

Adulticidal and oviposition- and hatching-altering activities of essential oil from Mexican oregano leaves (*Lippia graveolens* H.B.K.) against the cattle tick *Rhipicephalus microplus* (Acari: Ixodidae)

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Abstract. Adulticidal and oviposition- and hatching-altering activities of essential oil extracted from Mexican oregano leaves (*Lippia graveolens* H.B.K.) (OEO) were evaluated on engorged adult female *Rhipicephalus microplus* ticks using the adult immersion test bioassay. Two-fold dilutions of OEO were tested from a starting dilution of 10% down to 1.25%. Results showed 100% adulticidal activity at 10% OEO concentration and oviposition inhibition of 65.8% and 40.9% at 5.0% and 2.5% OEO concentration, respectively. Egg hatching inhibition was achieved by 26.0% and 11.5% at 5.0% and 2.5% OEO concentration, respectively. These effects could be attributed to OEO major components: thymol, carvacrol and p-cymene, which together account for more than 60.0% of the OEO chemical composition. Mexican oregano could represent a potential source for development of alternative tick control agents.

INTRODUCTION

The cattle tick *Rhipicephalus microplus* is found in tropical and subtropical regions around the world. This ectoparasite is of major importance to the livestock industry because of the damage it causes, its wide distribution and the number of cattle it affects (Castellanos *et al.*, 1996). Chemical acaricides have played an important role to control *R. microplus* infestations. However, their continuous and inappropriate use has led to the emergence of resistant tick populations in different parts of the world, including Mexico (Nolan & Schnitzerling, 1986). Plant extracts represent a viable alternative to chemical acaricides. Several researchers have obtained promising results with various plant components possessing toxicological effects against *R. microplus* (Fernandes & Freitas, 2007; Ribeiro *et al.*,

2007; Martínez-Velázquez *et al.*, 2011a, 2011b).

The oregano common name is used to designate several plant species, including *Origanum* (Lamiaceae) genus native from Europe, and *Lippia* (Verbenaceae) genus native from Mexico. It has been shown that plants of the *Origanum* genus have antibacterial, antifungal, insecticide, as well as acaricidal activity against *Rhipicephalus turanicus* ticks (Nostro *et al.*, 2007; Kordali *et al.*, 2008; Cetin *et al.*, 2009). Likewise, essential oils of *Lippia* genus have been shown to have insecticidal and mite repellent activity against *Aedes aegypti* larvae and *Varroa destructor*, respectively (Silva *et al.*, 2008; Ruffinengo *et al.*, 2005).

Previously, we reported a significant acaricide effect of Mexican oregano essential oil (OEO) from *Lippia graveolens* on 10-day-old *R. microplus* larvae (Martínez-

Velázquez *et al.*, 2011a). In the present study, we further investigated the OEO effect on adult stage of *R. microplus*, evaluating its impact on tick survival and on the oviposition and hatching processes.

MATERIALS AND METHODS

Purification of the essential oil

Leaves of *L. graveolens* were obtained commercially in a local market in Guadalajara, Jalisco, Mexico. The OEO was obtained by steam distillation using a semipilot extractor under the conditions described by Martínez-Velázquez *et al.* (2011a).

Chemical composition of the essential oil

Chemical composition of the OEO has been previously reported by Castillo-Herrera *et al.* (2007).

Rhipicephalus microplus ticks

Ticks used in this study were obtained from colonies maintained in CENID-PAVET-INIFAP Tick Research Unit, located in Jiutepec, Morelos, Mexico.

Adult Immersion Test

The adult immersion test (AIT) consisted of treat groups of 25 engorged adult females with each of 10%, 5%, 2.5% and 1.25% (v/v) dilutions of OEO. Dilutions were prepared starting from a 10% dilution of OEO using Tween 20 at a concentration of 5% with water, in order to facilitate the dispersion of the OEO in the aqueous medium. Control group was treated with Tween 20 5% in water. Each group was immersed for 1 min in 10 ml of each dilution, using plastic tubes of 15 ml capacity, with gentle shaking. After 1 min, the ticks were recovered, dried and placed in petri dishes with a filter paper at the base. These were incubated at 26°C and 80% humidity for two weeks. At two weeks post-treatment, the eggs laid were collected and weighed to calculate the percent inhibition of oviposition, as described by Drummond *et al.* (1973).

This was calculated as follows:

$$\text{Index of egg laying (IE)} = \frac{\text{weight of eggs laid (g)}}{\text{weight of females (g)}}$$

$$\text{Inhibition of egg laying (\%)} = \frac{\text{IE control group} - \text{IE treated group}}{\text{IE control group}} \times 100$$

Egg hatching inhibition (%)

$$= \frac{\text{Hatching of control group} - \text{Hatching of treated group}}{\text{Hatching of control group}} \times 100$$

All treatments were evaluated in triplicate.

Statistical Analysis

The mortality percentage was transformed through $2\sqrt{x}$ as described by Martínez-Velázquez *et al.* (2011b), given that data did not show homogeneous variances and normality according to Shapiro-Wilk test. The transformed data were analyzed by one-way ANOVA using the Statgraphics™ 5.1 software. The Tukey test was used to determine significant differences between different concentrations of oil. A p-value <0.05 was considered significant.

RESULTS

A high toxicity was observed on engorged adult *R. microplus* ticks treated with a 10% OEO concentration (Table 1). At this concentration all ticks died and presented a black cuticle (Fig. 1e). Additionally, the oviposition was inhibited by 65.84% and 40.92% at a 5% and 2.5% OEO concentration, respectively (Table 1 and Fig. 1 c, d). At a concentration of 1.25% OEO, there was no effect on oviposition nor on the viability of treated ticks (Table 1 and Fig. 1b). Nonetheless, it is noteworthy that at this concentration, the oviposition began approximately 24 h later, when compared to the control group. At the end of egg laying a similar egg production was reached by both groups.

Table 1. Effects of oregano essential oil on the viability and reproductive parameters of adult *R. microplus* ticks

OEO (%)	Mortality (%)	Tick weight (g)	Eggs weight (g)	Oviposition Index	Egg laying inhibition (%)	Egg hatching inhibition (%)
Control	0 ± 0.00a	6.50 ± 0.01	2.56 ± 0.11	0.39 ± 0.02	00.00 ± 0.00a	00.00 ± 0.00a
1.25	0 ± 0.00a	6.52 ± 0.01	2.44 ± 0.05	0.37 ± 0.01	04.98 ± 2.16a	07.00 ± 1.41a
2.50	0 ± 0.00a	6.49 ± 0.01	1.51 ± 0.17	0.23 ± 0.03	40.92 ± 9.33b	11.50 ± 0.71b
5.00	8 ± 1.41b	6.54 ± 0.05	0.88 ± 0.04	0.13 ± 0.01	65.84 ± 2.99c	26.00 ± 2.83c
10.0	100 ± 0.00c	6.57 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	100.0 ± 0.00d	–

^aMeans within a column (between oil concentration) followed by the same lowercase letter are not significantly different.



Figure 1. Effect of OEO on oviposition of engorged adult *R. microplus* ticks. a) Control group; b) Group treated with 1.25% OEO; c) Group treated with 2.5% OEO; d) Group treated with 5% OEO; e) Group treated with 10% OEO.

On the other hand, egg hatching was inhibited by 26% and 11.5% at 5% and 2.5% OEO concentration, respectively (Table 1).

DISCUSSION

This study shows that the OEO obtained from leaves of *L. graveolens* is highly toxic to adult *R. microplus* ticks and alters reproductive parameters such as oviposition and hatching. To our knowledge, this is the first report about the acaricidal activity of Mexican oregano against this ectoparasite in adult stage. These effects could be attributed to the presence of thymol, carvacrol, and p-cymene, which jointly represent over 60% of the chemical composition of the OEO (Castillo-Herrera *et al.*, 2007). Our findings are supported by recent evidence. Gomes *et al.* (2012) found that at concentrations of 60 and 80 µl/ml of *L. sidoides* essential oil, the *R. microplus* oviposition was fully inhibited, being thymol

the main constituent of the essential oil with a 67.60%. Lage *et al.* (2013) evaluated the acaricidal activity of *L. triplinervis* essential oil on engorged *R. microplus* females, showing an inhibition of egg laying (57.1% and 84%) and hatching (83.8% and 93.8%) at a concentration of 40 and 50 mg/mL, respectively. *L. triplinervis* essential oil was composed mainly of carvacrol (31.9%) and thymol (30.6%). *L. gracilis* essential oil has also shown acaricide properties against *R. microplus*, being its major components carvacrol and thymol (Cruz *et al.*, 2013). With regard to individual components, Silveira *et al.* (2007) have demonstrated that the application of thymol at a 1.0% concentration caused a mortality up to 100% on 15-day-old *R. microplus* larvae. Moreover, Monteiro *et al.* (2010) reported that thymol tested on engorged *R. microplus* females caused several alterations in egg mass weight, oviposition and hatching percentage. In another study, Monteiro *et al.* (2009)

evaluated different thymol concentrations, obtaining a 100% mortality on *R. sanguineus* nymphs at a 0.5% concentration. On the other hand, Silva *et al.* (2011) evaluated the thymol acaricidal efficacy on engorged *Amblyomma cajennense* nymphs, finding a mortality of 100% at 15 mg/ml concentration, while Cetin *et al.* (2010) tested a 10 ml/l carvacrol concentration against *Hyalomma marginatum* ticks. Parasites exposed to carvacrol-treated wicks produced up 93% knockdown at 3 h but after 24 h approximately 57% were dead.

Taken together, our results and the scientific accumulated evidence suggest that Mexican oregano and *Lippia* genus in general, may be an important source of metabolites to develop new tick control agents. Essential oils present few risks to the ecosystem due to its rapid dissipation and low level of residues left in the environment. Further studies are required to develop acaricide formulations and means to improve their efficiency and stability.

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