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[Comunicação]

In vitro efficacy of an association of carvacrol and a commercial acaricide (synthetic pyrethroid + organophosphate + citronellal) against *Rhipicephalus (Boophilus) microplus*

[*Eficácia* in vitro *da associação de carvacrol a carrapaticida comercial (piretroide sintético + organofosforado* + citronellal*) sobre* Riphicephalus (Boophilus) microplus)]

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Parasitism due to the cattle tick *Rhipicephalus (B.) microplus* is one of the most serious hindrances to the profitability of cattle-rearing, especially regarding dairy production. In addition to the damage that this tick causes directly, including retarded growth among young animals, weight loss and diminished milk production, it is also the vector of cattle tick fever. In some regions of Brazil, this disease gives rise to high mortality among cattle herds. From recent data, the economic losses ascribed to tick parasitism among cattle in Brazil are around 3.24 billion dollars per year, regarding lost milk and meat production alone (Grisi *et al*., 2014).

The control method that is most recommended consists of application of acaricides. However, resistance to the chemical products used has been growing (Reginato *et al*. 2017; Vilela *et al*. 2020). This has led to a need to decrease the interval between treatments, which has ended up increasing cattle production costs, thus compromising the profitability of this activity. Moreover, excessive use of acaricides has led to environmental contamination, with impairment of the soil and water resources, which has especially given rise to residues in foods and, in the case of dairy activity, in milk.

One of the alternatives for dealing with this situation is to investigate phytotherapy with acaricidal activity. In this regard, essential oils from plants have been shown to be effective against *R. (B.) microplus*, both *in vitro* (Santos *et al*., 2012 Kumar *et al*., 2016) and *in vivo* (Santos *et al*., 2017; Pereira *et al*., 2022). Certain constituents of these essential oils, such as carvacrol, have been reported to be effective against larvae and engorged female cattle ticks (Novato *et al*., 2019; Cardoso *et al*., 2020; Pereira Junior *et al*., 2019).

The aim of the present investigation was to evaluate the efficacy of carvacrol used in association with a commercial chemical acaricide (187.5 ppm cypermethrin + 312.5 ppm chlorpyrifos + 12.5 ppm citronellal).

Samples of *R. (B.) microplus* (n = 180) were collected from naturally infected cattle that had been routinely treated with ivermectin. At the time of the experiment, around 35 days had elapsed since their last treatment. The ticks were divided into six experimental groups, each with three replications: 1) control (DMSO solution 10,000 ppm + water) ; 2) carvacrol 1 (Sigma-Aldrich®, EUA) (200 ppm + DMSO 10,000 ppm + water); 3) carvacrol 2 (400 ppm + DMSO 10,000 ppm + water); 4) commercial acaricide (187.5 ppm cypermethrin + 312.5 ppm chlorpyrifos + 12.5 ppm citronellal); 5) commercial acaricide + carvacrol 200 ppm; and 6) commercial acaricide + carvacrol 400 ppm. The groups were subjected to Adult Immersion Test - AIT (Drummond *et al*. (1973). After immersion in the respective solution, the ticks were weighed individually, transferred to Petri dishes, immobilized dorsally using double-sided adhesive tape

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and maintained at a temperature of 27 °C and relative air humidity of more than 85%, for egg-laying and hatching of larvae to take place. The egg production index - EPI (Bennett, 1974) and AIT - Treatment Efficacy (Drummond *et al*., 1973) were evaluated. The EPI results were submitted to the Kruskal Wallis Test and means compared using the Student Newman Keuls Test ($p<0.01$).

The data obtained through the immersion test on the engorged female ticks are presented in Table 1. The results showed that using carvacrol in association with the commercial acaricide (a combination of pyrethroid + organophosphate + citronellal) reduced egg-laying 4.2-fold ($p<0.01$) and increased the efficacy of the commercial acaricide 4.8-fold, independent of the concentration of carvacrol used (200 or 400 ppm). Although the performance of the commercial acaricide alone (i.e. without association with carvacrol) was better than that of the control group, its performance was poor, with only around a 20% reduction in egg-laying. The efficacy of acaricides formulated using associations of organophosphates and pyrethroids has become compromised through parasite resistance to these compounds. Several studies have shown this trend in Brazil (Kemmer, 2020; Torres Santos *et al*., 2021). In the same way as seen with the commercial acaricide, carvacrol used alone at the concentrations of 200 and 400 ppm did not show satisfactory performance, and the results presented were practically the same as those from using the commercial acaricide. On the other hand, used at higher concentrations, carvacrol was shown to be effective against *R. (B.) microplus* in *in vitro* tests on larvae (Costa Júnior, 2016; Cardoso, *et al*., 2020) and on engorged females (Pereira Júnior *et al.* 2019). A synergistic effect between carvacrol and cypermethrin on larvae was described previously by Novato et al. (2022).

Table 1**.** Egg Production Index (EPI) and Efficacy (AIT) on engorged females *R. (B.) microplus* ticks exposed to Comercial Acaricide (CA), Carvacrol (CV) and Experiments combining Carvacrol + Comercial Acaricide. Paraíba Valley, Sao Paulo, Brazil

Treatments	EPI (%) (Mean ±SD)	Reduction EPI (%)	Efficacy (AIT) (%)
Control	$55.10 \pm 10.93^{a*}$	-	-
Carvacrol (CV) 200	44.06 ± 16.31^{ab}	20.04	17.80
Carvacrol (CV) 400	39.09 ± 21.98^{b}	29.06	28.03
Commercial Acaricide (CA)	42.42 ± 15.59^{b}	23.01	20.70
CA + CV 200	1.20 ± 6.56^{c}	97.82	99.86
CA + CV 400	1.30 ± 4.95^{c}	97.64	100.00

*Means within column with different letters are significantly different ($p < 0.01$) - Kruskal Wallis – Student Newman Keuls' test

Use of carvacrol (200 ppm and 400 ppm) in association with a commercial acaricide (cypermethrin + chlorpyrifos + citronellal) was shown in the present *in vitro* study to have high potential for controlling cattle ticks. However, it is extremely important to perform field tests on parasitized animals to confirm the efficacy of this association and the safety of using the product on animals, and to ascertain whether any presence of residues in meat and milk remains at low levels that ensure the healthiness of these foods.

Keywords: Carvacrol, cattle tick control, commercial acaricide, in vitro conditions

RESUMO

Foram realizados testes in vitro *sobre fêmeas ingurgitadas do carrapato dos bovinos* Riphicephalus (B.) microplus *(AIT), para determinar a eficácia da associação do carvacrol a carrapaticida comercial. Os testes foram realizados em seis grupos experimentais, com três repetições: 1) controle (água + DMSO 10.000ppm); 2) carvacrol 1 (Sigma-Aldrich®, EUA) (200ppm + DMSO 10.000ppm); 3) carvacrol 2 (400ppm + DMSO 10.000ppm); 4) carrapaticida comercial (187,5ppm cypermethrin + 312,5ppm chlorpyrifos + 12,5ppm citronellal); 5) carrapaticida comercial + carvacrol 200ppm; 6) carrapaticida comercial + carvacrol 400ppm. A associação de carvacrol ao carrapaticida comercial potencializou cerca de cinco vezes a sua eficácia.*

Palavras-chave: carvacrol, controle carrapato dos bovinos, carrapaticida comercial, testes in vitro

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