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Efficacy of *Tagetes minuta* (Asteraceae) essential oil against *Rhipicephalus sanguineus* (Acari: Ixodidae) on infested dogs and in vitro

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Abstract Ticks from *Rhipicephalus sanguineus* complex are widely distributed in the world and one species from this complex is the most common tick on dogs in Brazil, notably in urban areas. This tick is a vector of several diseases. Among others it transmits the agent of canine Ehrlichiosis, a major dog infectious disease and the agent of Rocky Mountain spotted fever. This tick can spread rapidly and develop intolerable infestations within no time. Currently tick control is done with acaricides and demand for such drugs has grown fast. However, *R. sanguineus* has already developed resistance to the main active compounds and the development of new acaricides is necessary. Many essential oils of plants have acaricidal effect and may be an important source of molecules for the synthesis of new acaricide products. In this study, we evaluated the effectiveness of a new herbal phytotherapic, consisting of the essential oil of *Tagetes minuta* L., against *R. sanguineus* in vitro and on dogs undergoing experimental infestations. The product displayed 100% efficacy against larvae, nymphs and adults of the tick on all tested conditions.

Keywords Control · Infestations · Phytoterapics · *Rhipicephalus sanguineus* · *Tagetes minuta*

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Introduction

Ticks are a major vector of infectious disease agents to both humans and domestic animals (Jongejan and Uilenberg 2004; Estrada-Peña and Jongejan 1999). Among ticks, the *Rhipicephalus sanguineus* complex has the broadest distribution in the world (Walker et al. 2000; Nava et al. 2015). This complex transmit major dog disease agents such as those of canine ehrlichiosis and babesiosis, (Walker et al. 2000; Guglielmone et al. 2006) and several possibly lethal human pathogens such as, *Rickettsia conorii* (Estrada-Peña and Jongejan 1999) and *Rickettsia rickettsii* (Demma et al. 2005).

In Brazil a yet undefined species of the *R. sanguineus* complex is the main dog tick, especially in urban areas (Labruna and Pereira 2001; Szabó et al. 2001, 2010). Dogs are an adequate host for all parasitic stages (Labruna 2004), and dogs do not develop an efficient immune response against the tick (Szabó et al. 1995).

Thus, the wide distribution of *R. sanguineus* within the country, its capacity to attain high infestation levels of both dogs and in household environments and the frequent tick-borne diseases of dogs prompt an ever increasing need for tick control. Currently such control relies almost exclusively on chemical acaricides, but resistance of *R. sanguineus* ticks to these chemicals has been reported (Guglielmone et al. 2004). For these reasons new tick control products are in demand.

Phytotherapy is treatment based on the use of plants and herbs. *Tagetes minuta* L. (Asteraceae), known in Brazil as cravo-de-defunto, is an aromatic plant from South America (Lovatto et al. 2013). The essential oil of this plant has already been shown to control in vitro this and other tick species (Garcia et al. 2012). We herein evaluated the efficacy of a phytotherapic compound from *T. minuta,* essential oil against *R. sanguineus* both in vitro and on dogs undergoing experimental infestations.

Materials and methods

Ticks

Ticks from a laboratory *R. sanguineus* (tropical lineage) colony were used throughout the experiments. Ticks from the colony were fed on tick-bite naive New Zealand rabbits; however, the colony was periodically renewed with engorged females that fed on healthy dogs from Uberlândia, Minas Gerais, Brazil.

Hosts

For the evaluation of the efficacy of the *T. minuta* essential oil acaricide on ticks in parasitic phase, male and female mixed-breed dogs were used as hosts. Dog puppies were obtained from private owners, vaccinated and dewormed according to routine veterinary procedures and held for at least one week in a kennel at Federal University of Uberlândia, prior to experimental infestations. Dogs were fed with commercial ration twice per day and water ad libitum. All procedures with animals were approved by the Institutional Committee for Animal Experimentation of the University (protocol 009/13 final document 031/13).

Phytotherapic acaricide

A 20% solution of *T. minuta* oil in 2% Tween was used an acaricide, as described previously (Garcia et al. 2012).

Efficacy evaluation against ticks feeding on dogs

Ticks were placed inside a feeding chamber consisting of a plastic tube with 5 cm diameter and 3 cm height, glued, on the previous day, to the shaved back of the hosts as described elsewhere (Szabó et al. 1995). Two feeding chambers at least 15 cm apart were glued to each animal; the cranial one for acaricide testing and the caudal as control (2% Tween). Test with each instar was replicated on five animals and a sixth dog infested similarly but with non-sprayed feeding chambers (acaricide or control solution) used as a control for tween solution. Infestations of each feeding chamber consisted of either 25 adult couples, 40 nymphs or 250 tick larvae. 24 h after tick infestation, each chamber was opened and the interior thereof was sprayed once with 20 μL of either control solution or acaricide, after spraying the chambers were closed again.

Chambers were thereafter inspected daily between 08:00 and 09:00 a.m. and either dead or engorged ticks collected. Engorged ticks were counted, weighed (adults individually and immatures in daily batches) and kept at 27 °C and 80% humidity for the evaluation of biological parameters.

In vitro efficacy on unfed ticks

Efficacy of acaricide upon unfed ticks by the immersion test as described by Drummond et al. (1971). Briefly, tick groups of 250 larvae, 40 nymphs or 25 adult couples were immersed in acaricide solution (*T. minuta* 20%) in Petri dishes for 5 min and then dried in filter paper, the ticks were kept at 27 °C and 80% humidity for evaluation. Control groups with similar numbers of ticks were immersed in 2% Tween solution without the acaricide. Evaluations with each tick stage were performed in triplicate included one control group.

The formula used for calculating the efficacy in both tests was according to Aguirre et al. (2015).

Results

All larvae, nymphs and adults from acaricide sprayed feeding chambers died within 24 h without engorging whereas most ticks from control feeding chambers in either test or control animals engorged, oviposited or underwent molting to the next stage. Biological parameters of these ticks are presented in Tables 1 and 2. Lack of activity against ticks in control chambers indicates that this phytotherapic acts by contact and does not have systemic effect.

In vitro efficacy on unfed ticks: All unfed larva, nymphs and adults died within 24 h after immersion in the phytotherapic whereas mortality of control larvae, nymphs and adults within 24 h was of 5, 5 and 8%, respectively.

Table 1 Biological parameters (mean ± SD) of *Rhipicephalus sanguineus* larvae and nymphs fed on dogs inside feeding chambers sprayed with a 20% solution of *Tagetes minuta* oil diluted in 2% Tween (test dog, test feeding chamber) or feeding chambers on the same animal sprayed with a 2% Tween solution (test dog, control feeding chamber) or in feeding chambers on non-sprayed dog (control dog)

Host	Chamber	Stage	Yield (%)	Weight (mg)	Feed (days)	Molt (days)	Molt (%)
Test	Test	Larva	0	–	–	–	–
	Control	Larva	53.1 ± 46.0	0.30 ± 0.1	5.9 ± 1.3	7.2 ± 1.5	85.9 ± 23.3
Control	–	Larva	92	0.33 ± 0.4	5.11 ± 0.9	5.4 ± 0.5	97
Test	Test	Nymph	0	–	–	–	–
	Control	Nymph	70.3 ± 15.2	3.5 ± 0.3	5.7 ± 0.9	10.2 ± 2.2	53.7 ± 26.2
Control	–	Nymph	67.5	3.7 ± 0.1	5.6 ± 0.6	8.4 ± 0.6	63

Table 2 Biological parameters (mean ± SD) of *Rhipicephalus sanguineus* females fed on dogs inside feeding chambers sprayed with a 20% solution of *Tagetes minuta* oil diluted in 2% Tween (test dog, test feeding chamber) or feeding chambers on the same animal sprayed with a 2% Tween solution (test dog, control feeding chamber) or in feeding chambers on non-sprayed dog (control dog)

Host Feeding chamber	Test Test	Test Control	Control –
Engorged weight (mg)	–	116.9 ± 23.4	115.6 ± 28.2
Feeding period (days)	–	8.4 ± 0.95	8.3 ± 0.8
Egg mass weight (mg)	–	58.3 ± 12.6	69.4 ± 24.3
Pre-oviposition (days)	–	3.3 ± 0.6	2.66 ± 0.9
Yield (%)	0	41.7 ± 23.9	80
IECO (%)	–	47.4 ± 6.1	56.6 ± 16.1
Egg hatching (%)	–	75.9 ± 37.0	88.5 ± 28.0

Discussion

The essential oil of *T. minuta* at a 20% concentration was lethal to all three *R. sanguineus* stages. Those experiments in vitro proved the efficacy of the phytotherapic upon unfed ticks. Such an effect is important for routine control against unfed ticks on the host prior to attachment as well as in the off-host environment. The same lethality was observed during experimental infestations on ticks that were already attached to dogs and the overall efficacy was 100% under the experimental conditions. Thus, this product would be acceptable by the Agricultural Ministry of Brazil which demands an efficacy above 95% for acaricides.

The observed results were not surprising because the *T. minuta* essential oil tested against other tick species in Brazil displayed a similar efficacy. Garcia et al. (2012) tested in vitro the same concentration of the oil against *Rhipicephalus microplus*, *R. sanguineus*, *Amblyomma sculptum* and *Argas miniatus*, and in all cases, the efficacy was above 95%. Andreotti et al. (2013) tested the product as an oil with a concentration of 20% in vivo against ticks on bovines kept in stalls. The cattle were experimentally infested with *R.*

microplus larvae on days 0, 9 and 18 and treated on day 20 to evaluate effect upon all tick stages. These authors obtained an overall efficacy of 99.98% for the oil against *R. microplus* under such experimental conditions.

Several recent works evaluated the in vitro efficacy of plant extracts against *R. microplus,* but only a few evaluated the efficacy against *R. sanguineus*. Additionally, a broad array of experimental conditions was used, but only a few reported high tick lethality with low extract concentrations. Olivo et al. (2008) evaluated citronella [*Cymbopogon nardus* (L.) Rendle] oil at concentrations of 1, 10 or 25% and observed an efficacies of, 92.1, 85.6 and 87.8% respectively. Sousa et al. (2008) observed that the extract of unripe *Melia azedarach* fruit induced a 100% mortality of *R. microplus* ticks at a concentration of 0.25%. Ribeiro et al. (2008) evaluated the efficacy of *Calea serrata* extracts against *R. microplus* and *R. sanguineus* larvae as well as *R. microplus* engorged females using four organic hexane extract concentrations (50, 25, 12.5, and 6.25 mg/ml), and observed an overall efficacy of 100%. Broglio-Micheletti et al. (2009) evaluated alcoholic extracts of four plant species (*Annona muricata*, *Syzygium malaccensis*, *Cymbopogon citratus*, *Azadirachta indica*) at a concentration of 2% and the hexane extract of *A. indica* at a concentration of 2% against *R. microplus.* Among these, only the *A. muricata* extract displayed a 100% efficacy against engorged females. Broglio-Micheletti et al. (2010) observed an efficacy of 96% against engorged females of *R. microplus* using an *A. indica* seed extract and emulsifiable oil at a concentration of 2% (weight/volume). Terassani et al. (2012) used an *A. indica* extract against *R. microplus* and observed a decrease in egg hatching and an increase in female tick mortality of 30%, but this extract had an overall efficacy of only 14%. Santos and Vogel (2012) used very high concentrations (50 and 100%) of basil (*Ocimum basilicum* L.) essential oil and obtained 100% efficacy against fully engorged *R. microplus* females. However, at a lower concentration (25%), the efficacy decrease to 50%. Santos et al. (2013) evaluated the extracts of 21 plant species against *R. microplus* larvae and observed that 18 had efficacies greater than 95%. The same authors reported an efficacy of 88% against engorged females of the same tick species with an extract of *Angelonia hirta* at a concentration of 40%.

Vegetable products are biodegradable and may provide acaricide products that are safer and less harmful towards the environment and humans (Roel 2001; Viegas Junior 2003; Campos et al. 2012). In fact, plant essential oils have long been used for many years for pest control, and they display excellent efficacy in in vitro studies. However, in Brazil, only a few studies were conduction the efficacy of plant essential oil against ticks on animals, and most of those studies involved *R. microplus*, because of its importance in the cattle industry (Andreotti and Koller 2013). Borges et al. (2011) estimated that extract of 55,000 plant species from 26 families have already been tested against this tick species. It is possible that many of the extracts that were efficacious against *R. microplus* have similar efficacies against *R. sanguineus*, as the case in the present study.

In addition to the toxicity of a plant against arthropods, other aspects should be evaluated to create a commercial product. Such a product should have efficacy against pests at low concentrations, should not be toxic to mammals, its harvesting, manipulation and application should be easy and commercially feasible (Viegas Junior 2003). Furthermore, because the acaricide compounds of plants may vary according to the plant species, cultivation and extraction forms, its chemical constitution should be determined to enable control upon such variations (Knaak and Fiuza 2010). In conclusion, the essential oil of *T. minuta* at 20% in Tween at 2% displayed a high acaricidal effect. But further studies are needed to be able to say that the essential oil of *T. minuta* is an alternative to control *R. sanguineus* tick.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Animal Experimentation Ethics Committee of the Federal University of Uberlândia). Permits and Approvals (protocol 009/13 final document 031/13) are on file in the office of M.P.J.S.

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