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In vitro activity of the *Schinus terebinthifolius* (R.) extract on engorged female tick of *Rhipicephalus Boophilus microplus* and on larvae hatchability

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Abstract. Ticks are the main ectoparasites responsible for losses in cattle livestock due to spoliation, hematozoa transmission, and destruction of the leather; *Rhipicephalus Boophilus microplus* is the main agent in tropical countries. The control of these agents is done basically with chemical products, but this procedure has presented some challenges, such as the rapid development of ticks' resistance against them. In this context, the phototherapy is an important alternative for controlling these agents. *Schinus terebinthifolius,* known as Brazilian peppertree, presents antimicrobial, antifungal, and anti-inflammatory activities. Many organic molecules are reported as having effect on organisms extracted from this plant. This paper aimed to test the efficient activity of the *S. terebinthifolius* extract under different concentrations on the egg mass formation of *R. B. microplus* and on larvae hatchability *in vitro* assay. Engorged females of *R. B. microplus* were collected in a bovine herd and were taken to laboratory and submitted to immersion test into alcoholic extract at 100%, 50%, 25%, 10%, 5%, and 2.5% dilutions. Test in impregnated papers was carried out under the same concentrations to verify the extract efficacy that causes larval mortality. Control groups were composed, one with water, one with alcohol, and one with a commercial formulation containing cypermethrin, chlorpyrifos, and piperonyl butoxide. It was found that the extract at 70% hydroalcoholic solution showed acceptable efficacy in 50% and 100% dilutions. Result of the impregnated papers test showed 100% extract efficacy at 50% and 100% concentration. These results suggest this phytotherapic extract could be used for *R. B. microplus* control.
Keywords: Tick, Control, Phytotherapy, *Schinus terebinthifolius*, *Rhipicephalus Boophilus microplus.*

Introduction

The economic losses caused by ectoparasites in cattle herds in Brazil exceed US $ 2 billion per year (Peixoto et al., 2013; Barbosa et al., 2016; Chagas et al., 2016; Velez et al., 2017; Villarreal et al., 2017), of which 75% are assigned to ticks. The remaining 25% losses are assigned to horn fly, berne, myiasis, fly of the stables parasites (Domingues et al., 2013; Chagas et al., 2016; Rosado-Aguilar et al., 2017). Among ticks, the *Riphicephalus Boophilus microplus*, also known as cattle tick, is the main determinant agent as an obstacle to cattle livestock (Domingues et al., 2013; Rosado-Aguilar et al., 2017; Velez et al., 2017); besides being the most prevalent tick responsible for decreasing beef and milk production, it is also responsible for leather destructing and hematozoa transmitter agent as *Babesia* spp. and *Anaplasma* spp. species; these hematozoa are responsible agents for tick fever, another disease of high prevalence in the field, sometimes very severe, causing abortion and mortality of animals (Chagas et al., 2016; Csordas et al., 2018).

This parasite's control has basically been accomplished with chemicals, which cause harm to parasitized organisms; harming man, when consuming products of animal origin, and harming animals, cases of poisoning and death (Chagas et al., 2016; Kumar et al., 2016; Rajabpour et al., 2018). In addition, there is the development of resistance to the used chemicals, especially in dairy cattle herds (Singh et al., 2015; Sharifi-Rad et al., 2017). In this context, the phytotherapy is an important alternative for controlling parasites and may reduce the economic and environmental impacts rather than using synthetic pesticides (Shyma et al. 2014; Singh et al., 2015). It is also emphasized that the use of phototherapic products to control parasites can extend the useful life of chemicals (Kumar et al., 2016; Vinturelle et al.,

2017; Sharifi-Rad et al., 2017). The use of phytotherapic products could help slow development of resistance, which, being biodegradable, do not cause damage to environment (Madzimure et al., 2011; Singh et al., 2015). Furthermore, these products are safer and have a low cost compared to chemicals (Rosado-Aguilar et al., 2017; Sharifi-Rad et al., 2017).

Schinus terebinthifolius Raddi (Brazilian peppertree) is popularly known in Brazil as *aroeira*, *aroeira-da-praia*, *aroeira-vermelha*, *aroeira-mansa*, *aroeira-precoce*, *aroeira-pimenteira*, among others (Ceruks et al., 2007); it belongs to the plant kingdom, family origin or Tracheophyta division, Magnoliopsida class, Sapindales order, Anacardiaceae family (Lenz & Orth, 2004; Azevedo, Quirino & Bruno, 2015). This plant is originating from South America (Brazil, Paraguay, Uruguay, and eastern Argentina). In Brazil, its occurrence extends from Pernambuco State (Northeast region) to the Rio Grande do Sul State (South region) (Ceruks et al. 2007; Azevedo, Quirino & Bruno, 2015).

The Brazilian peppertree has commercial importance because it is a plant with medicinal and phytochemical properties (Lenz & Orth, 2004; Ceruks et al. 2007). The morpho-histological features and chemical composition of *S. terebinthifolius* species, aiming at its laboratory recognition as a drug, are related to the leaves and bark that have proven to be rich in tannins and essential oils; saponins are restricted to barks (Azevedo, Quirino & Bruno, 2015). The bark phenolic compounds are different from the leaf phenolic compounds (Azevedo et al., 2015). Antimicrobial, antifungal, and anti-inflammatory activities can be highlighted among its pharmacological importance (Bendaoud et al., 2010; Azevedo, Quirino & Bruno, 2015). Many organic molecules are reported that have effect on organisms extracted from this plant (Bendaoud et al., 2010). However, there are no records in literature, in which the use of Brazilian peppertree for controlling ticks has been tested.

This study aimed to test the Brazilian peppertree (*S. terebinthifolius*) extract efficacy under different concentrations on the egg mass formation of *R. B. microplus* and on *in vitro* larvae hatchability.

Methods

A sample of hydroalcoholic extract of the *Schinus terebinthifolius* (Brazilian peppertree) leaf and bark was used for the experimental trial. Part of the plant was extracted from trees belonging to rural area of Castelo municipality, Espírito Santo State, Brazil, and the sample was sent to the Parasitology Laboratory of Castelo College. The extract was done in a 70% hydroalcoholic solution and maintained for three days in amber glass.

The collection of five hundred and forty engorged female ticks of *Rhipicephalus Boophilus microplus* species were carried out from cattle of a livestock farm in Castelo municipality. The animals of the control group of parasitic tick were treated previously to 70 days before the female tick collection with association of organophosphate and pyrethroid.

Hydroalcoholic extract (to 20% plant extract) was evaluated with serial dilutions to 100%, 50%, 25%, 10%, 5%, and 2.5% from matrix solution. Control groups were composed by 100% water and 70% hydroalcoholic solution. The effect of a commercial formulation of cypermethrin (5g/100ml), chlorpyrifos (2.5g/100ml), and piperonyl butoxide (1.0g/100ml) in 20% water was also tested to compare with the chemical control effect in relation to phytotherapic extract.

To evaluate the effectiveness of the hydroalcoholic extract of Brazilian peppertree leaves and bark, immersion test of engorged female ticks was carried out in accordance with the procedures recommended by Chagas et al. (2011). The tests were carried out with six replicates of ten engorged female ticks per plate, in which:

Estimated reproduction: ER

$$ER = \frac{\text{egg weight} \times \%\ \text{hatching} \times 20.000^*}{\text{Female weight}}$$

**Constant that indicates the number of eggs present in 1g of stance*

Product efficacy: PE

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

To be considered effective, phytotherapic treatment should present 95% efficiency, similar to efficiency to chemicals acaricide (Kumar et al., 2016; Barbosa et al., 2016; Souza et al., 2017).

Larval test was also carried out with impregnated paper in which the engorged female ticks were placed in Biochemical Oxygen Demand (BOD) (27 °C and RH>80%) to produce eggs and larvae. For the tests, larvae from 14 to 21 days old were used. In accordance with Chagas et al. (2011), the extracts were tested with about a hundred larvae placed between 2x2 cm filter papers newly impregnated with extracts immersed into 100%, 50%, 25%, 10%, 5%, and 2.5% concentrations, and in the control groups under 100% water, 70% hydroalcoholic solution, and an associated commercial formulation of cypermethrin to chlorpyrifos and piperonyl butoxide in 20% water. This test was carried out with 3 replicates.
The following calculation method was used:

Calculation of mortality:

$$\text{Mortality} = \frac{\%\ \text{control mortality} - \%\ \text{treated mortality}}{\text{Control mortality}} \times 100\%$$

$$\text{Mean Mort. (\%)} = \frac{\text{mort.of repetition 1} + \text{mort. of repetition 2} + \text{mort. of repetition 3}}{3}$$

The mortality rates of the groups were considered in the interpretation of impregnated papers test of larvae.

The average temperature and humidity in the laboratory were recorded during the experimental test.

Results and discussion

It was found that the mortality of *Rhipicephalus Boophilus microplus* female ticks ranged from 0% to 20% (Table 1); mortality different from 0% was observed in chemical control groups and in the groups in which plant extract formulation of *Schinus terebinthifolius* was used at 50% to 100% concentrations.

The groups of ticks treated with the commercial solution of chemical insecticide and the maximum concentration of the phytotherapic formulation showed efficacy above 99.9% (Table 2). The *S. terebinthifolius* extract at 70% hydroalcoholic solution showed acceptable efficacy without water dilution (100%) and diluted to 50%.

S. terebinthifolius extract showed efficacy in mortality of engorged female ticks (Table 1), 15% at 50%; and 20% at 00% concentration.

It is verified that the hydroalcoholic extract formulations of Brazilian peppertree presented relevant efficacy in relation to the hatching control of viable larvae in larval test with impregnated papers.

It was verified that larval mortality in 100% hydroalcoholic formulation of Brazilian peppertree extract occurred at 50% and 100% concentrations (Table 3). At 25% extract concentrations, there were 93.67% efficacy on larvae, and still 75.5% larvae inactivation when the extract was at 10% concentration.

Table 1. Weight medium of engorged females eggs, of the ticks and mortality of *Rhipicephalus Boophilus microplus* of the treatments with Brazilian peppertree extracts (*Schinus terebinthifolius* Raddi) at concentrations 100, 50, 25, 10, 5 and 2.5% and cypermethrin association and chlorpyrifos (20% concentration in water) and of the control groups: with water (100%) and 70% alcohol.

Repetitions	Eggs weight (grams) - 15 days	Ticks Weight (grams) - 15 days	Mortality (%)
Water C1	0.9785	2.2346	0.00 ± 0.00
Alcohol 70% C2	0.7236	2.2546	0.00 ± 0.00
Cipermetrin (0.5%) e clorpyriphos (0.25%) associated at piperonil butoxid (0.1%) (20% water) T1	0.0227	2.2436	15.00 ± 5.48
Brazilian peppertree extract 2.5% T2	0.6904	2.2493	0.00 ± 0.00
Brazilian peppertree extract 5% T3	0.4294	2.2701	0.00 ± 0.00
Brazilian peppertree extract 10% T4	0.4854	2.2553	0.00 ± 0.00
Brazilian peppertree extract 25% T5	0.4999	2.2336	0.00 ± 0.00
Brazilian peppertree extract 50% T6	0.0293	2.2603	15.00 ± 5.48
Brazilian peppertree extract 100% T7	0.0074	2.2527	20.00 ± 6.32

Table 2. Estimated reproduction (ER) and product efficacy (PE)% of *Rhipicephalus Boophilus microplus* of the treatments with Brazilian peppertree extracts (*Schinus terebinthifolius* Raddi) at concentrations of 100, 50, 25, 10, 5 and 2.5% and of the control groups: with water (100%) and with 70% alcohol.

Repetitions	Estimated reproduction: RE	Product efficacy: EP (%)
Water C1	873,660.71	-
Alcohol 70% C2	257,280.00	70.55
Cipermetrin (0.5%) e clorpyriphos (0.25%) associated at piperonil butoxid (0.1%) (em 20% em água) T1	2,026.79	99.97
Brazilian peppertree extract 2.5% T2	185,757.85	78.74
Brazilian peppertree extract 5% T3	94,581.50	89.17
Brazilian peppertree extract 10% T4	86,293.33	90.12
Brazilian peppertree extract 25% T5	88.477.88	89.87
Brazilian peppertree extract 50% T6	2,616.07	99.71
Brazilian peppertree extract 100% T7	654.87	99.93

Table 3. Larval mortality (mean ± standard deviation) of *Rhipicephalus Boophilus microplus* caused by the impregnation after 24 hours by Brazilian peppertree extracts (*Schinus terebinthifolius* Raddi) in concentrations of 100, 50, 25, 10, 5 and 2.5% and of association of cypermethrin and chlorpyrifos (20% in water) and control groups with 100% water and 70% alcohol.

Repetitions	Mortality (%)
Water C1	0.00 ± 0.00
Alcohol 70% C2	0.00 ± 0.00
Cipermetrin (0.5%) e clorpyriphos (0.25%) associated at piperonil butoxid (0.1%) (em 20% em água) T1	100.00 ± 0.00
B extract 2.5% T2	0.00 ± 0.00
Brazilian peppertree extract 5% T3	40.33 ± 5.75
Brazilian peppertree extract 10% T4	75.50 ± 3.73
Brazilian peppertree extract 25% T5	93.67 ± 2.58
Brazilian peppertree extract 50% T6	100.00 ± 0.00
Brazilian peppertree extract 100% T7	100.00 ± 0.00

The average temperature in the laboratory was 26.5 °C and the relative humidity was 78%, respectively. These variables favorably collaborated for laying eggs of female ticks and larval hatching (Fouche et al., 2017).

The *Schinus terebinthifolius* extract at 50% and 100% is in accordance with the criteria established by rules (Madzimure et al., 2011; Fouche et al., 2017), which requires 95% minimum effectiveness of acaricides.

The animals that offered the tick sample were not resistant to the commercial formulation tested in the laboratory. This was the first trial in which the formulation of Brazilian peppertree extract was tested to the bovine tick control. This plant is common in the Brazilian Atlantic forest and easily colonize another environment (Ceruks et al. 2007; Azevedo, Quirino & Bruno, 2015).

Since a phytotherapic extract has satisfactory efficacy against parasites tested *in vitro,* other tests are carried out aiming to isolate the molecules present in extracts of this plant (Chagas et al., 2016, Souza et al., 2017; Vélez et al., 2017). The indiscriminate use of acaricides contributed in some way to increase chemical resistance, and this fact encouraged increasing researches on phytotherapic plant extracts. Brazilian peppertree presented promising efficacy for tick control *in vitro* and presents effective molecules against bacteria and pathogenic fungi to man, in accordance with some authors (Lenz & Orth, 2004; Bendaoud et al., 2010; Azevedo, Quirino & Bruno, 2015). Due to the high potential of the Brazilian peppertree extract *in vitro* control against ticks, it is suggested that more refined investigations may be carried out to identify the presence of organic compounds responsible for combating *R. microplus*. Rosado-Aguilar et al., 2017, Souza et al. (2017) suggests that these plants undergo an evaluation to identify molecules that may have effect against *R. microplus*.

Efficiency of 93.3% was verified by Kumar et al. (2016) with *Carica papaya* extract against *R. microplus*, but the test was carried out with more polar solvent (methanolic extract compared to those used in this work with *S. terebinthifolius*). The essential oils of *Mentha arvensis* at 100% (20% extract) showed 73% efficacy on *R. microplus* larvae (Chagas et al. 2016). The ethanol extract of *Monsonia angustifolia* showed of 97.8% of mortality (Fouche et al., 2017). Chagas et al. (2011; 2016) report that some phytotherapic extracts have similar efficacy to those verified by commercial chemical formulations.

In this test, it was verified that Brazilian peppertree extract showed similar efficacy to the tested commercial chemical formulation; the chemical compound was diluted to 20% in water; the extract concentrations were tested to 20% phytotherapic extract (crude extract) in hydroalcoholic solution. In addition, the Brazilian peppertree extract also showed 100% efficacy on larval mortality, at the 10% final extract concentration (50% hydroalcoholic solution plus 50% in distilled water) and presented 15% in the average weight reduction of engorged female ticks, showing to be effective in reducing the oviposition. Superior effect on hatchability of eggs was verified in *S. terebinthifolius* compared to *Murraya koenigii* (Singh et al., 2015) which did not showed this activity.

According to Shyma et al. (2014), Villarreal et al. (2017) when crude extracts showed satisfactory results (good acaricidal properties), as *S. terebinthipholius* showed, this could be a potential component of alternative *R.* (*B.*) *microplus* tick control strategy.

Conclusion

The formulation of the *Schinus terebinthifolius* plant extract showed satisfactory activity at 50% and 100% concentrations, efficacy higher than 99.7% on engorged female ticks of *Rhipicephalus Boophilus microplus* species, and satisfactory efficacy in larval mortality.

Conflict of Interest Statement

We have verified that we have no conflict of interest.

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