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Short communication

Effect of aqueous extracts of *Baccharis trimera* on development and hatching of *Rhipicephalus microplus* (Acaridae) eggs

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ABSTRACT

This study evaluated the effects of aqueous extracts of *Baccharis trimera* (Less.) DC (Asteraceae), *colloquially* known as carqueja, on egg production, and hatching rate of larvae of *Rhipicephalus microplus*. Plant samples were collected in Montes Claros, north of Minas Gerais, Brazil. Adult female ticks were distributed into 24 homogeneous groups of 10. The *in vitro* test was performed by immersing each group in 10 ml solutions of aqueous extracts at 50, 100, 150, or 200 mg of fresh leaves ml^{-1}. These concentrations were compared with distilled water as negative control and a commercial product as positive control and the tests were repeated four *times*. The carqueja extract at concentrations of 150 and 200 mg of fresh leaves ml^{-1} showed 100% efficacy in inhibiting egg hatching and therefore could have potential as an acaricide.

1. Introduction

Infestation by *Rhipicephalus* (*Boophilus*) *microplus* can reduce bovine productivity through anemia associated with blood loss, the release of toxins at the bite location, and transmission of possibly lethal parasitic diseases (Grisi et al., 2002; Campos Júnior and Oliveira, 2005; Mans et al., 2004).

The climate in Brazil is suitable for completion of the life cycle and dispersion of the tick in most regions throughout the major part of the year (Magalhães and Lima, 1992; Grisi et al., 2002; Campos Júnior and Oliveira, 2005). *Rhipicephalus microplus* is the cause of almost 75% of losses associated with bovine ectoparasitism, which totals two billion dollars annually in Brazil (Grisi et al., 2002). The losses can be still higher for *Bos taurus* and its crossbreeds, which are notoriously susceptible to ticks (Campos Júnior and Oliveira, 2005).

Alternatives to conventional acaricides should be evaluated, as the resistance of ticks to synthetic compounds is widespread. This is related to indiscriminate use, which contributes to the selection of resistant populations and reduces the useful life of the available compounds (Campos Júnior and Oliveira, 2005; Clemente et al., 2008; Fernandez-Sala et al., 2012). Developing new drugs is expensive, and it is not always possible to keep pace with the evolution of resistance. Conventional products can also promote contamination of the environment and of food for human consumption (Clemente et al., 2008; Santos et al., 2009).

Therefore, research on alternative methods of control with plant substances as potential acaricides is of scientific and practical relevance (Prates et al., 1993; Chagas, 2004; Fernandes et al., 2005). Effective plant extracts could reduce drug resistant tick populations, preserving commercial acaricides (Chagas, 2004).

The genus *Baccharis* comprises 150 species, colloquially known as carqueja, bacárida, and bacórida cacália (Lorenzi and Abreu, 2008). Allelopathic, analgesic, anti-fungal, anti-inflammatory, antimicrobial, anti-mutagenic, anti-oxidant, anti-viral, and insecticidal actions have been described in these species (Nakasugi and Komai, 1998; Abad et al.,

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Table 1
Mean egg mass (g), number of hatched larvae (*n*), and the reproductive efficacy of cattle tick treated with varying concentrations of *Baccharis trimera* aqueous extract. These are compared with efficacy using distilled water or commercial acaricide (12.5 mg l^{-1} cypermethrin combined with 150 mg l^{-1} dichlorvos).

Treatments	Eggs mass (g)	Hatched larvae, *n*	Reproductive efficacy	Effectiveness (%)[a]
Baccharis trimera				
50 mg ml^{-1}	0.55 [ab]	815.00 [ab]	38,389.96	64.89
100 mg ml^{-1}	0.53 [ab]	749.00 [ab]	35,580.08	67.47
150 mg ml^{-1}	0.47 [ab]	0 [c]	0.00	100.00
200 mg ml^{-1}	0.42 [bb]	0 [c]	0.00	100.00
Positive control[b]	0.24 [cc]	20.00 [c]	941.16	99.14
Negative control	0.61 [aa]	2,246.63 [a]	109,355.79	0.00
Variation coefficient (%)	20.51	37.32		

Means followed by same letter in columns are similar statistically by Duncan's test at 5% probability ($P \le 0.05$).
[a] Means obtained by the equation from Drummond et al. (1973).
[b] Commercial acaricide, containing 12.5 mg ml^{-1} cypermethrin and 150 mg ml^{-1} dichlorvos-phosphate.

1999; Feresin et al., 2003; Paul et al., 2009). The objective of this study was to investigate the effects of carqueja aqueous extract (CAE) to egg production and hatching of *R. microplus*.

2. Materials and methods

The plant was grown in the medicinal garden of the Institute of Agricultural Sciences, Federal University of Minas Gerais (ICA/UFMG), Montes Claros, Brazil. The plants were collected in July and August 2010, and cataloged in the herbarium of this institution. Fresh leaves were chopped into approximately 2 cm^2 pieces, and the aqueous extract was prepared by adding 20 g of fresh leaves to 100 ml boiling distilled water, according to the method by Siqueira (1956). This mixture was placed in a beaker and maintained at boiling point for 15 min. The initial volume was maintained by adding sterile distilled water, followed by hot filtration through a cotton and gauze funnel. The CAE at a concentration equivalent to 200 mg of fresh leaves ml^{-1} was diluted with sterile distilled water, to make 150, 100, 50 mg ml^{-1} solutions.

Engorged adult females of *R. microplus* were collected from naturally infested Holstein cows in Bocaiúva city of Minas Gerais State, Brazil. Ticks were packed in aerated plastic containers and sent to the ICA/UFMG. Females larger than 4 mm were selected, washed with distilled water, placed on paper towels, and divided into 24 homogeneous groups of 10 females, based on the degree of engorgement and weight. Sterile distilled water was used as negative control, and a commercial product containing the mixture of 12.5 mg ml^{-1} of cypermethrin and 150 mg ml^{-1} of dichlorvos-phosphate (Flytick Plus, Valleé S.A., São Paulo, SP, Brazil), diluted as recommended by the manufacturer, was the positive control. This commercial product was selected because it was successfully used for tick control in the farm. The collection of engorged female ticks was conducted at the beginning of the rainy season between December and February after at least 60 days of last acaricide application.

The acaricidal effectiveness was evaluated by an immersion test according to Drummond et al. (1973). The 24 groups were immersed in 10 ml of test solution for 5 min. Excess solution was removed with a paper towel, and ticks were placed in a Petri dish and maintained at 28 °C and 70% relative humidity in an environmental chamber BOD incubator.

Fifteen days after the initiation of tick egg laying, the mass of eggs for each group was determined on an analytical scale and transferred to 3 ml plastic syringes. Thirty days after the start of hatching, the syringe contents were transferred to Petri dishes, and the larvae and eggs were counted under a stereoscopic microscope to determine the hatching rate in each group.

The efficacy of treatment (product efficacy) was estimated using the equation of Drummond et al. (1973):

$$\text{ER (reproductive efficacy)} = \frac{\text{Egg weight} \times \%\ \text{hatching} \times 20,000^{*}}{\text{Tick weight}}$$

*Constant of 20,000 eggs g^{-1} of mass.

$$\text{EP (efficacy of product)} = \frac{(\text{ER control group} - \text{ER treated group})}{\text{ER control group}} \times 100$$

A randomized design was used to compare the four carqueja concentrations with distilled water control and a commercial acaricide control and the tests were repeated four times. The data were log transformed ($x + 10$) and subjected to analysis of variance. The means were compared by Duncan's test ($P \le 0.05$). The concentration of the extract sufficient to inhibit 90% of the hatching (LC_{90}) was calculated by probit analysis using the statistical package Saeg 9.1 (Saeg, 2007).

3. Results and discussion

The average egg mass produced by ticks treated with 200 mg ml^{-1} of CAE was significantly lower than that of the negative control with distilled water ($P \le 0.05$). There was no hatching at concentrations of 150 and 200 mg ml^{-1} (Table 1). Thus, carqueja aqueous extract at these concentrations produced 100% inhibition of egg hatching, higher than the 95% minimum efficacy required for conventional acaricides (Brasil, 1990). The LC_{90} of CAE on hatching inhibition of *R. microplus*, estimated by probit analysis was 121.84 mg of fresh leaves ml^{-1} (Fig. 1).