DOI: 10.5216/cab.v14i1.19393

IN VITRO EFFECT OF THE ASSOCIATION OF CITRONELLA, SANTA MARIA HERB (*Chenopodium ambrosioides)* AND QUASSIA TINCTURE ON CATTLE TICK *Rhipicephalus (Boophilus) microplus*

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ABSTRACT

The tick *Rhipicephalus* (*Boophilus) microplus* is an ectoparasite which causes high financial losses to the production of Brazilian cattle. The control of this parasite is accomplished by the administration of chemical products, but they are not adequately used, leading to high costs and stimulating the selection of resistant parasites. Thus, the objective of this experiment was to assess the *in vitro* efficiency of different formulations containing an association of citronella (*Cymbopogon nardus*), Santa Maria herb (*Chenopodium ambrosioides)* and quassia (*Quassia amara)* tinctures on the bovine tick *R. microplus*. For this, engorged females of the parasite were submitted to the laboratory immersion test. The solutions containing all three plants were tested in different concentrations. Approximately 84% of the treatments presented efficiency equal or superior to 95%. The treatments with citronella in concentration equal or superior to 5% showed better results and the concentration of 10% showed maximum efficiency, independently of the concentrations of the other two plants. The high efficiency of the solutions was attributed mainly to the reduction of egg laying and hatching rate, and, in a minor scale, to death of the engorged females. It suggests that the phytotherapic solutions are able to induce alterations on the system and reproductive capacity of the females of *R. microplus* in *in vitro* tests, besides presenting a tickicide action.

KEYWORDS: *Chenopodium ambrosioides; Cymbopogon nardus*; phytoterapic; *Quassia amara.*

EFEITO *IN VITRO* DA ASSOCIAÇÃO DE CITRONELA, ERVA DE SANTA MARIA E QUÁSSIA SOBRE O CARRAPATO BOVINO *Rhipicephalus (Boophilus) microplus*

RESUMO

O carrapato *Rhipicephalus* (*Boophilus) microplus* é o ectoparasita que mais causa prejuízos à pecuária brasileira. O controle desse parasita é realizado por meio da administração de produtos químicos, sendo que estes são utilizados de forma inadequada, gerando alto custo e estimulando a seleção de parasitas resistentes. Tendo em vista essa situação, o objetivo deste experimento foi avaliar a eficácia *in vitro* de diferentes formulações contendo tintura de citronela (*Cymbopogon nardus)*, erva de Santa Maria (*Chenopodium ambrosioides)* e quássia (*Quassia amara)* sobre o carrapato bovino *R. microplus*. Para isso, fêmeas ingurgitadas do parasita foram submetidas ao teste laboratorial de biocarrapaticidograma. As soluções testadas continham as três plantas em diferentes concentrações e utilizadas em associação. Aproximadamente 84% dos tratamentos apresentou

eficiência igual ou superior a 95%. Os tratamentos que continham citronela em concentração igual ou superior a 5% apresentaram melhores resultados e a concentração de 10% apresentou máxima eficiência, independente das concentrações da quássia e da erva de Santa Maria. A alta eficácia das soluções foi atribuída principalmente à redução na postura dos ovos e na taxa de eclosão da postura, e em menor escala à morte das teleóginas. Isso sugere que as soluções contendo os três fitoterápicos provocam alteração no sistema e na capacidade reprodutiva das fêmeas de *R. microplus* e também apresenta ação carrapaticida em testes *in vitro*.

PALAVRAS-CHAVE: *Chenopodium ambrosioides; Cymbopogon nardo*; fitoterapia; *Quassia amara.*

INTRODUCTION

Worldwidely, the tick *Rhipicephalus (Boophilus) microplus* generates high damages and costs to cattle raising. These damages are mainly attributed to weight loss, low food conversion, decreasing milk production, leather quality loss, toxicosis, skin lesions that favors myiasis, anemia and transmission of intracellular pathogens. High tick infestation can also lead animals to death (BRESCIANI et al., 2003). The control of this parasite is based on intensive use of chemical products, resulting in high costs and residues in flesh and milk, which are harmful to the animal and to the environment. Besides these aspects, the use of chemical products in large scale can harm the future control of this parasite, because of quick selection and multiplication of resistant strains (FRAGA et al., 2003). The resistance to these drugs is one of the most serious problems of the animal productive chain (MOLENTO, 2004). The development of chemoresistance to organophosphorated substances, pyretroids and amidines, impairs cattle production, thus the research of alternative ways of control is necessary. Herbal medicine has been shown as a promissory and feasible alternative, due to the great variability of plant species, low cost and easy availability in certain regions (AGNOLIN et al., 2010).

Citronella is a medicinal plant from the Poacea family whose compounds, geraniol, eugenol, citronellal and limonene, act on insects (SHASANY et al., 2000). The medicinal plant quassia is from the Simarubaceas family and it is constituted of resin, mucilage, pectin and tannins, sugar, alkaloids, essential oil, resinous and pectinous matter, being used in popular culture in digestive disorders and as food additive (MELLO et al., 2009). Santa Maria herb (*Chenopodium ambrosioides*) is known in popular medicine for its possible anthelmintic effect.

The objective of this study was to evaluate the efficiency of associations containing citronella (*Cymbopogon nardus*), Santa Maria herb (*Chenopodium ambrosioides*) and quassia (*Quassia amara*) tinctures on the tick *R. microplus*, in *in vitro* experimentations.

MATERIAL AND METHODS

The *in vitro* tests were conducted at the Laboratory of Parasitic Diseases of the Federal University of Santa Maria (UFSM), State of Rio Grande do Sul (RS), Brazil. The engorged females of *R. microplus* were collected in naturally infested cattle from a farm located in Santa Maria, RS. The animals had not been treated with antiparasitic drugs for 60 days. Previously to this experiment, in this farm, engorged female immersion tests had already been carried out with amitraz and cypermethrin and the ticks were considered resistant (efficiency inferior to 95%, according to the Brazilian law) to both of the drugs.

The engorged females collected from the cattle were taken to the laboratory, where they were washed in water, dried in towel paper and separated into 14 treatments. The treatments were constituted by different concentrations of the tinctures, adapted from a commercial formulation available for use against the tick *Rhipicephalus sanguineaus* in dogs. The tinctures of citronella, Santa Maria herb and quassia came from the Schraiber Laboratory, Carapicuíba, São Paulo. We used different concentrations of the three plants in association for the treatment groups, according to Table 1, which contains the concentration of each plant individually. To complete the total volume, alcohol 70º was added to the solutions. It was used only distilled water for the control group.

The engorged female immersion test was performed following the technique described by DRUMMOND et al. (1973). For each individual treatment, 10 engorged females were used. They were immersed in 20 mL of the solution correspondent to each treatment for 5 minutes. After the immersion, the engorged female were dried in towel paper and fixed using adherent tape in Petri plates, previously identified. After that, they were taken to an acclimatized incubator, regulated at the temperature of 27 ºC and relative humidity superior to 80%, for 14 days. After the egg laying period (Figure1), the total amount of eggs was weighted and 0.3 grams of this content was transferred to test

tubes, sealed with hydrophobic cotton and taken again to the incubator, where they were kept for 26 days. After the incubation period of the eggs, the reading of the percentage of hatchability was performed (Figure 2).

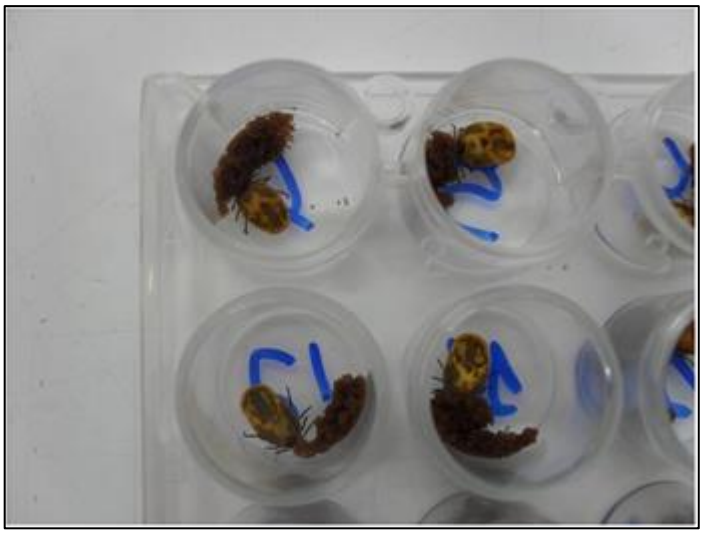

Figure 1. Engorged females of *Rhipicephalus (Boophilus) microplus* performing complete egg laying in *in vitro* tests.

Figure 2. Larvae of *Rhipicephalus (Boophilus) microplus* in *in vitro* tests.

All the treatments were performed in triplicate and the results were obtained through their averages. The data obtained during the *in vitro* test was used to calculate the Reproductive Efficiency (ER) and the Efficiency of Treatment (ET), using the following formulas, described by DRUMMOND et al. (1973).

$$ER = \frac{\text{Egg weight x\% hacthing} \times 20000}{\text{Engorged female weight}}$$

$$ET = \frac{(\text{ET control} - \text{ET treated}) \times 100}{\text{ET control}}$$

Statistical analysis was accomplished by the polynomial regression test and nonparametric test by Kruskal-Wallis with comparison of the treatments by Student-Newman-Keuls. Difference of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The laying inhibition, larvae eclosion and efficiency rate of the treatments are described on Tables 1 and 2.

Table 1. Percentage averages (%) of laying inhibition (LI), larvae eclosion (LE), and efficiency of the treatment (ET) of the formulations containing different concentrations of the tinctures of citronella, Santa Maria herb (SM) and quassia used in association, according to treatment on the tick *Rhipicephalus (Boophilus) microplus* in *in vitro* tests

TREATMENT	CITRONELLA	SM	QUASSIA	LI	LE	ET
I	10	20	20	45	0	98
II	20	40	40	100	0	100
III	20	20	20	100	0	100
IV	10	40	20	96	0	100
V	10	20	40	100	0	100
VI	5	10	10	90	0	100
VII	5	20	20	70	28	95
VIII	10	10	20	100	0	100
IX	10	20	10	96	0	100
X	2.5	5	5	23	80	36
XI	2.5	20	20	13	79	31
XII	10	5	20	93	0	100
XIII	10	20	5	100	0	100
XIV	0	0	0	0	100	0

The result of F test for the linear regression model was significant (P=0.003) for citronella tincture evaluated separately, according to the laying inhibition data, as in the equation $y = 32.7 + 4.55x$, showing that the increment on the laying inhibition can be obtained with growing dosages up to 20% of this plant. When the effect of the tinctures of Santa Maria herb and quassia were evaluated separately, no polynomial regression adjustment was verified, indicating that the laying inhibition in this case cannot be statistically explained by the isolated effect of these medicinal plants.

The result of the F test for the polynomial regression model indicated that solutions containing up to 10% of citronella demonstrated a linear increase in the efficiency of treatment ($y = 9.03205 + 14.6881x – 0.513573x^2$ P=0.0001). From 10 to 20% of citronella the efficiency of treatment remains from 98 to 100%. These results indicate that 10% of citronella would be enough in order to obtain 100% of efficiency in *in vitro* evaluations. The result of F test for the polynomial regression model indicates that it was the cubic model that best adjusted to estimate the efficiency of Santa Maria herb ($y = – 0.51 + 18.4879x – 1.00088x^2 + 0.015X^3$ P=0.04 R^2=59.3%). These data show that the increase in the concentration of this plant shows an oscillator result, indicating that the efficiency of this product depends on the concentrations of the other tinctures evaluated together.

The result of F test for the analysis of the efficiency of the quassia tincture showed the same prediction model as Santa Maria herb ($y = -0.51 + 18.4879x – 1.00088x^2 + 0.015X^3$ P=0.04 R^2=59.3%). It occurred due to the inclusion levels and the results obtained were the same for both tinctures, even though they were in different combinations with citronella (Figure 3).

In this experiment, 84% (11/13) of the treatments presented efficiency equal to or above 95%, and only 16% (2/13) presented efficiency below 90%. Approximately 69% (9/13) of the engorged female had 90% of their laying inhibited and 76% (10/13) of the larvae did not eclode, thus new larvae population was not generated. These results are significant, evidencing the *in vitro* tickicide action of citronella, Santa Maria herb and quassia tinctures. Treatment VI was considered the most effective, because it had lower concentration of the tinctures (25% of tincture and 75% of alcohol) and maximum efficiency. Treatments X and XI presented efficiency inferior to 40%, and it can be attributed to the lower citronella concentration (2.5%).

Table 2. Probabilities of significant difference among treatments with different formulations containing citronella, Santa Maria herb (SM) and quassia tincture and for the variable Efficiency of Treatment on the tick *Rhipicephalus (Boophilus) microplus* in *in vitro* tests

	Citronella	SM	Quassia	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I	10	20	20	x	0.57	0.57	0.57	0.57	0.57	0.28	0.57	0.57	0.10	0.12	0.57	0.57	0.04*
II	20	40	40		x	1	1	1	1	0.10	1	1	0.02	0.03	1	1	0
III	20	20	20			x	1	1	1	0.10	1	1	0.02	0.03	1	1	0
IV	10	40	20				x	1	1	0.10	1	1	0.02	0.03	1	1	0
V	10	20	40					x	1	0.10	1	1	0.02	0.03	1	1	0
VI	5	10	10						x	0.10	1	1	0.02	0.03	1	1	0
VII	5	20	20							x	0.10	0.10	0.5	0.6	0.1	0.1	0.3
VIII	10	10	20								X	1	0.02*	0.03*	1	1	0
IX	10	20	10									x	0.02*	0.03*	1	1	0
X	2.5	5	5										x	0.9	0.02*	0.02*	0.6
XI	2.5	20	20											x	0.03*	0.03*	0.6
XII	10	5	20												x	1	0
XIII	10	20	5													x	0
XIV	0	0	0														x

* Difference of $p<0.05$ were considered significant.

The results of this experiment demonstrated high *in vitro* efficiency of the association of citronella, Santa Maria herb and quassia on engorged females of *R. microplus*. They also suggest that citronella plays a more relevant role than the other plants, altering mainly egg laying and larvae eclosion. The females that succeeded in laying eggs showed high rate of infertile laying, indicating a harmful activity of the plants on the reproduction of the engorded female, with consequent reduction of the offspring. Moreover, most of the females that did not lay were dead, indicating tickicide activity as well.

There are few reports using citronella, Santa Maria herb and quassia in association against cattle tick; however, there are many works presenting evidence of use of these plants separately in herbal medicine. The main uses of quassia, as a medical plant, are in the treatments of ascariasis, arthropods, and skin lesions caused by *Leishmania* (*Viannia*) *braziliensis* (FRANÇA et al., 1996), in the control of domestic plagues, in the inhibition of the soil fungi and insects development, such as *Scrobipalpula absoluta* and *Spodoptera frugiperda,*and in the relief of abdominal pain and the flu (MOREIRA et al., 2002). Quassia presents medicinal action on several diseases, including parasitical (LEV & AMAR, 2002), stimulating researches about its use against ectoparasites. This plant was widely used in the elaboration of natural insecticides in Europe and the United States; however, its use decreased with the forthcoming of synthetic insecticides. A long time ago, LE COINTE (1947) was already aware of the insecticide action of the quassia extracts impregnated in fly-catcher paper, and also of its use as substitute for hop in brewing. The property to kill flies stimulated researches about its action on other ectoparasites.

The Santa Maria herb stands out because it presents repellent activity (NOVO et al., 1997; MAZZONETTO & VENDRAMIN, 2003) and insecticide activity (MAZZONETTO & VENDRAMIN, 2003). Its use is due to the presence of high rates of ascaridol in the seeds, leaves and stem. The essential oil of this plant has 90% of ascaridol, and it seems that this compound is responsible for its action against insects.

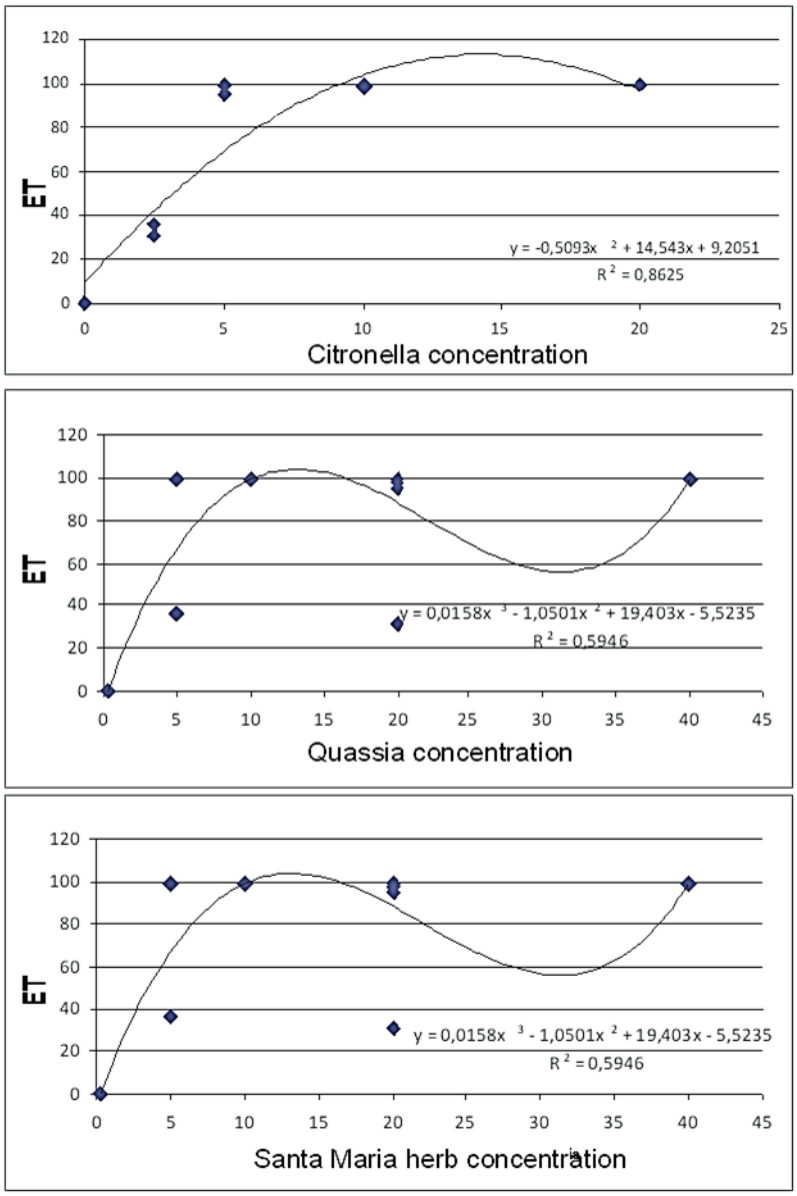

Figure 3. Adjustment of the regression equation to estimate the efficiency of the treatment (ET) on engorged female of *Rhipicephalus (Boophilus) microplus*, according to different concentrations of citronella, quassia and Santa Maria herb tinctures in association.

Studies made with distilled substances from citronella leaves demonstrated high tickicide action, both in larvae and adult females (CHUNGSAMARNYART & JIWAJINDA, 1992). Researchers conducted with citronella oil demonstrated its action as insecticide and repellent to mosquitoes and flies (RAJA et al., 2001). VERÍSSIMO & PIGLIONE (1998) observed that the natural citronella essence can act as a repellent to *R. microplus* larvae. In the present study, solutions with higher concentrations of citronella obtained superior efficiency rates, indicating a greater action of this medical plant on the cattle tick. The concentration with 10% of this plant obtained 100% of efficiency. The statistical tests support the idea that the action of citronella can be explained separately in the treatments, contrary to the other two plants.

Regarding the *in vitro* use of citronella, OLIVO et al. (2008) tested its oil obtained from fresh leaves by the distillation process and concluded that this plant had acaricidal activity. Most of the treatments obtained high efficiency related to the increase of citronella concentration. COSTA et al. (2008) found in their *in vitro* experiments that there was 100% of mortality of the engorged female immersed for 10, 20 and 40 minutes in citronella extract at 20%. In the study, the authors evaluated treatments with significant quantity of citronella in order to obtain high tickcide action and similar results were obtained in the present experiment that also points to the tickcide activity of this plant. These properties are attributed to the presence of volatile substances in its leaves such as citronellal, eugenol, geraniol and limonene, among

others, denominated in general as monoterpenes (SHASANY et al., 2000).

CONCLUSION

The different formulations containing association of citronella, Santa Maria herb and quassia tincture presented high *in vitro* efficiency on engorged female of *Rhipicephalus (Boophilus) microplus*. Citronella had greater acaricidal effect when compared to herb of Santa Maria and quassia. These associations are a potential alternative to use in the bovine tick control. *In vivo* researches must be performed aiming to prove the efficacy of these phytotherapics on the field.

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Submitted on: July, 18, 2012. Accepted on: November, 30, 2012.