

Acaricides efficiency on *Rhipicephalus (Boophilus) microplus* from Bahia state North-Central region

Eficiência de acaricidas sobre *Rhipicephalus (Boophilus) microplus* na região Centro-Norte, Bahia

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Abstract

The cattle tick *Rhipicephalus (Boophilus) microplus* is responsible for major losses in the Brazilian livestock, mainly due to reduction in cattle productive performance. Resistance development to major classes of acaricide widely used nowadays has been extensively reported, as well as the occurrence of residues from these compounds in animal products and the environment. This study aimed to evaluate the efficiency of acaricides on *R. (B.) microplus* collected from rural properties in the North-Central region of Bahia State. Ticks were collected in several cattle farms in the cited region, and an *in vitro* acaricide efficiency assay was performed based on the immersion of ticks in acaricide solutions made according to manufacturers' recommendations. The results obtained in the experiments indicated varying degrees of efficiency of the several bases studied, with the products benzofenilurea, macrocyclic lactone and fipronil showing the highest levels of efficiency, 100%, 100% and 97.34%, respectively. It was possible to conclude that, for the region studied, there are different degrees of commercial acaricides efficiency, and many present less than 95% effectiveness, value determined as acceptable by the Brazilian legislation.

Keywords: Ticks, cattle, drug resistance, acaricide, Brazilian Northeast.

Resumo

O carrapato *Rhipicephalus (Boophilus) microplus* é responsável por grandes perdas na pecuária brasileira, principalmente devido à redução no desempenho produtivo dos animais. A resistência do parasita a diferentes classes de acaricidas tem sido amplamente relatada, bem como os problemas de ocorrência de resíduos desses compostos em produtos de origem animal e no ambiente. Esse estudo teve como objetivo avaliar a eficiência de diversos acaricidas sobre populações de *R. (B.) microplus* provenientes de propriedades rurais da Região Centro-Norte do Estado da Bahia, Brasil. Teleóginas ingurgitadas foram colhidas em diferentes propriedades da região e submetidas a ensaio *in vitro* de eficiência de acaricidas, baseado na imersão dos carrapatos em soluções de acaricidas feitas seguindo as recomendações dos fabricantes. Os resultados obtidos indicam níveis variados de eficiência das bases estudadas, com os acaricidas benzofenilureia, lactona macrocíclica e fipronil apresentando os índices mais altos de eficiência, 100%, 100% e 97.34%, respectivamente. Pode-se concluir que, para a região estudada, os acaricidas apresentam níveis diversos de eficiência, e muitos deles possuem eficácia de menos de 95%, como recomendado pela legislação brasileira.

Palavras-chave: Carrapatos, bovinos, resistência, carrapaticidas, Nordeste.

Introduction

Several damages caused by parasites in cattle herd lead to drop in production in tropical and subtropical countries (PATARROYO et al., 2002; PEREIRA, 2006). The tick *R. (B.) microplus* (Canestrini, 1887) (Acari: Ixodidae) is a major ectoparasite of cattle, causing many problems in national livestock,

which causes efficiency and productivity losses in these animals, besides being vectors of pathogens causing various diseases (BROWN et al., 2006; JONSSON, 2006; JONSSON et al., 2008).

In Brazil, parasite-related losses are estimated at about 1 billion dollars per year, resulted from the direct action of the parasite and the cost of control systems, the latter representing annual expenditures of approximately US\$ 800 million in chemicals to combat parasites (FURLONG et al., 2004). The Brazilian cattle herd is composed of taurine, zebu and crossbred races, each one presenting different

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susceptibilities to the development of ticks' parasitic stages, and there are climatic conditions favorable to the survival and development of its non-parasitic stages (CORDOVÉS, 1997).

Chemical control of ticks has been characterized by continuous increase of resistance, with consequent increase in the frequency of acaricides application and the presence of residues of these products in milk and meat (VARGAS et al., 2003; CAMPOS JUNIOR; OLIVEIRA, 2005; MENDES, 2005). A chemical base tick must present more than 95% efficacy on a sensitive strain of *R. (B.) microplus* to be licensed by the Brazilian government (BRASIL, 1987). Thus, tick control strategies are considered as being a combination of prudent and rational use of the chemical bases currently available and the frequency of its application, leading to the maintenance of parasite populations below their economic damage threshold, and with a minimal environmental impact (FAO, 2003).

In the North-Central region of Bahia State, there is no data record demonstrating acaricides efficacy for *R. (B.) microplus* control. The detection of the resistance of ticks is essential to choose a proven effective product for the control of ectoparasites. Obtaining a regional profile of the problem of resistance to acaricides could serve as background for the planning of parasite control measures. Based on the assumption that there are populations of *R. (B.) microplus* in the North-Central region of Bahia State presenting varying levels of resistance against the acaricides available in the market, this study aimed to evaluate the in vitro efficacy of various drugs on *R. (B.) microplus*.

Materials and Methods

Engorged *R. (B.) microplus* females, identified as suggested by Aragão and Fonseca (1961), with length equal to or greater than 4 mm, were collected directly from several body parts of naturally infested cattle of various breeds and blood levels, in rural properties in the North-Central region of Bahia State. These procedures were conducted forty-five days after the last acaricide treatment. The Figure 1 depicts the North-Central region of Bahia State and the municipalities studied, which were chosen because they presented the largest cattle herds in the region (IBGE, 2009). Three hundred ticks were collected in each property at two different moments. Ten groups of twenty ticks each were separated, with the size and

weight of the gravid females observed for this separation to make it as equitable as possible.

After that, the tick immersion assay (PATARROYO et al., 2002) was performed with slight modifications. Briefly, each group of twenty ticks was wrapped in surgical gauze and immersed in acaricide solution. The nine pharmacological bases used and their concentrations are listed in Table 1. All dilutions were made in accordance with the manufacturers' recommendations, with final volume of 100 mL for each group, using distilled water as control. A positive control of the fipronil solution was prepared by diluting 0.031 g of Regent 800 WG (BASE, California) in 40 mL acetone and 60 mL of distilled water, obtaining a final concentration of 25 µg/mL; also, a negative control was made for this group, where ticks were immersed in a solution of 40 mL acetone and 60 mL of distilled water. The neem extract was composed by a commercial solution of neem plant (*Azadirachta indica* – Meliaceae Family) extract at a 1.2% concentration. After five minutes of immersion, ticks were dried with absorbent paper and individually placed in 24 well plates (TPP), and incubated in a BOD chamber at 27 °C and 80% humidity for a 15-day period. After that, the egg mass of each female was weighted. Each oviposition was replaced at the BOD chamber (27 °C and 80% humidity) in test tubes sealed with hydrophilic cotton and incubated for another 30 days, the eggshells were then weighted to predict the larvae weight (DE LA FUENTE, 1995).

The efficiency of acaricides was calculated as described by Patarroyo et al. (2002), taking into account the weight of eggs and larvae of the experimental groups compared to the control group, as described next:

$$\text{Reduced Oviposition (RO)} = \left(1 - \left(\frac{\text{oviposition weight of the test}}{\text{oviposition weight control}} \right) \right) \times 100$$

$$\text{Reduced Efficiency (RE)} = \left(1 - \left(\frac{\text{larval weight of the test}}{\text{larval weight control}} \right) \right) \times 100$$

$$\text{Final Efficiency} = (\text{RO} \times \text{RE}) \times 100$$

Statistical analyses were performed using SPSS v-18 software. The differences between the efficiencies of each acaricide tested and the control group were identified by variance analysis (ANOVA) with subsequent application of the Tukey test, with significance set at $p < 0.05$.

Table 1. Acaricides used in the experiment, with their respective chemical groups, and the work concentrations recommended by the manufacturers, which were used in this study.

Acaricide	Group	Concentration used
Cypermethrin	Pyrethroid	0.15 mg/mL
Deltamethrin	Pyrethroid	0.5 mg/mL
Amitraz	Amitrazin	0.25 mg/mL
Dichlorvos and Clorpirifos	Organophosphate	1.5 mg/mL and 0.5 mg/mL
Dichlorvos and Cypermethrin	Organophosphate and Pyrethroid	1.25 mg/mL and 0.12 mg/mL
Fluazuron	Benzofenilureas	25 mg/mL
Ivermectin	Avermectin	5 mg/mL
Fipronil	Fenilpirazoles	0.25 µg/mL
Extract of the neem plant (extracted from <i>A indica</i> – Meliaceae Family – 1.2%)	Tetranortriterpenoids	1:200

Results

The average acaricides efficiency recorded in the municipalities in the North-Central region of Bahia is described in Table 2. Cypermethrin, deltamethrin, amitraz, dichlorvos and chlorpyrifos association, dichlorvos and cypermethrin association and neem plant (*A. indica*) extract showed efficiency equal to zero in some sampled counties, and below the values determined as acceptable by the Brazilian legislation in others. Ivermectin, fipronil and fluazuron showed better results, sometimes higher than 96.14% efficiency, in most of the counties recorded.

The efficiencies of the tested pharmacological bases in a specific property in the municipality of Miguel Calmon are shown in Table 3, where it is possible to observe low efficacy of acaricides, also revealing resistance to fipronil.

Figure 2 shows the average efficiency values obtained in all the municipalities studied in the North-Central region of Bahia State.

It shows that fipronil, ivermectin and fluazuron reached the highest levels of efficiency across the region, with no statistical difference between them in the statistical test (Tukey), with $p < 0.001$. Amitraz appeared as the basis that presented intermediate levels of performance, different from control (0%) with $p < 0.05$. None of the other acaricides (cypermethrin, deltamethrin, dichlorvos and chlorpyrifos, dichlorvos and cypermethrin and neem extract) showed statistical differences, when compared to the control group.

Discussion

The North-Central region of Bahia State has a 1,642,583 cattle herd, and the number of animals in the studied municipalities corresponds to 221,631 heads (IBGE, 2009), equivalent to 13.49% of the cattle herd in the region. These data demonstrate that the above-cited region is of great importance in the cattle breeding scenario of the State of Bahia.

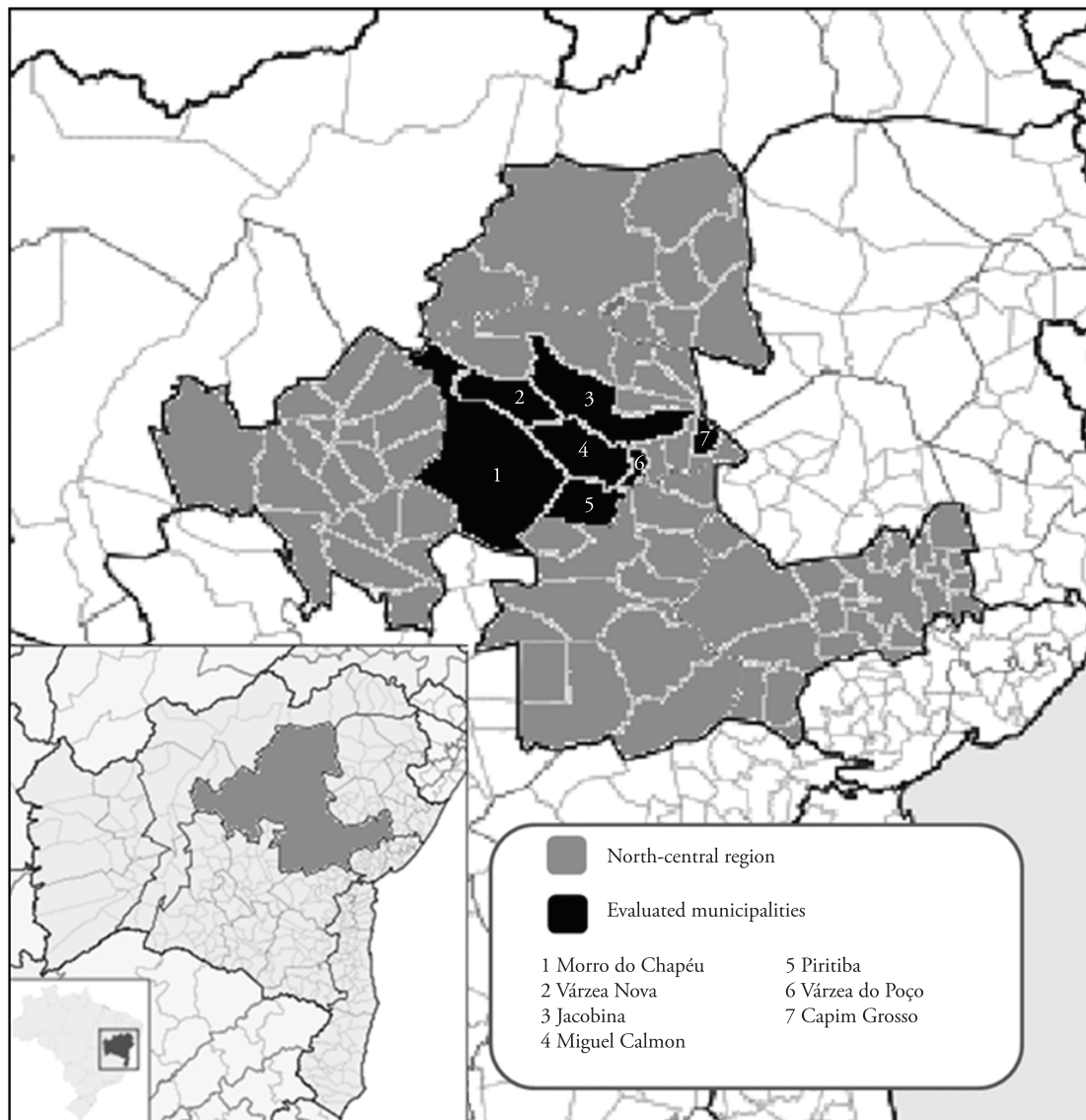


Figure 1. Sampled area in the North-Central region of Bahia State. The studied municipalities are detached in bold.

Table 2. Efficiency levels of several acaricides on *R. (B.) microplus* from the North-Central region of Bahia State, Brazil. Results are expressed in percentage (%), followed by the confidence interval (5%).

Acaricide	Municipalities						
	Miguel Calmon	Jacobina	Piritiba	Capim Grosso	Várzea do Poço	Morro do Chapéu	Várzea Nova
Cypermethrin	30.49 (28.97-32.02)	28.10 (26.70-29.51)	0	19.18 (18.22-20.14)	0	19.49 (18.52-20.47)	10.99 (10.44-11.53)
Deltamethrin	22.57 (21.44-23.69)	4.83 (39.73-43.92)	0	21.45 (20.38-22.52)	0.07 (0.07-0.07)	26.15 (24.84-27.45)	14.82 (14.08-15.56)
Amitraz	41.33 (39.26-43.39)	31.17 (29.61-32.73)	59.53 (56.55-62.50)	40.00 (38.00-42.00)	11.02 (10.47-11.57)	36.15 (34.34-37.96)	1.66 (1.57-1.74)
Dichlorvos and Clorpirifos	51.20 (48.64-53.76)	55.15 (52.39-57.90)	0	48.18 (45.77-50.59)	63.46 (60.29-66.64)	43.99 (41.79-46.19)	50.17 (47.66-52.68)
Dichlorvos and Cypermethrin	18.34 (17.42-19.26)	29.87 (28.38-31.37)	0	38.08 (36.18-39.99)	9.79 (9.30-10.28)	37.62 (35.74-39.50)	3.57 (3.39-3.75)
Fluazuron	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)
Ivermectin	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)	100.00 (95.00-100)
Fipronil	96.14 (91.33-100)	98.09 (93.19-100)	100.00 (95.00-100)	100.00 (95.00-100)	99.48 (94.51-100)	99.22 (94.26-100)	97.99 (93.09-100)
Extract of the neem plant	6.49 (96.16-96.81)	25.97 (24.68-27.27)	0	22.84 (21.70-23.99)	8.57 (8.14-9.00)	22.85 (21.71-23.99)	12.69 (12.05-13.32)

Table 3. Acaricides efficiency levels on *R. (B.) microplus* from a private property in the municipality of Miguel Calmon, Bahia State. Results are expressed in percentage (%), followed by the confidence interval (5%).

Acaricides	efficiency of the Acaricide (%)
Cypermethrin	0.67 (0.63-0.70)
Deltamethrin	1.35 (1.28-1.42)
Amitraz	15.80 (15.01-16.59)
Dichlorvos and Clorpirifos	22.15 (21.04-23.26)
Dichlorvos and Cypermethrin	2.82 (2.68-2.96)
Fluazuron	100 (95.00-100)
Ivermectin	100 (95.00-100)
Fipronil	86.54 (82.22-90.87)
Extract of the neem plant	2.83 (2.69-2.97)

Ours results showed it is possible to observe a variation of resistance to acaricides in each evaluated municipality, which is justified on the basis of the ways and times of various applications; random use of chemical bases; and inappropriate acaricide management. This can consequently select ticks that naturally possess the resistance gene for to the pharmacological product, and such feature is transmitted to future generations.

In other studies, cypermethrin showed an efficacy ranging from 41.27% to 64.16% in the municipality of Ibiúna (São Paulo State) (MENDES et al., 2007) and, also in São Paulo, it was showed that 82.6% and 86.36% of the *R. (B.) microplus* population showed resistance to cypermethrin and deltamethrin (MENDES et al., 2011), respectively. In the State of Bahia, in the municipalities of Ilhéus and Itamaraju, it was found that deltamethrin showed

65.04% (CAMPOS JUNIOR; OLIVEIRA, 2005) and 33.90% (SPAGNOL et al., 2010) effectiveness, respectively. In a study conducted in the Vale do Paraíba, State of Sao Paulo, it was demonstrated that deltamethrin treatment presented efficiency of about 28.24% (PEREIRA, 2006). These data show that the resistance of *R. (B.) microplus* to two pyrethroids in the present study can vary in different parts of the country, and the treatment with these bases may be ineffective for the control of parasitic tick populations in different localities.

In already published studies with amitrazin conducted in other Brazilian states, such as Pernambuco (FAUSTINO et al., 1995) and Rio Grande do Sul (VIEIRA et al., 1998); it was found efficacy rates of 81.94% and 91.50%, respectively. In Bahia State, 49% efficiency (SPAGNOL et al., 2010) was found in the municipality of Itamaraju. Although this study demonstrated low efficiency of the amitrazin acaricide, it still presents good efficiency in other states and regions of Brazil, so it should still be considered as an option in tick control. In this way, chemical treatments should be preceded by a tick test.

Tests of efficiency with the association of two organophosphates presented variable effectiveness levels. Mendes et al. (2007) observed efficiencies ranging from 85.31% to 100% in Ibiúna, in the State of Sao Paulo, and 81.4% efficacy (SPAGNOL et al., 2010) was found in Itamaraju, State of Bahia. In the Brazilian State of Minas Gerais, studies found a general efficacy of 96.63% (ARANTES et al., 1995) and 76.7% (DAHER et al., 2012). In the State of Sao Paulo, Coumaphos efficacy ranged from 57.01% to 70.28% (MENDES et al., 2001). In this study, low levels of efficacy were observed for the combination of the two

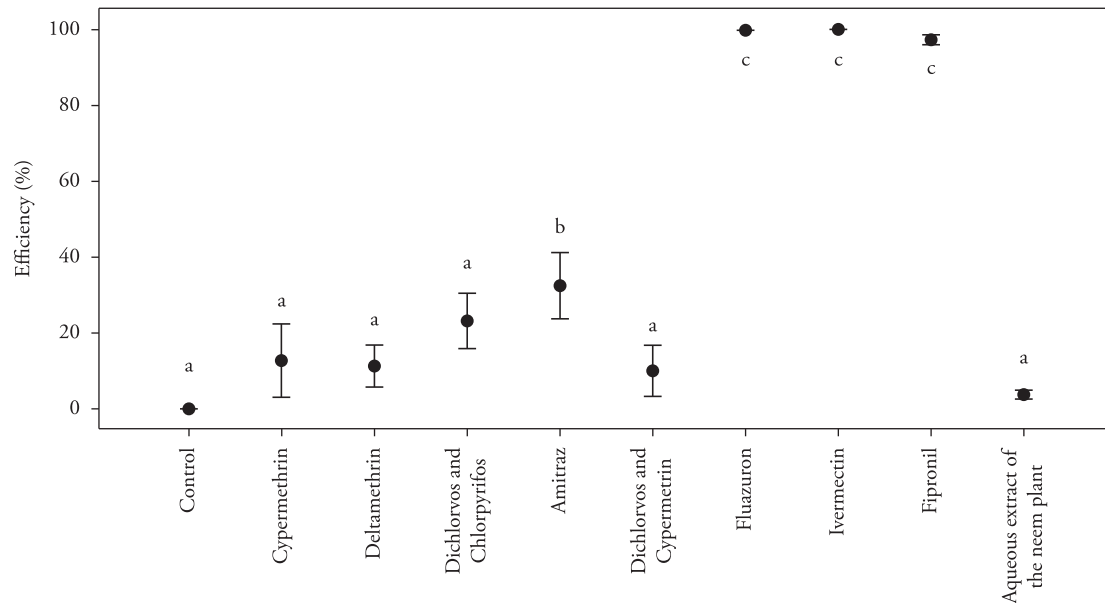


Figure 2. Average efficiency of acaricides on *R. (B.) microplus* from the North-Central region of Bahia State, Brazil. Results express the average efficiency obtained from all samples collected in the region after each *in vitro* acaricide treatment, and bars indicate the standard errors. Different superscript letters indicate statistically significant difference among the efficiency rates, by the ANOVA test, followed by Tukey test. Between a and b – $p < 0.05$; between c, and a and b – $p < 0.001$.

organophosphates, which can be caused due to inappropriate and continuous use of these bases in the studied region.

One option for the control of the *R. (B.) microplus* tick is the association of two different pharmacological bases, and this presentation is largely found at the commercial level. The association evaluated in this test involved an organophosphate and a pyrethroid. Some published reports concerning the efficacy of this type of association can be found, as it could be seen that in Bahia State, municipality of Ilhéus, the efficacy was of about 75.73% (CAMPOS JUNIOR; OLIVEIRA, 2005) and efficiency ranged from 40.38% to 76.81% in Ibiúna, State of Sao Paulo (MENDES et al., 2007). In addition, in the present study, this association was not able to produce good levels of efficiency.

In this study, fluzaron presented high levels of efficiency. No reports were found regarding the resistance of *R. (B.) microplus* to the fluzaron pharmacological basis, but attention must be paid to the fact that if any product is not applied to the animals in an appropriate way and at a suitable time, the resistance will tend to appear.

Regarding ivermectin, reports described resistance in Mexico (PEREZ-COGOLLO et al., 2010) and the field experiment reported by Martins and Furlong (2001) discovered a strain resistant to avermectins in the State of Rio Grande do Sul; also, this same situation was already described in reports in the states of São Paulo and Mato Grosso do Sul (CASTRO-JANER et al., 2010b).

In this study, fipronil showed high degree of efficiency, being fully considered within the parameters recommended by the Brazilian Ministry of Agriculture. In Uruguay (CASTRO-JANER et al., 2010a), in the Brazilian states of Rio Grande do Sul, Mato Grosso do Sul and São Paulo, the existence of fipronil resistant strains (CASTRO-JANER et al., 2010b) was also shown. This situation points to the fact that this drug must be carefully used by Bahia

State herd owners, since the inadequate application can lead to the development of resistant ticks.

The extract of the neem plant did not show appropriate results in this study; other reports have shown 30.0% efficacy in the semi-arid region of Paraíba State (SILVA et al., 2007). In India, it was found 31.2% to 76.1% effectiveness against *R. (B.) microplus* (SRIVASTAVA et al., 2008; MAGADUM et al., 2009). The biochemical effects of this extract over the cattle tick remains unclear, but Habluetzel et al. (2007) found that the extract produced a reduction in the survival of the larvae and adults and also interfered with oviposition and oogenesis, behavior, dietary process, and growth of arthropods (SCHMUTTERER, 1990; MORDUE; NISBET, 2000; BROGLIO-MICHELETTI et al., 2009, 2010).

The results reported herein can serve as a basis for direct tests of acaricide resistance in the region, and also to guide farmers on what type of base to use in their region. Even so, several bases presented different degrees of effectiveness depending on the municipality, and should be considered and added in efficacy trials in each city or production unit to better understand the type of control to be conducted, and also to base the decision about the cost-benefit relationship. Moreover, it is also necessary to consider the indices of relative humidity, rainfall and temperature in the region studied, as a basis for drawing a map, which will advise on what would be the best time for the application of high efficiency bases, and also guide on the correct application frequency of these drugs. With these actions, a decrease in the number of applications would be expected, as well as a reduction in personnel expenses, and losses in animal performance. It should be also considered the production of milk and meat without or with less chemical waste, reduced environmental impact and lower risk of animals and employees poisoning.

Conclusions

Rhipicephalus (Boophilus) microplus from the Bahia State North-Central region presents a high degree of resistance to organophosphates (23.18% efficiency), pyrethroids (cypermethrin and deltamethrin, 12.67% and 11.13% efficiency, respectively), association organophosphate-pyrethroid (23.18% efficiency), amitrazin (32.35% efficiency) and neem plant aqueous extract (3.74% efficiency). Acceptable levels of efficiency associated to the use of benzofenilurea, macrocyclic lactones (avermectins) and fipronil were observed.

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