pulverização sobre os parâmetros reprodutivos de uma população susceptível de *Rhipicephalus (Boophilus) microplus*

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Abstract

The number of studies emphasizing the possible damage that acaricidal spray formulations can cause on engorged female ticks' reproductive parameters is small. The present study evaluated the deleterious effects of a spray formulation (dichlorvos 60% + chlorpyrifos 20%) on the reproductive parameters of a susceptible population of *Rhipicephalus (B.) microplus* females, using the Stall Test. The ticks were allocated randomly to treatments according to the mean numbers of females detached from each cow on days -3, -2 and -1 and the cattle pen location. The numbers of engorged female ticks that naturally detached from the cattle were counted daily from day 1 to day 30. For each group, 20 detached engorged female ticks or the available number collected daily were evaluated regarding reproductive parameters. Associations of organophosphates demonstrated elevated acaricidal efficacy, as well as deleterious effects on the reproductive parameters of *R. (B.) microplus* females. The engorged female weight (days 1 to 7), weight of egg masses (days 5 to 10) and larval hatching percentage (days 5 to 19) were decreased ($P \leq 0.05$). It is possible that a formulation can lead to deleterious effects on *R. (B.) microplus* females when the tick population analyzed shows elevated sensitivity towards a particular formulation. However, further studies need to be conducted.

Keywords: Cattle tick, dichlorvos 60% + chlorpyrifos 20%, susceptible population, reproductive efficiency, *R. (B.) microplus*.

Resumo

É relativamente pequeno o número de estudos que enfatiza os danos que uma formulação acaricida spray pode desencadear sobre os parâmetros reprodutivos das teleóginas. O presente estudo teve como objetivo avaliar os efeitos deletérios de uma formulação spray comercial (dichlorvos 60% + Clorpirifós 20%), sobre os parâmetros reprodutivos de uma população susceptível de *R. (B.) microplus*, desprendidas de bovinos experimentalmente infestados, utilizando-se o teste de estábulo. Os animais foram alocados aos grupos de tratamentos de acordo com a contagem média de fêmeas desprendidas dos bovinos nos dias -3, -2 e -1. O número de teleóginas desprendidas foi quantificado do dia 1 ao 30. Para cada grupo, diariamente 20 fêmeas, ou a quantidade disponível, foram selecionadas e submetidas à avaliação dos parâmetros reprodutivos. A associação de organofosforados demonstrou elevada eficácia acaricida e também apresentou efeitos deletérios sob os parâmetros reprodutivos de *Rhipicephalus (B.) microplus*, diminuindo ($P \leq 0,05$) o peso das teleóginas (dos dias 1 ao 7), o peso da massa de ovos (dos dias 5 ao 10) e a eclodibilidade das larvas (dos dias 5 ao 19). Talvez uma formulação pode apresentar efeitos deletérios sobre os parâmetros reprodutivos de fêmeas de *R. (B.) microplus*, quando existe um elevado grau de sensibilidade dessa cepa de carrapato a um determinado composto. De qualquer maneira, futuros estudos devem ser realizados.

Palavras-chave: Carrapato bovino, dichlorvos 60% + chlorpyrifos 20%, população susceptível, eficiência reprodutiva, *R. (B.) microplus.*

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Introduction

Rhipicephalus (*Boophilus*) *microplus* is considered to be the most important ectoparasite in cattle industry because of the direct and indirect damage it can cause to animal health, thereby considerably limiting bovine productivity (CALLIGARIS et al., 2013; FAZA et al., 2013). It possesses a wide geographical distribution, encompassing tropical and subtropical regions situated between the parallels 32° latitude north and 35° latitude south, comprising countries in Latin America, Africa, Asia and Oceania (WHARTON, 1974).

Control over these parasites is essentially based on using chemical products (FURLONG, 2005; KLAFKE et al., 2006). Pereira et al. (2008) reported that in order to control *R. (B.) microplus,* it is necessary to remember that only 5% of the parasites are located in the host, meaning that the remaining 95% stay in the environment. Based on this idea, several studies (GONZALES et al., 1993; GEORGE & DAVEY, 2004; PEREIRA, 2009; LOPES et al., 2013) have emphasized that successful control over a tick population on a given farm is related not only to great efficacy of a certain acaricide, but also to the deleterious effects that these active agents have on tick populations in the field, especially in relation to the reproductive parameters of engorged *R. (B.) microplus* females. This effect, caused by some formulations, is called "pasture cleansing" (PEREIRA, 2009; SANTANA et al., 2013).

On the other hand, the number of studies emphasizing the possible damage that acaricidal spray formulations can cause to engorged females' reproductive parameters is relatively small. On this basis, the present study aimed to evaluate the deleterious effects of a spray formulation available on the market (Ectofós® spray, Vallée, which contains 60% dichlorvos (DDVP) + 20% chlorpyrifos 20%), in relation to the reproductive parameters of engorged *R. (B.) microplus* females, detached from experimentally infested cattle.

Materials and Methods

Location, animals and pretreatment infestations

This experiment was conducted at the Animal Health Research Center (Centro de Pesquisas em Sanidade Animal, CPPAR), at the School of Agrarian and Veterinary Sciences (FCAV), UNESP, Jaboticabal, state of São Paulo, Brazil.

Sixteen crossbred cattle, aged approximately seven months, which had not received any antiparasitic treatment for the last 90 days, were selected and identified (numbered ear tags). In this study, all the procedures using animals complied with the Ethical Principles for Animal Research adopted by the College of Animal Experimentation (COBEA) and were approved by the Ethics Committee for Animal Welfare, Institute of Animal Health Research (IPESA), Formiga, Minas Gerais, Brazil, under procedural number PP 003G1/2012. All the animals were kept in individual suspended stalls and were infested with approximately 5000 *R.* (*B.*) *microplus* larvae (0.25 grams of eggs) of mean age 14 days, on days -24, -21, -19, -17, -14, -12, -10, -7, -5, -3 and -1, taking day 0 as the treatment date (Brasil, 1997). This strain of *R. (B.) microplus* was obtained from fields at a farm in Formiga, Minas Gerais) and is now kept at CPPAR, using cattle and a BOD incubator. All procedures using infestations of animals in order to maintain colonies also complied with the Ethical Principles for Animal Research adopted by the College of Animal Experimentation (COBEA) and were approved by the Ethics Committee for Animal Welfare, IPESA, Formiga, Minas Gerais, Brazil, under procedural number PP 001B1/2011.

Counting engorged R. (B.) microplus females detached from cattle before treatment and allocating animals to treatment groups

On days -3, -2 and -1, fully engorged *R. (B.) microplus* females that detached from each animal were counted. All counts were performed daily in the mornings (between 08:00 and 09:00 am). Animals were allocated randomly to treatments in accordance with a randomized complete block design. The block formation was based on the arithmetic mean number of female ticks detached from each animal prior to treatment (days -3, -2 and -1) and the cattle pen location. The cattle were divided into eight blocks of two animals each and, within each block, animals were randomly placed in one of the treatment groups: in T01, the animals were kept as controls; and in T02, the cattle received 60% dichlorvos + 20% chlorpyrifos via spray. The blocks were assigned to sets of two nearby cattle pens, and the animals within each block were randomly allocated to cattle pens within the set. The experimental unit was the animal. The formulation was administered in accordance with the manufacturer's recommendations.

Counting engorged R. (B.) microplus females detached from each animal and post-treatment infestations

Engorged female ticks that naturally detached from the cattle were counted daily from day 1 until the end of the experiment (day 30). During the post-treatment period, all the cattle were infested with approximately 5000 viable unfed larvae twice a week (every Tuesday and Thursday of each week), up to the end of the study, as recommended by Holdsworth et al. (2006).

Assessment of the reproductive parameters of the preselected engorged female ticks

The following reproductive parameters of the engorged female ticks were analyzed: female weight, egg mass weight, percentage hatchability, percentage reduction in oviposition, percentage reduction in hatchability, reproductive efficiency and percentage control/efficacy of formulations with regard to reproductive parameters.

For this analysis, all the engorged female ticks that detached from the animals were collected every morning from day 1 until the end of the experiment (day 30), as described above. For each group, 20 detached engorged female ticks or the available number collected daily were randomly selected, weighed, fixed

on Petri plates and moved to a BOD incubator at 27 °C and approximately 85% relative humidity to stimulate oviposition. Twenty days after oviposition in the BOD incubator, the egg mass weight of the engorged female ticks was recorded for each group and post-treatment day. Subsequently, the egg mass of each group and post-treatment day was transferred to adapted 3-mL syringes and was returned to the BOD incubator at 27 °C and approximately 85% relative humidity to stimulate larval hatching. After 20 days of larval hatching in the BOD incubator, the percentage hatchability was calculated in accordance with the methodology described by Gonzales et al. (1993). The percentage hatchability of each sample group (derived from the daily sample of detached preselected engorged female ticks) was visually estimated using a stereomicroscope with an ocular grid, by comparing the proportion of larvae in relation to the proportion of unhatched eggs for each group and post-treatment day (DRUMMOND et al., 1973; GONZALES et al., 1993; GEORGE & DAVEY, 2004; HOLDSWORTH et al., 2006).

Percentage efficacy

Reduction in the number of engorged females counted

The acaricidal efficacy of the formulation was calculated using arithmetic means from the counts of engorged female ticks that detached from the animals of each group. The data collected were grouped into three-day intervals.

$$\text{Percent efficacy} = \left[1 - \frac{Ta \times Cb}{Tb \times Ca}\right] \times 100 \quad (1)$$

where Ta = average number of engorged female ticks counted on treated animals post-medication;
Tb = average number of engorged female ticks counted on treated animals during the 3 days prior to treatment;
Ca = average number of engorged female ticks counted on control animals after the experiment began;
Cb = average number of engorged female ticks counted on control animals during the 3 days prior to treatment.

Reproductive parameters of pre-selected engorged female ticks

To assess the impact of treatment on the reduction of oviposition and hatching, the following equations were used:

$$\textbf{\% reduction in oviposition} = \frac{\text{average egg mass weight of control group} - \text{average egg mass weight of treated group}}{\text{average egg mass weight of control group}} \times 100 \quad (2)$$

$$\textbf{\% reduction in hatching} = \frac{\text{average hatchability of control group} - \text{average hatchability of treated group}}{\text{average hatchability of control group}} \times 100 \quad (3)$$

To estimate the extent of reproduction and percentage of control/efficacy, the following equations were used:

$$\textbf{Estimate of reproduction (ER)} = \frac{\text{egg weight (g)}}{\text{female weight (g)}} \times \%\ \text{hatching} \times 20{,}000^{1} \quad (4)$$

$$\textbf{\% control or efficacy} = \frac{\text{ER of control group} - \text{ER of treated group}}{\text{ER of control group}} \times 100 \quad (5)$$

Data analysis

The data pertaining to the reproductive parameters of the naturally detached and pre-selected engorged female ticks from the experimental groups (engorged female weight, egg mass weight and percentage hatchability) were analyzed using a completely randomized design. The differences between mean values were compared using Tukey's test at a confidence level of 95%.

For the raw counts on all the detached engorged female ticks, the data were grouped into three-day intervals and log transformed [ln(tick count + 1)]. A generalized linear mixed model for repetitive sampling was applied using SAS (SAS Institute, 1996) to assess the fixed effects of the treatment, the tick counts, the interaction between treatment and counts, the random block effects and the random residual effects.

Results and Discussion

From analysis on the results from the statistical analysis, grouped at three-day intervals, on the reproductive parameters of the *R. (B.) microplus* females that were exposed to whole-body spraying of the dichlorvos + chlorpyrifos combination, it could be seen that the fully engorged females that detached and were preselected from animals that received the aforementioned formulation were statistically lighter ($P \leq 0.05$) than the female ticks of the control group, from the 1st to the 7th day post-treatment (DPT) (Table 1).

The females in the group treated with the organophosphate association (dichlorvos + chlorpyrifos) presented a deleterious effect regarding egg mass weight, such that the egg mass was lighter than that of the control group between the 5th and 10th DPT (Table 1). The action of dichlorvos + chlorpyrifos producing lower-weight female ticks and egg masses may have diminished the hatchability values of the treated group, which were lower than the values obtained for the control group from the 5th to the 19th DPT (Table 1).

Table 2 shows the grouped results from the 30 post-treatment days. Based on data from this table, it can be seen that the dichlorvos + chlorpyrifos combination led to deleterious effects on the reproductive parameters of the detached and pre-selected female ticks (egg mass weight and hatching percentage) after the respective treatment, in comparison with *R. (B.) microplus* females detached from the control group.

The acaricidal efficacy of the dichlorvos + chlorpyrifos combination, grouped in intervals of three days, reached levels superior to 95% (arithmetic means) starting right between the 1st and 4th DPT. The maximum efficacy of 99.31% was reached by this formulation between the 5th and 7th DPT. Efficacy values

[1] Constant corresponding to an estimate of the number of larvae contained in 1 g of eggs.

Table 1. Results of multiple comparisons of the reproductive parameters of engorged *Rhipicephalus (Boophilus) microplus* females naturally detached and pre-selected from control and treated cattle, CPPAR , Jaboticabal, São Paulo State, Brazil.

Experimental Period	Reproductive Parameter	Experimental Groups/Means and Standard Deviation[1]		Variance Analysis	
		Control	Dichlorvos 60% + Chlorpyrifos 20%*	Value of F	Pr < F
1 to 4	Total number of engorged female	20.0 A	20.0 A	1.74	0.2201
5 to 7		20.0 A	5.0 B	7.54	0.0226
8 to 10		20.0 A	20.0 A	0.05	0.8310
11 to 13		20.0 A	20.0 A	0.00	1,0000
14 to 16		20.0 A	20.0 A	0.43	0.5264
17 to 19		20.0 A	10.0 B	4.35	0.0465
20 to 22		20.0 A	18.0 A	2.04	0.1871
23 to 25		20.0 A	20.0 A	0.00	1.0000
26 to 28		20.0 A	20.0 A	0.00	1.0000
29 to 30		20.0 A	20.0 A	0.00	1.0000
Value of F		**0.00**	**2.00**		
Pr < F		**1.0000**	**0.1582**		
1 to 4	Engorged female weight (g)	4.3 ± 0.2 A	2.2 ± 0.2 B	3.2	0.0410
5 to 7		5.2 ± 0.1 A	1.3 ± 0.0 B	8.53	0.0170
8 to 10		4.1 ± 0.4 A	4.3 ± 0.2 A	0.02	0.8907
11 to 13		4.6 ± 0.7 A	4.5 ± 0.9 A	0.00	0.9635
14 to 16		4.8 ± 0.5 A	4.1 ± 0.7 A	0.37	0.5555
17 to 19		4.5 ± 0.2 A	3.1 ± 1.2 A	1.07	0.3271
20 to 22		4.8 ± 0.1 A	3.3 ± 0.8 A	2.27	0.1660
23 to 25		4.6 ± 0.2 A	4.8 ± 0.2 A	0.02	0.8907
26 to 28		4.7 ± 0.3 A	5.2 ± 0.1 A	0.00	0.9635
29 to 30		5.0 ± 0.3 A	5.1 ± 0.3 A	0.37	0.5555
Value of F		**0.24**	**2.22**		
Pr < F		**0.9779**	**0.1248**		
1 to 4	Egg mass weight (g)	0.7 ± 0.2 A	0.7 ± 0.1 A	0.00	1.0000
5 to 7		1.4 ± 0.2 A	0.2 ± 0.1 B	4.16	0.0317
8 to 10		1.9 ± 0.2 A	1.0 ± 0.0 B	1.4	0.0377
11 to 13		1.8 ± 0.2 A	0.6 ± 0.2 A	1.95	0.1961
14 to 16		1.5 ± 0.3 A	0.6 ± 0.2 A	2.6	0.1416
17 to 19		1.9 ± 0.2 A	0.6 ± 0.1 A	3.74	0.0852
20 to 22		1.4 ± 0.4 A	0.7 ± 0.2 A	1.4	0.2677
23 to 25		1.3 ± 0.4 A	1.7 ± 0.5 A	0.42	0.5354
26 to 28		2.1 ± 0.1 A	2.2 ± 0.1 A	0.18	0.6776
29 to 30		1.9 ± 0.0 A	2.0 ± 0.3 A	0.00	1.0000
Value of F		**1.10**	**3.05**		
Pr < F		**0.4460**	**0.0560**		
1 to 4	Hatching percentage (%)	85.7 ± 4.2 A	66.7 ± 8.9 A	0.48	0.5076
5 to 7		97.3 ± 1.2 A	22.5 ± 2.1 B	7.39	0.0237
8 to 10		98.0 ± 2.0 A	42.0 ± 5.6 B	4.14	0.0425
11 to 13		93.7 ± 4.2 A	41.7 ± 2.8 B	1.35	0.0451
14 to 16		94.7 ± 4.0 A	32.0 ± 1.4 B	5.18	0.0489
17 to 19		92.0 ± 8.2 A	36.0 ± 5.6 B	4.14	0.0425
20 to 22		81.7 ± 1.8 A	60.3 ± 9.8 A	0.6	0.4584
23 to 25		84.0 ± 3.4 A	73.3 ± 5.7 A	0.15	0.7075
26 to 28		94.3 ± 1.5 A	96.7 ± 3.2 A	0.01	0.9343
29 to 30		93.5 ± 2.5 A	93.0 ± 3.0 A	0.00	0.9859
Value of F		**0.09**	**1.68**		
Pr < F		**0.9994**	**0.2264**		

1: Means followed by the same letter in the line do not differ (P>0.05); * Commercial formulation purchased in the local market.

superior to 95% lasted until the 22nd DPT. Between days 23 and 25 post-treatment, the efficacy decreased to 93.53%. These values continued to decrease between the 26th and 28th DPT (11.97%) and the 29th and 31st DPT (0.00%), when the trial was concluded (Table 3).

The formulation containing 60% dichlorvos + 20% chlorpyrifos, administered as a whole-body spray, demonstrated elevated acaricidal efficacy and also presented deleterious effects on the reproductive parameters of *R. (B.) microplus* females, significantly diminishing ($P \leq 0.05$) the number of fully engorged female ticks collected (between the 5th and 7th and the 17th and 19th DPT), the weight of these females (between the 1st and 7th DPT), the weight of egg masses obtained (from day 5 until day 10 post-treatment) and the larval hatchability (from the 5th to the 19th DPT). The absence of studies conducted with the same active agents used on the present study, regarding the effects of these agents on the reproductive parameters of fully engorged *R. (B.) microplus* females, makes it impossible to make comparisons with the results obtained. The majority of the studies that have been conducted using spray formulations aimed to detect the resistance of *R. (B.) microplus* towards these compounds using *in vitro* methodologies, such as the Adult Immersion Test (AIT), Larval Packet Test (LPT) or Larval Immersion Test (LIT). Of these, the second test is recognized by the Food and Agriculture Organization (FAO) as a standard for evaluating the efficacy or resistance (CASTRO et al., 1997; FAO, 2003; ALONSO-DIÁZ et al., 2006; LOVIS et al., 2013; LOPES et al., 2014).

The first studies evaluating the reproductive parameters of female fully engorged ticks, detached from experimentally infested animals that had been treated with macrocyclic lactones, were conducted by Gonzales et al. (1993) and George & Davey (2004), using doramectin administered as a pour-on (500 mcg/kg) and subcutaneously (200 mcg/kg). Pereira (2009) evaluated the effects of ivermectin, abamectin and doramectin, all administered subcutaneously at a dosage of 200 mcg/kg, on the average and the reduction in oviposition among engorged females collected from experimentally infested animals that were kept in fields. More recently, Lopes et al. (2013) evaluated the effect of ivermectin,

Table 2. Results of multiple comparisons of the reproductive parameters of engorged *Rhipicephalus (Boophilus) microplus* females naturally detached and pre-selected from control and treated cattle during the 30-day post-treatment period, CPPAR , Jaboticabal, São Paulo State, Brazil.

Parameter	Mean value*/Experimental groups		Test F	
	Control	Dichlorvos 60% + Chlorpyrifos 20%**	Value	Significance probability
Nº. of engorged female	20.00 A*	17.30 A	6.78	0.5662
Engorged female weigth (g)	5.2 A	4.0 A	3.12	0.0532
Egg mass weight (g)	1.6 A	1.1 B	0.69	0.0304
Hatchability (%)	91.5 A	58.4 B	4.33	0.0311
Reduction in oviposition (%)	-	31.3	Not applicable	
Reduction in hatchability (%)	-	36.2	Not applicable	
Reproductive efficiency	632115.00	318572.00	Not applicable	
Efficacy (%)	-	49.6	Not applicable	

* Means followed by the same letter in the line do not differ (P>0.05); ** Commercial formulation purchased in the local market.

Table 3. Average number of engorged *Rhipicephalus (Boophilus) microplus* females naturally detached from control and treated cattle and percent efficacy (arithmetic means), CPPAR , Jaboticabal, São Paulo State, Brazil.

Experimental Period	Experimental Groups/Means and Standard Deviation[1]		Efficacy (%)	Variance Analysis	
	T01: control	T02: 60% DDVP + 20% Clorpirifós*	T02	Value of F	Pr < F
0	40.17 A	40.67 A	-	0.00	0.9878
1 to 4	39.61 A	1.94 B	95.15	104.03	<0.0001
5 to 7	39.56 A	0.28 B	99.31	150.27	<0.0001
8 to 10	39.44 A	1.27 B	95.83	95.39	<0.0001
11 to 13	39.61 A	2.44 B	93.90	72.23	<0.0001
14 to 16	40.89 A	1.33 B	96.78	98.41	<0.0001
17 to 19	43.17 A	0.61 B	98.60	135.19	<0.0001
20 to 22	45.28 A	1.00 B	97.82	134.60	<0.0001
23 to 25	47.39 A	3.11 B	93.52	80.80	<0.0001
26 to 28	61.83 A	55.11 A	11.97	0.860	0.3562
29 to 30	39.42 A	39.42 A	0.00	0.010	0.9274
Value de F	**0.28**	**48.71**			
Pr < F	**0.9848**	**<0.0001**			

1: Means followed by the same letter in the line do not differ (P>0.05); * Commercial formulation purchased in the local market.

abamectin, doramectin and moxidectin on the reproductive parameters of fully engorged females of *R. (B.) microplus*.

For doramectin (700 mcg/kg), the results found by Lopes et al. (2013), regarding the reduction in oviposition among female ticks exposed to this formulation (62.17%), were slightly inferior to those obtained by Gonzales et al. (1993) (99.9%), George & Davey (2004) (91.43%) and Pereira (2009) (83.51%), all of whom used doramectin administered as a pour-on (500 mcg/kg) or subcutaneously (200 mcg/kg), respectively. For abamectin administered subcutaneously (200 mcg/kg), Pereira (2009) found that this formulation provided a percentage reduction of 37.11% in the oviposition among engorged females exposed to this active agent. These results were similar to those obtained by Lopes et al. (2013), who used abamectin as a pour-on (500 mcg/kg). Regarding ivermectin, Pereira (2009) observed a 63.92% reduction in oviposition among ticks exposed to 200 mcg/kg ivermectin, while Lopes et al. (2013), evaluating the same agent at a dosage of 630 mcg/kg, attained a 46.31% reduction in oviposition among engorged *R. (B.) microplus* females exposed to ivermectin. These authors explained that this difference may be related to the degree of resistance of different tick strains against ivermectin. However, it is important to emphasize that the average acaricidal efficacy values obtained from ivermectin during the whole experimental period were similar to those in the studies conducted by Lopes et al. (2013), in which 630 mcg/kg ivermectin reached 77.88% efficacy, and by Pereira (2009), in which 200 mcg/kg ivermectin reached an efficacy of 72.70%.

Among all the macrocyclic lactones evaluated (abamectin, ivermectin, doramectin and moxidectin), Lopes et al. (2013) found that doramectin and moxidectin were the ones that presented superior acaricidal efficacy and deleterious effects over the reproductive parameters of fully engorged *R. (B.) microplus* females. These authors mentioned that the differences in effect can be explained by variations between the formulations, caused by absorption of the active agent, which is subject to interference from the quality of each molecule and/or components of the vehicles used (TOUTAIN et al., 1997; AGUILAR-TIPACAMÚ & RODRÍGUEZ-VIVAS, 2002). Furthermore, few commercial products based on doramectin and moxidectin are yet available on the Brazilian veterinary market (SINDAN, 2008: Compendium of Veterinary Products).

Martins et al. (1995) studied the effect of fluazuron on the reproductive performance of *R. (B.) microplus*. Using different fluazuron concentrations (1 mg/kg and 2 mg/kg), these authors found that there was either no oviposition among the engorged females on days 7-11 or it occurred at very low percentages (< 15%). In another study, Mendonça (2010) evaluated a formulation of 3.0 mg/kg of fluazuron + 0.5 mg/kg of abamectin on a different strain of *R. (B.) microplus*. They observed that this compound had deleterious effects on the reproductive parameters of fully engorged *R. (B.) microplus* females, showing 100% efficacy on several post-treatment days. Despite the results obtained, Mendonça (2010) emphasized that because of the high sensitivity of the *R. (B.) microplus* strain that was evaluated, it was not possible to collect a minimum of 10 fully engorged females detached from treated animals (3.0 mg/kg of fluazuron + 0.5 mg/kg of abamectin) on some post-treatment days. This interfered directly with the total number of preselected females, the weights of these females and the weights of the egg masses during this period. Similar results were obtained with different compound containing fluazuron by Cruz et al. (2014). According to these authors, a formulation can lead to deleterious effects on *R. (B.) microplus* females if this compound reaches elevated percentages (above 95-99%). In other words, this can occur when the *R. (B.) microplus* strain analyzed presents elevated sensitivity. The results from the 60% dichlorvos + 20% chlorpyrifos combination in this study can possibly be correlated with these authors' assertion. However, further studies need to be conducted with the objective of verifying this hypothesis.

Conclusions

Based on the data and results from the present study, it is possible to conclude that this association of organophosphates (60% dichlorvos + 20% chlorpyrifos), administered to animals as a whole-body spray, demonstrated elevated acaricidal efficacy, as well as deleterious effects on the reproductive parameters of *R. (B.) microplus* females, thereby significantly decreasing ($P \leq 0.05$) the number of ticks collected (from the 5th to the 7th and from the 17th to the 19th DPT), the weight of egg masses (between days 5 and 10 post-treatment) and the larval hatching percentage (between the 5th and the 19th DPT). The results from this combination of 60% dichlorvos + 20% chlorpyrifos can possibly be correlated with the high sensitivity of the strain used. However, further studies need to be conducted with the objective of verifying this hypothesis.

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