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Efficacy of commercial synthetic pyrethroids and organophosphates associations used to control *Rhipicephalus (Boophilus) microplus* in Southern Brazil

Eficácia de associações comerciais entre piretroides sintéticos e organofosforados utilizados no controle de *Rhipicephalus (Boophilus) microplus* na Região Sul do Brasil

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Abstract

This study evaluated the acaricidal efficiency of synthetic pyrethroids (SP) associated with organophosphates (OP) against *Rhipicephalus (Boophilus) microplus* populations in the state of Rio Grande do Sul, Southern Brazil. Ticks were collected in 54 cattle farms and tested using *in vitro* adult immersion test against four commercially available acaricide mixtures of SP and OP. Only one of four acaricides, comprising a mixture of cypermethrin, chlorpyrifos, and fenthion, had a mean efficiency higher than 95%, and was effective in 94.44% (51/54) of the cattle farms tested. The acaricide with a mixture of cypermethrin, chlorpyrifos, and citronelall had the lowest mean efficiency (62.11%), and was effective in 29.62% (16/54) of cattle farms tested. Furthermore, *R. (B.) microplus* in Southern Brazil exhibited differing degrees of resistance to SP and OP mixtures. The results suggest that the presence of fenthion contributes to the higher efficiency of the formulation with this active principle. This is possibly due to its recent commercial availability, as tick populations have been lower challenged with this product. Monitoring the resistance of ticks to carrapaticides is essential to maximize the efficiency of these products in the control of *R. (B.) microplus*.

Keywords: Tick, cattle, chemical control, resistance, *Boophilus*.

Resumo

Este estudo avaliou a eficiência acaricida de piretroides sintéticos (SP) associados a organofosforados (OP) sobre populações de *Rhipicephalus (Boophilus) microplus* no estado do Rio Grande do Sul, Brasil. Carrapatos foram coletados em 54 fazendas de bovinos, testados *in vitro* pelo teste de imersão de teleóginas, contra quatro associações acaricidas comercialmente disponíveis de SP e OP. Apenas um dos quatro acaricidas, constituído por associação de cipermetrina, clorpirifós e fenthion, teve eficiência média superior a 95%, sendo eficaz em 94,44% (51/54) das fazendas testadas. O acaricida com associação de cipermetrina, clorpirifós e citronelal teve menor eficiência média (62,11%), sendo efetivo em 29,62% (16/54) das fazendas testadas. Além disso, o *R. (B.) microplus* no sul do Brasil, apresentou diferentes graus de resistência às associações de SP e OP. Os resultados sugerem que a presença do fenthion contribui para a maior eficiência da formulação com esse princípio ativo. Possivelmente, isso ocorre em função da sua recente introdução no mercado, já que as populações de carrapatos foram pouco desafiadas com este produto. Monitorar a resistência dos carrapatos aos carrapaticidas é fundamental para maximizar a eficiência desses produtos no controle de *R. (B.) microplus*.

Palavras-chave: Carrapatos, bovinos, carrapaticida, resistência, *Boophilus*.

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Introduction

Rhipicephalus (Boophilus) microplus represents a serious economic threat to the cattle industry in tropical and subtropical areas, particularly in South America. The negative economic impact of tick infestation is caused by a combination of direct and indirect effects on infected bovines, including blood loss, reduced weight gain and milk production, increased risk of myiasis, and transmission of tick-borne diseases (ROCHA et al., 2011; RECK et al., 2014). Losses caused by tick infestation can be minimized by treating cattle with acaricides, but resistance is widespread in countries where *R. (B.) microplus* is well established (FERNÁNDEZ-SALAS et al., 2012; KLAFKE et al., 2017). The use of chemical control against ticks is characterized by a continuous increase of resistance, proportional to the frequency of the application of acaricides (VARGAS et al., 2003; CAMPOS & OLIVEIRA, 2005). To be licensed in Brazil, a chemical product to be used in the control of tick populations must be at least 95% effective in a sensitive strain of *R. (B.) microplus* (BRASIL, 1997). Control of cattle ticks primarily depends on treatment with synthetic acaricides (MILLER et al., 2007); however, the intensive use of chemical formulations leads to a loss of effectiveness of these molecules with the consequent spread of resistance against them, making essential the strategic administration of these products (FAO, 2004).

Indiscriminate use of incorrect concentrations of acaricides has likely contributed to the development of resistance in tick populations, which is aggravated by the increasing numbers of products that combine two or more active ingredients (FAO, 2004). The inefficacy of chemical products in tick control is a major challenge for the cattle industry, and resistance to the main classes of acaricides has been

reported in several regions (FERNÁNDEZ-SALAS et al., 2012; RECK et al., 2014; GHOSH et al., 2015). The combination of two different pharmacological bases in a single acaricide is a common practice found at commercial level. The gradual loss of efficacy to acaricides is commonly reported by farmers in the Brazilian state of Rio Grande do Sul, that report the failure of tick control in their herds. Thus, this study aimed to evaluate the acaricide efficiency of synthetic pyrethroids (SP) associated with organophosphates (OP) used to control of *R. (B.) microplus* populations in Rio Grande do Sul, Southern Brazil.

Materials and Methods

Engorged female *R. (B.) microplus* were collected from dairy and beef cattle in different regions of Rio Grande do Sul from November 2015 to August 2016. Ticks were collected directly from animals in 54 cattle farms located in 28 cities of Rio Grande do Sul in southern Brazil: Alegrete (two farms), Caçapava do Sul (eight farms), Cacequi (two farms), Cachoeira do Sul (two farms), Dilermando de Aguiar (two farms), Encruzilhada do Sul, Faxinal do Soturno (two farms), Jaguarí, Jari, Júlio de Castilhos, Lavras do Sul, Nova Esperança do Sul, Nova Palma (two farms), Paraíso do Sul, Pinhal Grande, Restinga Seca, Rosário do Sul, Santa Barbara do Sul, Santa Maria (two farms), Santiago (three farms), São Francisco de Assis (two farms), São Gabriel (five farms), São João do Polêsine, São Martinho da Serra, São Pedro do Sul, São Sepé (five farms), São Vicente do Sul (two farms), and Vila Nova do Sul (Figure 1). In vitro adult immersion tests (AIT) were used to detect susceptibility or resistance to different acaricide mixtures. Farms were selected based

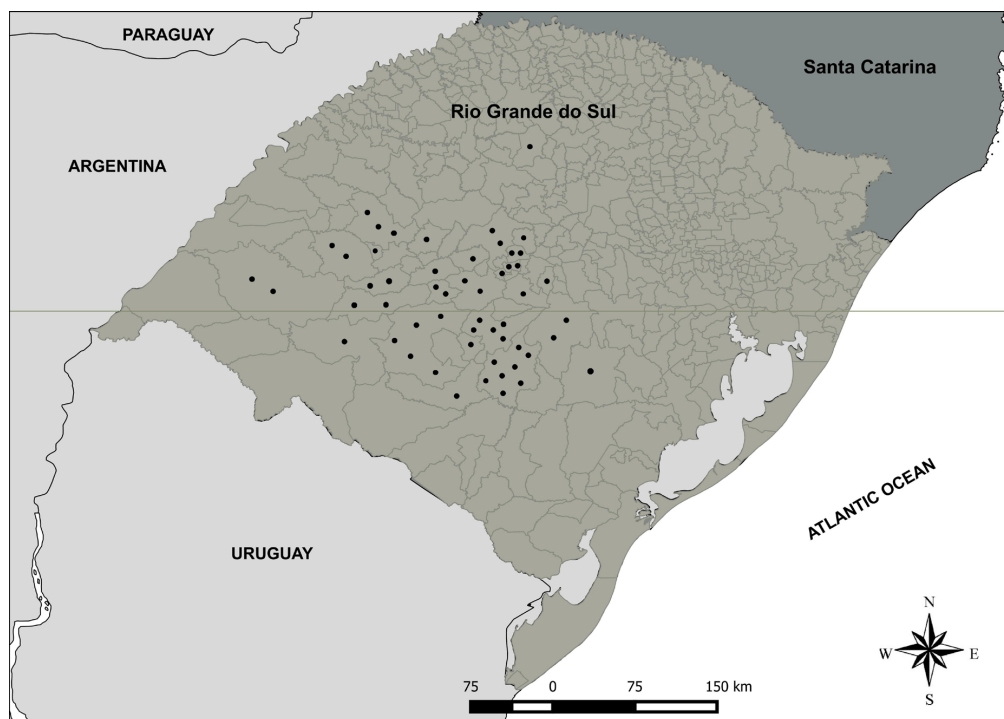


Figure 1. Locations of 54 cattle farms in 28 cities of Rio Grande do Sul, southern Brazil, at which four commercially formulations of synthetic pyrethroids and organophosphates were tested against *Rhipicephalus (B.) microplus* using *in vitro* Adult Immersion Test. Spheres indicate the locations of the farms.

on a historical failure of tick chemical control measures, and ticks were only taken from animals that had not received acaricides in the 30 days preceding collection.

Four commercial acaricide mixtures of SP and OP were tested by AIT, according to the methods set out by Drummond et al. (1973). The chemical compounds belonging to the OP chemical group used in this study were chlorpyrifos, ethion, and fenthion. The chemical compounds belonging to the SP chemical group used in this study were cypermethrin, cypermethrin high-cis, and alpha-cypermethrin. At each farm, approximately 200 engorged females were taken directly from infested cattle. *Ectoparasiticide A* (Colosso®, OuroFino Animal Health) comprised 15% cypermethrin, 25% chlorpyrifos, and 1% citronellal diluted with distilled water to final concentrations of 0.15 mg mL⁻¹, 0.25 mg mL⁻¹, and 0.01 mg mL⁻¹, respectively; *ectoparasiticide B* (Colosso FC30®, OuroFino Animal Health) comprised 15% cypermethrin, 30% chlorpyrifos, and 15% fenthion diluted with distilled water to final concentrations of 0.188 mg mL⁻¹, 0.375 mg mL⁻¹, and 0.188 mg mL⁻¹, respectively; *ectoparasiticide C* (Flytion®, Clarion Biosciences Ltda.) comprised 50% chlorpyrifos and 6% cypermethrin high-cis diluted with distilled water to final concentrations of 0.833 mg mL⁻¹ and 0.1 mg mL⁻¹, respectively; and *ectoparasiticide D* (Potenty®, MSD Animal Health) comprised 16% ethion, 8.5% chlorpyrifos, and 5% alpha-cypermethrin, diluted with distilled water to final concentrations of 0.4 mg mL⁻¹, 0.212 mg mL⁻¹, and 0.125 mg mL⁻¹, respectively. All products were commercially available, were used according to recommendations and at concentrations specified by the manufacturers for immersion baths. Distilled water was used for the control group. Each test was performed in triplicate with 10 engorged females per group immersed for 5 min in 30 mL of test solution.

Data were analyzed using SAS software (SAS, 1985). Mean efficiency of each acaricide solution was determined using Fisher's exact test with the significance threshold set at 0.05.

Results and Discussion

Of the four commercial acaricides tested, *ectoparasiticide A* had the lowest mean efficiency and *ectoparasiticide B* had the highest (Table 1). Three of the products analyzed had a mean efficiency lower than 95% (*ectoparasiticides A, C, and D*; Table 1). Reports of cattle tick resistance to SP and OP have been recorded for decades (NOLAN et al., 1977). An alternative measure to increase acaricide treatments efficacy against tick populations is the combination of two different chemicals. Combinations of different chemical bases are generally more effective than single molecule formulations (VARGAS et al., 2003). However, these combinations do not always lead to efficiency levels of at least 95% (CAMPOS & OLIVEIRA, 2005; MENDES et al., 2007). For instance, in the present study, a mixture of cypermethrin, chlorpyrifos, and citronellal (*ectoparasiticide A*) and a mixture of ethion, chlorpyrifos, and alpha-cypermethrin (*ectoparasiticide D*) did not achieve satisfactory efficiency levels (Table 1).

Each of the analyzed ectoparasiticides exhibited both efficiency (≥ 95%) and acaricide resistance (<95%) in samples from at least one farm cattle (Figure 2). Ectoparasiticides B and C had satisfactory acaricidal efficiencies (≥ 95%) in 51 (94.44%) and

Table 1. *In vitro* efficiency, using Adult Immersion Test, of acaricide mixtures of synthetic pyrethroids and organophosphates used to control the *Rhipicephalus (B.) microplus* populations from state of Rio Grande do Sul in southern Brazil.

	A	B	C	D
Percentage of farms with efficacy ≥95% (%)	29.62 ^c (16/54)	94.44 ^a (51/54)	81.48 ^b (44/54)	40.74 ^c (22/54)
Mean efficacy (%)	62.11	98.76	92.14	72.06
Amplitude (%)	1.2 - 100	67 - 100	10.2 - 100	4.6 - 100
Standard Error	+9.31	+1.46	+5.4	+8.42
Lower 95% CI	52.8	97.3	86.74	63.64
Upper 95% CI	71.42	100	97.54	80.48

Distinct small letters indicate statistical differences by Fischer's exact test (*p*<0.05). *ectoparasiticide A* (cypermethrin 0.15 mg mL⁻¹, chlorpyrifos 0.25 mg mL⁻¹, citronellal 0.01 mg mL⁻¹); *ectoparasiticide B* (cypermethrin 0.188 mg mL⁻¹, chlorpyrifos 0.375 mg mL⁻¹, fenthion 0.188 mg mL⁻¹); *ectoparasiticide C* (chlorpyrifos 0.833 mg mL⁻¹, cypermethrin high-cis 0.1 mg mL⁻¹); and *ectoparasiticide D* (ethion 0.4 mg mL⁻¹, chlorpyrifos 0.212 mg mL⁻¹, alpha-cypermethrin 0.125 mg mL⁻¹). *CI*: confidence intervals.

44 (81.48%) of the 54 cattle farms, respectively. Conversely, the populations of ticks studied were resistant to ectoparasiticides A and D (efficiency < 95%) in 38 (70.37%) and 32 (59.26%) of the 54 cattle farms, respectively (Table 1). The efficiency of a given acaricide varies greatly between different tick populations, and this depends, among other factors, on the management system in a given farm and the frequency of the ectoparasiticide application (ROCHA et al., 2011). The higher efficiency of *ectoparasiticide B* can be attributed to the fact that the product contained fenthion in its formulation. However, to confirm this hypothesis, in the future we must test the active principle in isolation against these tick populations. This acaricide is only recently available for cattle tick control in Brazil; accordingly, tick populations have been subjected to lower selective pressures for resistance to this chemical than to the other acaricides tested (SINDAN, 2016).

Different levels of resistance to all ectoparasiticides tested were detected. It is common for commercial products to combine active ingredients from different chemical families owing to their inefficacy when used separately (ANDREOTTI et al., 2011). However, ectoparasiticides comprising chemicals of different families were satisfactorily effective against *R. (B.) microplus* at only 11 of the 54 farms tested. Resistance to products containing a combination of SP and OP is also reported in other regions of Brazil (CAMPOS & OLIVEIRA, 2005; MENDES et al., 2007; ANDREOTTI et al., 2011) and worldwide (FERNÁNDEZ-SALAS et al., 2012; GHOSH et al., 2015; PUERTA et al., 2015). Frequent use of chemical treatments increases the chances of selecting for resistance among ticks, which is one of the main factors in establishing a resistant population of *R. (B.) microplus* (RODRIGUEZ-VIVAS et al., 2006). Therefore, we consider important to emphasize the importance of constant monitoring of the acaricidal efficiency, through *in vitro* tests to contribute to the rational use of acaricides currently available in the market and to reduce the frequency of treatments. These measures can lead to the maintenance of parasite populations below the threshold of economic damage and contribute to a lower environmental impact (FAO, 2003).

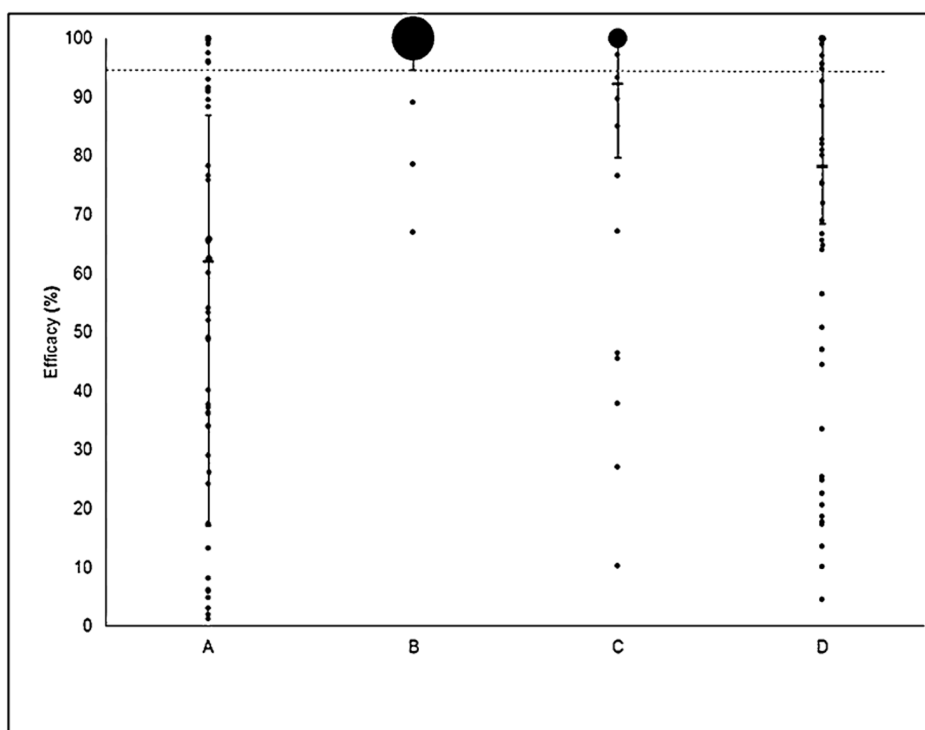


Figure 2. Distribution of efficiency of four acaricides used to control *Rhipicephalus (B.) microplus* on cattle farms in Rio Grande do Sul, southern Brazil. Lines indicate mean efficiency and standard error. *Ectoparasiticide A* comprises cypermethrin (0.15 mg mL⁻¹), chlorpyrifos (0.25 mg mL⁻¹), and citronellal (0.01 mg mL⁻¹); *ectoparasiticide B* comprises cypermethrin (0.188 mg mL⁻¹), chlorpyrifos (0.375 mg mL⁻¹), and fenthion (0.188 mg mL⁻¹); *ectoparasiticide C* comprises chlorpyrifos (0.833 mg mL⁻¹) and cypermethrin high-cis (0.1 mg mL⁻¹); and *ectoparasiticide D* comprises ethion (0.4 mg mL⁻¹), chlorpyrifos (0.212 mg mL⁻¹), and alpha-cypermethrin (0.125 mg mL⁻¹).

Conclusion

Only the combination of cypermethrin, chlorpyrifos, and fenthion had acceptable mean levels of efficiency in the tick populations studied. The higher efficiency of this mixture is possibly due to the presence of fenthion, probably owing to its recent commercial availability in Brazil, and consequently, lower levels of resistance established in tick populations.

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