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ORIGINAL PAPER

Acaricidal efficacy of *Origanum onites* L. essential oil against *Rhipicephalus turanicus* (Ixodidae)

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Abstract Essential oils that were extracted by steam distillation from *Origanum onites* L. from northwest Turkey were analyzed using an Agilent GC-MSD system. Carvacrol was the major component (64.3%) of the oil. Both pure commercial carvacrol and essential oil at concentrations of 1.5%, 3.2%, 6.25%, 12.5%, 25%, 50%, and 100% (*v*/*v*) were tested for their effects against engorging *Rhipicephalus turanicus*, which were collected from cattle breeding in the region. Pure carvacrol killed all the ticks following 6 h of exposure, while 25% and higher concentrations of the oil were completely effective in killing the ticks by the 24th-hour post-treatment ($p<0.05$). The concentrations of 6.25% and 12.5% killed all the ticks by day 2. Lethal concentrations for 50% (LC_{50}) and 90% (LC_{90}) of 2.34% and 7.12%, respectively, were detected 24 h post-treatment. These findings indicate that the essential oil of *O. onites* L. has potential to be utilized at reasonable concentrations to control tick infestations.

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Introduction

Origanum species are very common in Mediterranean countries. *Origanum onites* L. is the dominant species in the northwest of Turkey. Essential oils of *Origanum* species have antibacterial (Dadalioglu and Evrendilek 2004; Nostro et al. 2007), antifungal (Chami et al. 2005; Faleiro et al. 2005; Demirci et al. 2006), antioxidative (Faleiro et al. 2005), and antiparasitic (Panella et al. 2005; Dietrich et al. 2006; Cetin and Yanikoglu 2006) activities. These activities have been attributed to carvacrol, the major constituent of these oils. The amount of carvacrol in the essential oil of *Origanum* species growing in the different geographical regions of the country varies from 23.4% to 80.4% (Baser et al. 1993).

Currently, information on the antiparasitic activity of the essential oils of *Origanum* species is very limited although several studies have documented the activities of these compounds. The biocidal activity of carvacrol against ticks and mosquitoes was first demonstrated by Panella et al. (2005), with 50% lethal concentration (LC_{50}) values of 0.0068% and 0.0051% (*w*/*v*), respectively, after 24 h. The repellent concentration (RC_{50}) of this compound against nymphal *Ixodes scapularis* was detected with an RC_{50} value of 0.112% (*w*/*v*; Dietrich et al. 2006). Larvicidal activity of the essential oil of *O. onites* and *Origanum minutiflorum* against the third and fourth instar larvae of the mosquito, *Culex pipiens*, has been reported with LC_{50} and LC_{90} values of 24.8 and 61.3 ppm and 73.8 and 118.9 ppm, respectively (Cetin and Yanikoglu 2006).

In order to further our knowledge of the effects of the essential oil of these plants, *O. onites* was collected from northwest Turkey, and the composition of the oil and its acaricidal efficacy against engorging *Rhipicephalus turanicus*, a tick frequently found on cattle in the region, were analyzed.

Table 1 Composition of essential oil of *Origanum onites* L.

Main components	Percent
α-Pinene	1.0
Tymol	1.4
α-Terpinene	1.4
Mycene	1.6
γ-Terpinene	3.5
p-Cymene	7.1
Linalool	13.8
Carvacrol	64.3

Materials and methods

Extraction and analysis of essential oil

O. onites collected from northwest Turkey were air-dried at room temperature for 10 days. Oil extraction was accomplished through steam distillation. The oil was analyzed by capillary GC and GC/MS using an Agilent GC-MSD system (Baser et al. 2000).

Collection and identification of the ticks

Male and female *R. turanicus*, which are frequently found on cattle in northwest Turkey, were collected from a herd of 60 cattle without breaking their rostrum. A total of 520 ticks were collected. Forty ticks were selected randomly and fixed in 70% ethanol. The ticks were identified as *R. turanicus* according to Walker et al. (2002). The average weight of the engorging ticks was 0.5±0.1 g, and the average length was 6.5±0.4 mm. These two parameters were used as the reference measure to determine which ticks to be used in the experiments.

Preparation of the test compounds

Seven concentrations (1.5%, 3.2%, 6.25%, 12.5%, 25%, 50%, and 100% (*v*/*v*)) of the oil were prepared using 96% ethyl alcohol as solvent. Pure carvacrol was purchased from Frutarom Industries Ltd (Product No: 1114370, Lot No: 194519/8), Israel.

In vitro study

A total of 48 Petri dishes, six Petri dishes (three for experiments and three for control) for each concentration of the test compound, were prepared. Disks of Whatman No. 1 filter paper with surface areas of 21.25 cm^2 each were wet with 150 μl of the oil or with the same amount of alcohol as a control, and then the disks were spread into Petri dishes. Ten randomly selected ticks were placed in each Petri dish and covered with tulle curtain. The dishes were examined on the 1st, 3rd, 6th, 24th, and 48th hour of the trial to determine the numbers of dead ticks. The efficacy rate of the compounds was calculated as follows:

$$E = D_T - D_C/A_C \times 100$$

where D_T is the number of dead ticks in treated Petri dishes, D_C the number of dead ticks in control Petri dishes, and A_C the number of live ticks in control Petri dishes.

Statistical analysis

Groups were compared using one-way ANOVA for repeated measurements. Tukey test was used for post hoc analysis. A value of $p<0.05$ was considered significant.

Results

Analysis of *O. onites* L. essential oil

Relative percentages of the compounds that were separated from the oil are shown in Table 1.

In vitro study

All essential oil concentrations that were tested in this study proved acaricidal to the ticks. Efficacy rates increased as both time of exposure and concentration of oil increased. Pure carvacrol killed all the ticks by hour 6 of exposure (Table 2). Essential oil concentrations of 25% and higher were completely (100%) effective in killing the ticks at 24 h post-treatment. At 6.25% and 12.5%, the essential oil

Table 2 Efficacy of *Origanum onites* L. essential oil against *Rhipicephalus turanicus*

Time (h)	Concentrations of essential oil (%)							Pure carvacrol
	1.5	3.12	6.25	12.5	25	50	100	
1	0	0	0	7.9	25	30	33.3	30
3	0	0	3.3	21	50	60	63.3	75
6	3.3	5	26.7	41.7	65.4	78.9	80	100
24	38.9	61.1	88.9	96.8	100	100	100	
48	46.4	64.3	100	100				

treatment killed all the ticks by day 2. The doses of 1.5% and 3.12% were partly effective at the end of the trial. Median efficacy rates observed at 24 h post-treatment were significantly different ($p<0.05$) from that of 1, 3, and 6 h of exposure. The difference between efficacies at 24 and 48 h of treatment was not statistically significant ($p>0.05$). After 24 h, the LC_{50} and LC_{90} values were determined to be 2.34% and 7.12%, respectively.

Discussion

Essential oil extracted from *O. onites* that were collected from Bursa province and its vicinity in Turkey contains a high proportion (64.3%) of carvacrol, and thus, this plant appears to be a good natural source of carvacrol. In fact, 23 species of the genus *Origanum* and its intersection hybrids occur in natural flora in Turkey. Five commercially important *Origanum* species are cultivated and exported more than 4,500 tons in a year (Baser et al. 1993). Here, we demonstrated that pure carvacrol kills *R. turanicus* in vitro.

In addition, all the ticks treated with 6.25% and higher concentrations of the essential oil from *O. onites* were dead 48 h following exposure. Since most of the ticks in control Petri dishes died on the third day of the trial, efficacy rates of the lower concentrations could not be evaluated. LC_{50} and LC_{90} values were 2.34% and 7.12%, respectively, 24 h post-treatment while pure carvacrol killed all the ticks by 6 h of exposure. These findings indicate that carvacrol does not exhibit a knock down efficacy against ticks. Plant extracts that exhibit acaricidal efficacy against ticks act within a time from 90 min to 15 days (Gonzales et al. 1991; Williams et al. 1996; Abdel-Shafy and Zayed 2002; Pamo et al. 2005; Iori et al. 2005). Additionally, the effects of some plant extracts against different development stages of ticks vary significantly. For example, larvae, nymphs, and unfed adults are more sensitive than engorging adults (Abdel-Shafy and Zayed 2002).

In this study, significant efficacy against engorging *R. turanicus* was observed with a LC_{90} value of 7.12% of the compound 24 h post-treatment. Thus, carvacrol has potential as an agent to control tick infestations. Further studies are necessary, however, to elucidate its mode of action, side effects, and actual in vivo application.

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