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REGIONAL STUDY OF ACTION *IN VITRO* OF COMMERCIAL ACARICIDES ON THE CATTLE TICK

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RESUMO: To evaluate the effect of commercial acaricides for the control of *Rhipicephalus* (*Boophilus*) *microplus* in the region of Uberaba, Minas Gerais, engorged female cattle ticks were collected from five properties. These samples were immersed in previously prepared solutions containing five acaricide treatments and one control, as follows: T1, control (distilled water); T2, Amitraz (12.5%); T3, Cypermethrin (15%); T4, Deltamethrin (2.5%); T5, Cypermethrin (15%) + Chlorpyrifos (25%) + Citronellal (1%); and T6, Dichlorvos (60%) + Chlorpyrifos (20%). Ticks were then dried on absorbent paper, taped onto Petri dishes, and placed in an incubator at 27.0°C and humidity greater than 80%. After 20 days of fixation, the eggs of each group were collected, weighed, and placed in modified plastic syringes and again kept in an incubator until the larvae hatched. A greater reduction in egg mass was observed with Amitraz (12.5%) and the combination of Cypermethrin (15%) + Chlorpyrifos (25%) + Citronellal (1%) than with the other treatments. This combination proved superior to the other acaricides in controlling larvae hatchability. It was concluded that the combination Cypermethrin (15%) + Chlorpyrifos (25%) + Citronellal (1%) is efficient in combatting the R. (B.) *microplus* strain, unlike the isolated use of pyrethroids and organophosphorus associations.

Palavras-chave: Chemical control. Susceptibility to acaricides. *Rhipicephalus* (*Boophilus*) *microplus*.

ESTUDO REGIONAL DA AÇÃO *IN VITRO* DE ACARICIDAS COMERCIAIS SOBRE O CARRAPATO BOVINO

ABSTRACT: Para avaliar o efeito de carrapaticidas comerciais no controle do *Rhipicephalus* (*Boophilus*) *microplus* na região de Uberaba, Minas Gerais, foram colhidas teleóginas de cinco propriedades. Estas amostras foram imersas em soluções previamente preparadas contendo cinco tratamentos carrapaticidas e um controle, sendo eles: T1 – Controle (água destilada); T2 – Amitraz (12,5%); T3 – Cypermethrin (15%); T4 – Deltametrina (2,5%); T5 – Cipermetrina (15%) + Clorpirifós (25%) + Citronelal (1%); T6 – Diclorvós (60%) + Clorpirifós (20%). Em seguida, secadas em papel absorvente e fixadas com fita adesiva em placas de Petri e acondicionadas em estufa a 27,0ºC e umidade superior a 80%. Decorridos 20 dias da fixação as posturas de cada grupo foram recolhidas, pesadas e colocadas em seringas plásticas adaptadas e novamente levadas para a incubação em estufa até a eclosão das larvas. Maior redução da massa de postura foi observada com Amitraz (12,5%) e a associação Cipermetrina (15%) + Clorpirifós (25%) + Citronelal (1%), tendo esta associação se mostrado superior aos demais carrapaticidas no controle da eclodibilidade das larvas. Conclui-se que a associação Cipermetrina (15%) + Clorpirifós (25%) + Citronelal (1%) foi eficiente no combate da cepa de R. (B.) *microplus*; ao contrário do uso isolado de piretróides e da associação de organofosforados.

Keywords: Controle químico. Susceptibilidade a acaricidas. *Rhipicephalus* (*Boophilus*) *microplus*

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INTRODUCTION

Cattle production is one of the most important sectors of Brazilian agribusiness and consequently, the national economy (CEPEA, 2016). Brazil has the second largest cattle herd in the world, with 214,9 million head, producing 33,5 billion liters of milk, the state of Minas Gerais being the main producer (IBGE, 2017).

In Brazil, milk production provides approximately 170 liters of milk per inhabitant per year (BRASIL, 2014). Despite the high production of milk in the country the average productivity of the national herd is only approximately 1,963 liters (IBGE, 2017). The main reasons for the low productivity include the use of animals that are not good milk producers or of inappropriate genetic stock, inadequate food, poor handling of reproductive and health issues, and low educational level of the producers (57% have limited education), which makes the proper use of available technologies difficult (BRASIL, 2014).

The tick *Rhipicephalus* (*Boophilus*) *microplus* belonging to the *Ixodidae* family, is the main ectoparasite of cattle in Brazil, as well as all tropical and subtropical countries, and is a major impediment to national cattle farming (GRISI et al., 2002). This tick can lead to a reduction in milk production of up to 2.7%, equaling an average of 90.4 liters per lactating cow (RODRIGUES and LEITE, 2013). Brazilian climatic conditions are favorable for the survival and development of the free-living stages of this ectoparasite, which contributes to its predominance (FURLONG et al., 2007; CAMILLO et al., 2009).

The most effective way to control this ectoparasite is by using chemicals, but these have become less efficient (GRISI et al., 2002). The use of acaricide indiscriminately and without restriction, errors in solution preparation, mistakes in the manner, stage, and frequency of application, and inadequate management of parasite control may all favor the selection of ticks resistant to the chemical agents most often used by Brazilian cattle producers (FURLONG et al., 2007; CAMILLO et al., 2009).

The resistance of *Rhipicephalus* (*Boophilus*) *microplus* has been studied throughout the world, including in several regions of Brazil. The primary reason for the study of resistance (FURLONG et al., 2007; CAMILLO et al., 2009; AMARAL et al., 2011; CARNEIRO et al., 2015) is to find a proven product to control the ticks, thus reducing the number of acaricide baths needed (CARNEIRO et al., 2015). Therefore, an interesting tool for the identification of resistance to ectoparasites is the constant monitoring of herds in each region by visually counting ticks on the body of infected animals. Another method is the use of tests to detect the susceptibility of ticks to acaricides.

Because of the inefficiency of control efforts for *R*. (*B*.) *microplus*, the objective of this study was to determine the *in vitro* efficiency of five commercial acaricides in the city of Uberaba, state of Minas Gerais (MG).

MATERIAL AND METHODS

Samples of *Ixodidae* were collected manually from cattle at five different dairy cattle properties in the region of Uberaba, MG, from March to October 2014. Were collected approximately 300 engorged females per property. The absence of any ectoparasiticidal treatment of the cattle during the previous 45 days was confirmed before the collection and transport of samples. At the Parasitology Laboratory of the Federal Institute of Education, Science and Technology of Triangulo Mineiro, Uberaba IFTM Campus, ticks were washed in distilled water and dried on absorbent paper. From each sample, 120 engorged females of similar size were selected, weighed on an analytical balance, and ordered by decreasing weight (ARANTES and MARQUES, 1995). The final arrangement provided six groups with ten ticks each with two repetitions per treatment.

Next, six treatments were randomly distributed among twelve containers, which later received the engorged females. The treatments were as follows: T1, control (distilled water); T2, Amitraz (12.5%); T3, Cypermethrin (15%); T4, Deltamethrin (2.5%); T5, Cypermethrin (15%) + Chlorpyrifos (25%) + Citronellal (1%); T6, Dichlorvos (60%) + Chlorpyrifos (20%).

The groups of engorged females were immersed for 5 min in 50 mL of the test solution. They were then dried on absorbent paper, fixed with adhesive tape in Petri dishes (100 mm diameter × 20 mm height), labeled, and placed in an incubator at 27.0°C and humidity greater than 80%. The engorged females in the control groups were immersed in distilled water for the same duration as those treated with acaricide.

Twenty days after fixation of the engorged females, eggs from each group were collected, weighed, placed in modified plastic syringes, relabeled, and incubated until larval eclosion.

It was calculated reproductive efficiency (RE) with the aid of a stereoscopic microscope according to the methodology of Rocha et al., (1984). Three homogeneous samples were collected containing infertile eggs, larvae, and hatched eggs, and these were diluted in a 50% glycerin/alcohol solution. The reproductive efficiency (RE) was calculated using the equation 1:

$$RE = \frac{ew}{fw} \times eh \times 20{,}000$$

Where:
RE is the reproductive efficiency (%)
ew is the weight of the egg mass, in grams;
fw is the weight of engorged females, in grams;
eh is the percentage of larvae hatching;
and 20,000 is a constant that represents the estimated number of eggs in one gram of R. (B) microplus eggs.

Acaricide product efficiency (EP) was calculated using to the equation 2 below (Rocha et al., 1984), taking the arithmetic mean of the two repetitions for each of the experimental groups.

$$EP_i = \left(\frac{ERte - ERtr_i}{ERte} \right) \times 100$$

Where:
EP_i is the percentage of effectiveness of the ith treatment;
ERte is the reproductive efficiency for the control groups;
$ERtr_i$ is the reproductive efficiency for the ith treatment.

For comparison of treatments, the following characteristics were considered: mass of the eggs in grams, hatchability, and efficacy of treatment. The second and the third were calculated as percentages. These data were calculated as described above.

Regarding egg mass, data were subjected to an analysis of variance (ANOVA) at a 5% significance level for each of the five locations. Locations that were not significantly different were pooled (GOMES, 1990).

For hatchability, data were transformed prior to the ANOVA using the arcsine transformation of the square root (GOMES, 1990). For comparison between the means of the treatments, Tukey's test was used at a 5% level of significance.

Efficacy of treatments was compared graphically, and products were considered highly effective if they had a value greater than 95%, as recommended by the Ministry of Livestock Agriculture and Supply (BRASIL, 1990). All statistical analyses were performed using R Software (R CT, 2012).

RESULTS AND DISCUSSION

There was no interaction among treatments and egg mass ($p > 0.05$). The effect of treatment was significant ($p \leq 0.05$) for both mass and the transformed hatchability values. Thus, further evaluation regarding the effect of treatments was made with pooled collections of several locations.

When the effect of treatments was significant for both variables, Tukey's test was conducted at a 5% significance level to compare the means for egg mass and transformed hatchability.

Regarding egg mass (Table 1), the most effective treatments — the ones that resulted in the greatest reduction in egg mass — were T2 (Amitraz [12.5%]) and T5 (Cypermethrin [15%] + Chlorpyrifos [25%] + Citronellal [1%]). The strains subjected to these active ingredients would cause less contamination by eggs in pastures, thus reducing environmental infestation by the parasite.

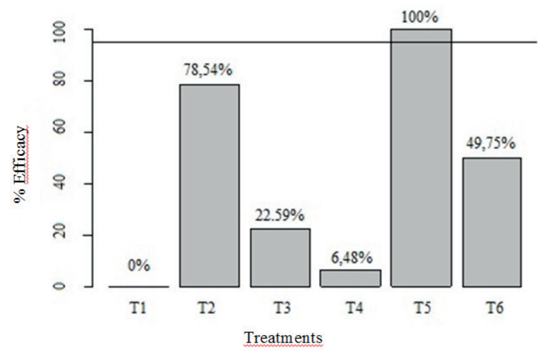
Table 1 Tests of multiple comparisons for *Rhipicephalus* (*Boophilus*) *microplus* egg mass immersed in commercial acaricides.

Treatments	Means (grams)
T1—Control (distilled water)	0.6531 ± 0.0581^{a}
T2—Amitraz (12.5%)	0.1797 ± 0.0581^{bc}
T3—Cypermethrin (15%)	0.5571 ± 0.0581^{a}
T4—Deltamethrin (2.5%)	0.6161 ± 0.0581^{a}
T5—Cypermethrin (15%) + Chlorpyrifos (25%) + Citronellal (1%)	0.0084 ± 0.0581^{c}
T6—Dichlorvos (60%) + Chlorpyrifos (20%)	0.2945 ± 0.0581^{b}

Means followed by the same letter in the columns did not differ statistically using Tukey's test at 5% probability.

Regarding the mean efficacy (%), the only treatment that reached an index higher than 95% efficacy as recommended by the MAPA (BRASIL, 1990) was T5 (Cypermethrin [15%] + Chlorpyrifos [25%] + Citronellal [1%]) (Figure 1).

Figure 1. Mean percentage of efficacy, based on productive efficiency, for each of the six treatments—T1, Control (distilled water); T2, Amitraz (12.5%); T3, Cypermethrin (15%); T4, Deltamethrin (2.5%); T5, Cypermethrin (15%) + Chlorpyrifos (25%) + Citronellal (1%); T6, Dichlorvos (60%) + Chlorpyrifos (20%).



Studies following methodologies similar to that developed during this research, found an efficiency of at least 95% for Amitraz in the control of *R.* (*B.*) *microplus* collected from dairy cattle properties in the northeastern part of the state of São Paulo (SOARES et al., 2001) and Santa Maria in the state of Rio Grande do Sul (OLIVO et al., 2009). These results differ from these experiment, where Amitraz attained an average efficiency of 78.54% for locations within the city of Uberaba, Minas Gerais, possibly due to the resistance of these parasites to this principle.

Despite the lack of information on the efficiency of Cypermethrin at 15% in the study area, most studies note low effectiveness of this agent in the control of *R.* (*B.*) *microplus* in the northeastern (CAMPOS JUNIOR, 2005), southeastern (PEREIRA, 2006), southern (CAMILLO et al., 2009), and midwestern (KOLLER et al., 2009) regions, confirming the low efficacy of this formulation throughout the country.

Spagnol et al. (2010), working in the city of Itamaraju in the state of Bahia, found the worst acaricide efficiency for chemical products in the pyrethroids group when used alone (Deltamethrin 33.9% and Cypermethrin 21.1%). This is in agreement with the 6.48% and 22.59% efficacy found in this experiment for T4 (Deltamethrin) and T3 (Cypermethrin), respectively. In Ilheus, Itabuna, in Bahia, even lower efficiencies (> 10%) were found for Deltamethrin (CAMPOS JUNIOR, 2005). The explanation is possibly related to the high residual capacity of its active ingredients, which favors the survival of naturally tolerant parasites, as well as the fact that it has cross-resistance with organochlorines (FURLONG et al., 2007), thus facilitating the development of resistance in populations of this parasite.

Nevertheless, recent research reported close to 100% efficiency for 15% Cypermethrin in regions such as the northern state of Rio de Janeiro (VITA et al., 2012), in the municipality of Brejo da Madre de Deus in the state of Pernambuco (MELO et al., 2010), and in four municipalities of Mato Grosso do Sul (ANDREOTTI et al., 2011).

The mean percentage of effectiveness of the T5 product (Cypermethrin [15%] + Chlorpyrifos [25%] + Citronellal [1%]), taking into account all the surveyed properties, was above 95%. This was similar to that found in the northwestern part of São Paulo (OLIVEIRA et al., 2013), where close to a 100% efficiency was reported with the use of the same combination of active ingredients. Furlong et al. (2007), performing a representative number of tests in regions of Minas Gerais, found that combinations of phosphorus and phosphorus /pyrethroids were significantly more effective. By evaluating six properties in northern Minas Gerais with animals with different blood levels, Carneiro et al. (2015) observed that the efficacy of Amitraz, Deltamethrin, and of the Cypermethrin, Chlorpyrifos and Citronellal combination varied significantly among cattle. The use of Deltamethrin was not effective in any of the evaluated herds. A higher efficiency of the pyrethroid and organophosphate combination has been described by various authors (PEREIRA, 2006; FURLONG et al., 2007; CAMILLO et al., 2009; CAMPOS JUNIOR and OLIVEIRA, 2005; MELO et al., 2010), and probably occurs because the chemical groups have different mechanisms of action and act on biochemically distinct sites of the Ixodidae, which leads to greater effectiveness when combined.

A mean efficiency of 100% in rural properties in the city of Uberaba, Minas Gerais, is valuable information for farmers in this region who face severe difficulties in the control of *R.* (*B.*) *microplus*.

CONCLUSIONS

The combination Cypermethrin (15%) + Chlorpyrifos (25%) + Citronellal (1%) is effective in controlling the regional strain of R. (B.) microplus in Uberaba, state of Minas Gerais, in contrast to the effect of pyrethroids alone, or in association with organophosphates.

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