



In vitro and *in vivo* acaricidal activity evaluation of organo-modified siloxanes in populations of *Rhipicephalus microplus*

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ABSTRACT

Infestations of *Rhipicephalus microplus* cause significant damage to cattle breeding and their control is primarily based on chemical products. There are extensive reports of efficacy losses of acaricidal products over time, as well as resistance of the parasites to them, thereby making it necessary to search for new alternatives. The present work aimed to determine the *in vitro* and *in vivo* acaricidal activity of organo-modified siloxanes with and without piperonyl butoxide (PBO) in southern Brazil. For the *in vitro* test, engorged females of *R. microplus* were collected and submitted to the immersion test. The formulation containing organo-modified siloxanes was tested at 4 different concentrations: 0.6, 1.0, 2.5, and 5.0%. The lowest dilutions (0.6, 1.0, and 2.5%) were also tested with the inclusion of 10% PBO. The *in vivo* test was performed by applying 2.5% organo-modified siloxanes, and the addition of 10% PBO was applied by spraying. The results showed high acaricidal activity (100%) *in vitro* in the concentration of 5% on non-associated forms, and in combinations of concentrations of 0.6, 1.0, 2.5% with PBO. The *in vivo* results also increased the efficacy with the association of PBO. In view of the current multi-resistance scenario of the *R. microplus* tick to the different commercially available acaricidal products, this study investigated the use of this product in association with PBO as an alternative to *R. microplus* control and found positive results. To the authors' knowledge, this is the first study to use organo-modified siloxanes against ticks.

1. Introduction

Rhipicephalus microplus is a monoxene tick, considered the main and most important ectoparasite that affects bovines between the 32° North and South parallels (Mathias, 2013). The damage caused includes injury in leather, decrease in reproduction rate and weight and production loss (Kessler and Schenk, 1998). Such damage leads to the estimated loss of US \$ 3.24 billion per year (Grisi et al., 2014). In order to alleviate this situation, acaricides have played an important role in the control of *R. microplus* since the beginning of the century when ixodidiosis was first considered a serious problem in cattle breeding (Chiminazzo et al., 2004).

In Brazil, there were registered populations of ticks resistant to most of the classes of drugs used, it is known that the indiscriminate use of acaricidal products contributed to the appearance of several cases resistance. Arantes et al. (1995) reported cases of resistance to organo-phosphates and synthetic pyrethroids. In the mid-1990s, the use of

macrocyclic lactones was intensified, serving as an alternative to the control of populations resistant to amitraz and synthetic pyrethroids, and also, by the ease of application. The results of this extensive use was the emergence of field populations resistant to this chemical group (Martins and Furlong, 2001), later, ivermectin resistance was detected through *in vitro* tests (Klafke et al., 2006). Castro-Janer et al. (2010) confirmed the occurrence of fipronil-resistant tick populations in Rio Grande do Sul, Minas Gerais, São Paulo, and more recently, resistance to fluzuron in the state of Rio Grande do Sul (Reck et al., 2014).

Organo-modified siloxanes are compounds that generate a specific contact action, resulting in dehydration, immobilization, and eventual suffocation of the parasite. They provide a physical mode of action and therefore, they become perfect candidates for a safe and effective alternative, delaying the development of resistance (Liszka and Dhang, 2014). Piperonyl butoxide (PBO) was semi-synthesized from safrole, the main component of camphor oil (Tozzi, 1999). It is a liquid of colorless or pale-yellow coloration and is moderately stable as it is resistant to

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hydrolysis, oxidation and exposure to sunlight (Kidd and James, 1994). Since its discovery and since pesticide resistance has emerged as a problem, PBO has often been used as an insecticidal synergist in over 1700 insecticides (United States Environmental Protection Agency, 2002). It is commonly associated with synthetic pyrethrins and pyrethroids, including in human and veterinary medicine (Tozzi, 1999). Considering the need for new alternatives for the control of *R. microplus*, this work was carried out to evaluate the *in vitro* and *in vivo* acaricidal activity of organo-modified siloxanes in populations of *R. microplus*, associated and unassociated with PBO.

2. Material and methods

The organo-modified siloxanes were administered using the product “Provecta ®” (ICBPharma) which more specifically, contains polyalkyleneoxide modified heptamethyltrisiloxane. Its Chemical Abstracts Service Number is 67674-67-3.

Protocols and procedures were approved by the animal experiment ethics committee of the Federal University of Santa Maria, Brazil, under protocol number 2881260318.

2.1. *In vitro* tests

Ten laboratory studies were conducted to evaluate the efficacy of the organo-modified siloxanes combined or not with PBO against *R. microplus*, a tick that commonly infests cattle. The tests were conducted in accordance with the adult immersion test (AIT) described by Drummond et al. (1973).

For each battery, approximately 200 female ticks were collected from animals that had not been treated with contact mode of action products for at least 21 days and also non treated with systemic mode of action products.

The collected ticks were kept refrigerated until the completion of the tests. The period between collection and testing did not exceed 24 h. The female ticks were weighed and divided into groups containing 10 ticks.

Each group was weighed, dipped in a different concentration of organo-modified siloxanes in water for 5 min., drained and allowed to dry on paper toweling (Table 1). The lowest concentrations tested were chosen according to guidance from other countries where it is already commercialized against bedbugs. The *in vitro* efficacy was established using the formulas described by Drummond et al. (1973):

RE = reproductive efficiency:

$$RE = (\text{eggs weight} \times \% \text{ of hatching eggs} \times 20,000) / \text{ticks weight}$$

(The 20,000 in this formula is an estimate of the number of larvae in 1 g of eggs.)

INR% = percentage of reproduction inhibition:

$$INR\% = (\text{RE of the control group} - \text{RE of the treated group}) / \text{RE of the control group} \times 100$$

Efficacy of the active ingredients was considered as the minimum value of 95%, according to relevant legislation for the commercial use

Table 1

Efficacy and lower and upper confidence intervals 95% (CI) of the *in vitro* organo-modified siloxanes concentrations against *Rhipicephalus microplus*.

Group	Efficacy % ^a	CI 95 %
Organo-modified siloxanes 5%	100	
Organo-modified siloxanes 2.5%	87.7	74.4; 101.1
Organo-modified siloxanes 2.5 + PBO 10%	100	
Organo-modified siloxanes 1.0%	83.9	74.1; 93.7
Organo-modified siloxanes 1.0 + PBO 10%	100	
Organo-modified siloxanes 0.6%	71.2	56.7; 85.7
Organo-modified siloxanes 0.6 + PBO 10%	100	

^a Estimated according Drummond et al. (1973) percentage of reproduction inhibition (INR%).

Table 2

Efficacy and lower and upper confidence intervals 95% (CI) of *in vivo* organo-modified siloxanes concentrations against *Rhipicephalus microplus*.

Group	Efficacy % ^a	CI 95 %
Organo-modified siloxanes 2.5%	93.7	73.6; 113.7
Organo-modified siloxanes 2.5 + PBO 10%	98.7	96.4; 101.1

^a Estimated according to Roulston and Wharton (1967) field acaricidal product test formula.

of tick pesticides in the country (Brazil MAPA, 1989). In addition, an acaricidal product currently available on the market (Chlorpyrifos 30%, Cipermetrin 15%, Fenthion 15%) was also tested with the sole intention of comparing the results with the of organo-modified siloxanes concentrations and associated forms with BPO10%.

2.2. Histopathology

The control group and the female ticks subjected to treatment with organo-modified siloxanes were used for histological evaluation. The selected concentration for the analysis corresponded to organo-modified Siloxanes 2.5% + PBO 10%. Female ticks were fixed in 10% formaldehyde solution for 15 days and then sectioned in a coronal plane. The two sections of each female tick were processed for histopathology and embedded in paraffin. Histological sections of 3 μm were then cut, stained using the hematoxylin and eosin technique and mounted on a microscope slide. Histopathological data were used for pre and post-treatment comparisons.

2.3. *In vivo* tests

For the inclusion in the experimental groups, animals from four rural properties in the central region of the state of Rio Grande do Sul were selected following the same protocols of the *in vitro* tests.

On day -2, the animals were contained and the ticks with a size of 4.5 mm–8.0 mm on one side of the animal were counted and multiplied by two, following Holdsworth et al. (2006). Only animals with a minimum count of 40 female ticks were selected to three experimental groups (Untreated control, organo-modified siloxanes 2.5% and organo-modified siloxanes 2.5% + PBO 10%) comprising 10 animals each.

The day of treatment application was designated as Day 0. It was performed by manual spraying with 5 L of the diluted product directed against the animal hair and throughout the body. On Day 7 each animal was examined to remove and count ticks.

Efficacy was determined by comparing pre-(day -2) and post-treatment (day 7) counts. All animals were submitted for reassessment and the live ticks in the animals' leather were recounted. A subsequent evaluation of the efficacy of the product used was then performed. The percentage of efficacy against *R. microplus* was calculated using arithmetic means, according to a formula proposed by Roulston and Wharton (1967) and recommended by Ordinance no. 48 of May 12, 1997, item 2.4.2, field test of acaricidal product (Brazil MAPA, 1997).

$$\text{Efficacy (\%)} = [1 - (Ta \times Cb / Tb \times Ca)] \times 100$$

Ta: average number of ticks post-treatment (day 7).

Tb: average number of ticks at day 2 before treatment

Ca: average number of control ticks at day 7.

Cb: average number of control ticks at day 2 before treatment.

3. Results and discussion

In this study, using the Adult Immersion Test, we demonstrated that all associated concentrations with BPO 10% reached 100% efficacy indexes against female *R. microplus*. However, when observing results from *in vivo* trials with naturally infested animals, the chosen

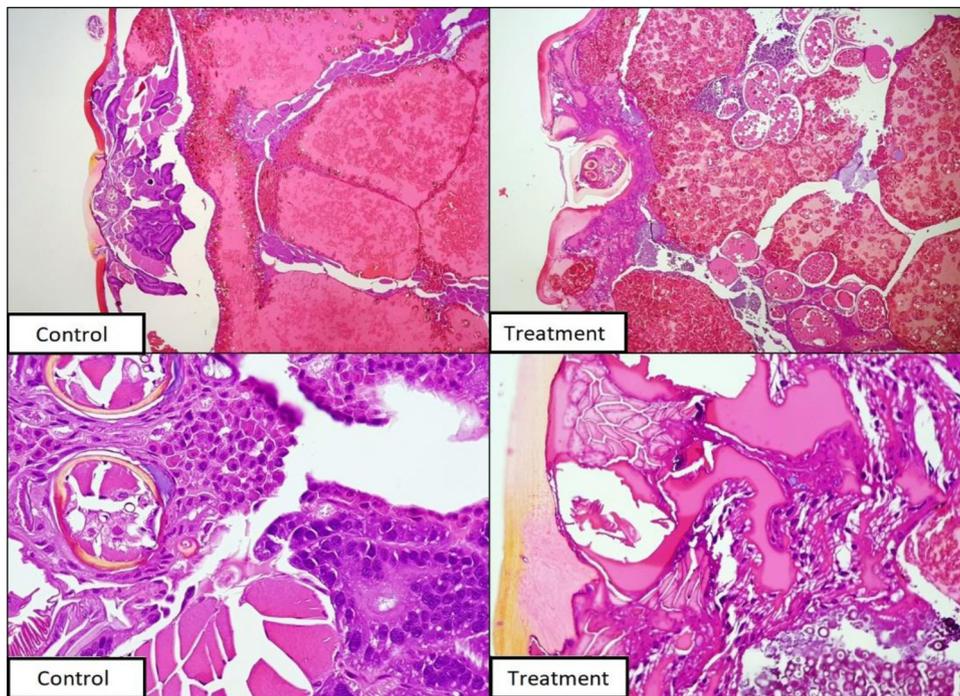


Fig. 1. Histological sections stained with HE of *Rhipicephalus microplus* (Control) and seven days after treatment with organo-modified Siloxanes 2.5% + PBO 10% (Treatment).

concentration of organo-modified siloxanes didn't reach the same index of acaricidal efficacy against the fully engorged female ticks, neither when associated with BPO10%.

As demonstrated (Table 1), the *in vitro* tests proved acaricidal activity of the organo-modified siloxanes. In groups submitted to organo-modified siloxanes without PBO, the 5% concentration was effective in all *R. microplus* samples tested (100%). The 2.5% concentration obtained 87.7% efficacy, while groups with concentrations of 1.0% and 0.6% efficacy of only 83.9% and 71.2% was achieved, respectively. These values were only improved with the association with PBO, where all concentrations resulted in 100% efficacy results.

A product already commercialized was also tested (Chlorpyrifos 30%, Cipermetrin 15%, Fenthion 15%), in order to make a comparison. As expected, the results of reproduction inhibition reached satisfactory values of 96.2%, allowing to be classified as an efficient acaricide. Despite the encouraging results of the organo-modified siloxanes, only the higher isolated concentration and associated forms of organo-modified siloxanes with PBO tested in this study reached higher parameters than the commercial product, evidencing the importance of the BPO.

For the *in vivo* tests (Table 2), organo-modified siloxanes 2.5% obtained 93.70% efficacy, while the result was 98.75% for organo-modified siloxanes 2.5% + PBO 10% formulation. Also showing a high synergistic effect of the PBO, as described by Tozzi (1999). It is important to note that in addition to the improve efficacy, the BPO also contributes to a lower margin of error of the obtained results, showing more concise data (Table 1 and 2).

This present study is the only that develops a description of the acaricidal activity of the organo-modified siloxanes on ticks. The findings are of great importance and may represent a new alternative and strategy of tick control in different production systems. Although the acaricidal activity of organo-modified siloxanes has not been previously tested in populations of *R. microplus*, the efficacy is not surprising given that already proved to be effective against bird mites and bedbugs (Liszka and Dhang, 2014). The development of a product with acaricidal activity is extremely important in view of the issue of tick resistance to acaricides already commercially available.

Little is known about the mechanism of action of the organo-modified siloxanes, however it is believed that they act specifically by contact, causing dehydration, immobilization, and eventual inactivation by suffocation of the parasite (Liszka and Dhang, 2014). The comparison of the tissues from pre and post-treatment ticks corroborate this hypothesis. Also, the tissues of engorged females pre- and post-treatment showed disintegration of tissues and cells after the treatment (Fig. 1). It is believed that lack of oxygenation may cause the cellular structure loss and damage.

Analyzing the results, we suggest that the use of organo-modified siloxanes can contribute to retarding the process of resistance development also to increasing the percentage of parasites susceptible to a certain active principle. This assumption is due to the mode of physical action, therefore, they become perfect candidates for a safe and effective alternative, delaying the development of resistance (Liszka and Dhang, 2014). Given that, we believe that the isolated use or association of the organo-modified siloxanes with other active principles will improve the general effectiveness.

4. Conclusion

The formulation of organo-modified siloxanes demonstrated high acaricidal activity (100%) *in vitro* in the concentration of 5% on non-associated form, and in combinations of 0.6; 1.0; 2.5% concentrations of organo-modified siloxanes with PBO 10%.

The non-associated concentration tested in the *in vivo* (organo-modified siloxanes 2.5%) also increased the efficacy when associated with PBO 10%, improving from 93.7%–98.7%. Providing results of efficiency, in compliance with the requirements and recommendations of the competent authorities (Brazil MAPA, 1997).

The findings demonstrated that in both tests (*in vitro* and *in vivo*), the organo-modified siloxanes showed acaricidal activity of organo-modified siloxanes on engorged females of *R. microplus*. Also, showed that the association with PBO potentiates the action of the organo-modified siloxanes, allowing to improve and use of a lower concentration. It is suggested that the use of organo-modified siloxanes associated or unassociated with PBO may be an excellent alternative for

the control of *R. microplus*, however, other studies need to be conducted to characterize both concentration/efficacy, forms of application, and interval between treatments.

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