Veterinary Parasitology 197 (2013) 341–345

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Protective action of *Tagetes minuta* (Asteraceae) essential oil in the control of *Rhipicephalus microplus* (Canestrini, 1887) (Acari: Ixodidae) in a cattle pen trial

Renato Andreotti[a,*], Marcos Valério Garcia[a], Rodrigo Casquero Cunha[b], Jacqueline Cavalcante Barros[a]

[a] *Embrapa Beef Cattle, Avenida Rádio Maia, 830, Vila Popular, Caixa Postal 154, CEP 79106-550 Campo Grande, MS, Brazil*
[b] *Doctor degree student in the Graduate Program in Animal Science, Federal University of Mato Grosso do Sul (UFMS) - Fellow CAPES/REUNI, Brazil*

ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form 25 April 2013
Accepted 30 April 2013

Keywords:
Rhipicephalus microplus
Cattle tick
Tagetes minuta
Control
Phytoterapics
Bovines

ABSTRACT

The *Rhipicephalus microplus* tick is globally regarded as the most economically important ectoparasite of livestock, and the evolution of resistance to commercial acaricides among cattle tick populations is of great concern. The essential oil derived from *Tagetes minuta* may be efficacious against cattle tick infestation, and the results of a cattle pen trial using this essential oil for the control of ticks are reported here. The chemical composition of the essential oil was determined by GC–MS and NMR spectroscopy analyses, which revealed the presence of four major components in the essential oil. These components represent more than 70% of the essential oil: limonene (6.96%), β-ocimene (5.11%), dihydrotagetone (54.10%) and tagetone (6.73%). The results of the cattle pen trial indicated significant differences among the average values of the analyzed biological parameters, including the number of ticks, the average weight of the ticks, the average egg weight per engorged female and larval viability. Treatment with the *T. minuta* essential oil prepared in this study promoted significant effects on all biological indicators analyzed. Based on the biological indicators, the essential oil showed 99.98% efficacy compared to the control group when used at a 20% concentration. The results obtained in this study suggest that the *T. minuta* essential oil is a potential *R. microplus* tick control agent and may be used to mitigate the economic losses caused by tick infestation.

1. Introduction

The southern cattle tick, *Rhipicephalus microplus*, inflicts severe economic losses to the livestock industry. Infestation by *R. microplus* causes losses in productivity directly through the effects of ectoparasitism and indirectly through its role as the vector for *Babesia bovis* and *Babesia bigemina*, which cause bovine babesiosis, and *Anaplasma marginale*, which causes anaplasmosis (Grisi et al., 2002; De la Fuente et al., 2008).

The control of *R. microplus* is achieved primarily through the use of chemical acaricides (Andreotti et al., 2011). However, chemical acaricides have not been utilized judiciously, which has led to the evolution of resistance among populations of *R. microplus* (Furlong, 2004; Rosario-Cruz et al., 2009).

The use of plant extracts as tick control agents has been an area of focused research in several countries (Chungsamarnyart et al., 1991; Williams, 1993; Vatsya et al., 2006; Álvarez et al., 2008). Studies using emulsifiable oils from eucalyptus (*Eucalyptus* spp.: Myrtaceae),

* Corresponding author. Tel.: +55 67 33682169; fax: +55 67 33682169.
E-mail address: renato.andreotti@embrapa.br (R. Andreotti).

0304-4017/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2013.04.045

carotenoids extracted from timbo (*Derris urucu*: Fabaceae) (Veríssimo, 2004), and azadirachtin A, which is present in plants of the family Meliaceae (*Melia azedarach*) (Borges et al., 2003; Sousa et al., 2008), have shown promise in controlling this parasite.

Tagetes minuta (Asteraceae) is an annual perennial herb that belongs to the Asteraceae family. This plant's leaves are slightly glossy, green and pinnately dissected into 4–6 pairs of pinnae (Abdel-Shafy and Zayed, 2002; Bastos et al., 2010). This plant is used in popular medicine and grows in temperate regions of South America (Chamorro et al., 2008). The major components of *T. minuta* oil have been previously found to be α-terpineol, (Z)-β-ocimene, dihydrotagetone, (E)-ocimenone, (Z)-tagetone, and (Z)-ocimenone, which is consistent with the composition observed in the present study (Moghaddam et al., 2007).

Previously, a 20% concentration of *T. minuta* essential oil was shown to be needed for its acaricidal effect and was over 95% effective for controlling the following tick species: *R. microplus*, *Rhipicephalus sanguineus*, *Amblyomma cajennense* and *Argas miniatus*. The efficacy of *T. minuta* essential oil has been assayed using the adult immersion test (AIT) and larval packet test (LPT) (Garcia et al., 2012).

Because the control of *R. microplus* using acaricides is problematic, there is a need to find new control strategies. Our hypothesis is that *T. minuta* essential oil is a potential tool for the control of the ectoparasite *R. microplus*. To support this hypothesis, the efficacy of *T. minuta* essential oil in controlling *R. microplus* infection was tested using a cattle pen trial. The *T. minuta* essential oil prepared in this study was analyzed with regard to its effects on tick biology, and the results of a cattle pen trial using this essential oil to control the *R. microplus* tick are reported here.

2. Materials and methods

2.1. Ticks

Ticks used for cattle infestation were obtained from a six year laboratory colony that was sensitive to pyrethroid (SP), organophosphate (OP) and amitraz (Am) and free of *Babesia* sp. and *Anaplasma* sp. The tick colony was maintained at Embrapa Beef Cattle (20°26′ S, 54°43′ W) in Campo Grande, MS, Brazil (Andreotti et al., 2012).

2.2. Plant material and extraction

T. minuta leaves and stems were cultivated at Embrapa Beef Cattle (20°26′ S, 54°43′ W) in Campo Grande, MS, Brazil, dried at 40 °C for 72 h and ground in a grinder with a 5-mm mesh. The total biomass was subjected to steam distillation to extract the essential oil. The plant material was placed on a perforated plate, which served as a support and also allowed for the homogeneous flow of steam. The plate containing the plant material was placed on an extractor, and a gooseneck pipe was attached to transfer the vapors to a condenser (Prakaso et al., 1999).

The steam carried the volatile organic compounds (essential oil) that were present in the plant material into the vapor phase. A container was placed at the end of the condenser to separate the essential oil from the water. The extraction was conducted for 2 h inside an extractor at normal atmospheric pressure and at a temperature of 96–97 °C. The residual water from the essential oil isolation was removed by filtration with anhydrous sodium sulfate. The essential oil was stored in amber flasks.

2.3. Oil extract chromatography (GC/MS)

The essential oil extract was analyzed qualitatively and quantitatively using a GCMS-QP2010 Plus (Shimadzu, Tokio, Japan) equipped with an Rtx®-WAX Crossbond-Carbowax-polyethylene glycol column (30 m × 0.25 mm i.d. × 0.25 μm film thickness – Restek, Bellefonte, PA, USA), a split injector (split ratio 50:1), an automatic injection system and a selective mass detector. The test was performed at 250 °C, and the oven temperature was programmed to increase the temperature from 50 °C to 210 °C at 10 °C/min using He as the carrier gas. The gas flow was 0.7 mL/min at a constant speed of 30 cm/s and an interface of 250 °C. The injector temperature was 200 °C, and the injection volume was 1.0 μL. The sample was prepared in $CHCl_3$. The peak area percentages were calculated without correction factors or internal standards. The peaks were identified by comparison of their mass spectra (MS) to the mass spectral data from the National Institute of Standards and Technology (NIST) and Wiley's FFNSC (Flavor and Fragrance Natural and Synthetic Compounds). The results obtained through this method were also based on an analysis of the fragmentation pattern obtained for each component and comparison of their retention indexes (IR) with the Shimadzu GCMS Solution Program, version 2.53.

2.4. T. minuta (Asteraceae) essential oil

T. minuta essential oil was prepared at a 20% concentration because previous studies showed that this concentration is sufficient for the acaricidal effect, and this concentration is also over 95% effective on the *R. microplus* tick in the adult immersion test (AIT) and larval packet test (LPT) (Garcia et al., 2012). The solution applied to the animals was prepared as a pour-on formulation (*T. minuta* essential oil 20%, isopropanol 72%, DMSO 8%, v/v).

2.5. Cattle pen trial and efficacy assessment

One-year-old Holstein calves were randomly distributed into two groups of six animals each. The bovines were infested with 5000 larvae at three separate time points, specifically, on days 0, 9 and 18. The infestations were performed on these days to produce larvae, nymphs and adults on the day of treatment. All of the animals were infested with ticks on the same day. On the 20th day after the beginning of infestation, one group was treated with 20% *T. minuta* (Asteraceae) essential oil. The solution was applied onto the skin of each animal using approximately 50 mL of solution. The application of the *T. minuta* essential oil was done using a 50 mL falcon tube. The oil formulation was distributed longitudinally on the dorsum of the animals, uniformly. Did not formed clumps in the hair coat, and was not there dripping. The second group (control group) was evaluated for tick production. The tick