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Repellency of the *Origanum onites* L. essential oil and constituents to the lone star tick and yellow fever mosquito

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**ABSTRACT**

The oregano, *Origanum onites* L., essential oil (EO) was tested in laboratory behavioural bioassays for repellent activity against *Amblyomma americanum* (L.) and *Aedes aegypti* (L.). The *O. onites* EO was characterised using GC-FID and GC-MS. Carvacrol (75.70%), linalool (9.0%), \( p \)-cymene (4.33%) and thymol (1.9%) were the most abundant compounds. At a concentration of 0.413 mg oil/cm\(^2\) of filter paper, *O. onites* EO repelled 100% of the ticks tested and at 0.103 mg oil/cm\(^2\) of filter paper, 66.7% of the ticks were repelled. At 0.075 mg oil/cm\(^2\) filter paper, thymol repelled 66.7% of the ticks compared to 28.7% by carvacrol at that same concentration. Against *Ae. aegypti*, *O. onites* EO was repellent at the minimum effective dosage (MED) of 0.011 (±0.00) mg/cm\(^2\) in the cloth patch assay compared to the reference control, \( N, N \)-dimethyl-3-methylbenzamide (DEET) with a MED = 0.007 ± (0.003) mg/cm\(^2\).

**KEYWORDS**

*Origanum onites*; thymol; carvacrol; \( (-) \)-linalool; terpinolene; \( \alpha \)-humulene; natural repellency; *Amblyomma americanum*; *Aedes aegypti*

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1. Introduction

Vector-borne diseases are mainly transmitted to humans and animals by the bites of arthropods, such as mosquitoes and ticks, infected with one or more pathogens. Arthropod transmitted illnesses cause serious problems for humans and domesticated animals in much of the habitable world. Lone star ticks, *Amblyomma americanum* (L.), long notorious in the United States as nuisance biters, are increasingly recognised as vectors of pathogens harmful to humans and domesticated animals (Childs & Paddock 2003; Mixson et al. 2006; Stafford 2007; Goddard & Varela-Stokes 2009; Stromdahl & Hickling 2012). They exhibit a hunting type of host-seeking behaviour (Waladde & Rice 1982) and where present are readily noticeable to the public (Armstrong et al. 2001). The widespread yellow fever mosquito, *Aedes aegypti* (L.), is recognised as a health hazard and nuisance biter (Cimmino et al. 2015).

Personal protection against tick and mosquito bites can be obtained by proper use of repellents, and their use has been recommended by the U.S.A. CDC (2002). For several decades, *N,N*-dimethyl-3-methylbenzamide (DEET) has been the mainstay among repellents approved for use on human skin (Demirci et al. 2013), as has permethrin for repellent products applied to clothing (Bernier et al. 2015). Growing public interest in repellents featuring non-synthetic chemicals from natural products has spurred research, development and marketing of a variety of plant-based repellents, e.g. Repel®, Off!® and Botanicals® (Tabanca et al. 2016). Most plants produce secondary compounds that not only foil some plant pathogens and herbivores, but also repel a broad spectrum of arthropods, including blood feeders, which pose no threat to plants. Oregano, *Origanum vulgare* L., has been reported to repel the tick *Am. americanum* (Meng et al. 2016) and another species of oregano, *Origanum onites* L. has been found to be toxic to the tick *Rhipicephalus turanicus* Pomerantsev (Coskun et al. 2008). Recently, the *Aedes*-borne Zika virus has aroused considerable attention as a threat to human health in a growing number of countries (Rasmussen et al. 2016). In addition to the clinical symptoms of Zika virus, the pathogen has been linked to cases of microcephaly in children born to women infected with Zika virus (Rasmussen et al. 2016). The host-seeking behaviours of *Am. americanum* and *Ae. aegypti* make them good subjects for testing repellents. Therefore, we tested *Origanum onites* (Syn: *O. smyrneum* L.) essential oil (EO) and its major compounds against these medical and veterinary pests as a potential arthropod repellent and source of beneficial compounds.

2. Results and discussion

*O. onites* EO was analysed on a GC-FID and a GC-MS system. Thirty-nine components were identified and these constituted 99.63% of the essential oil with carvacrol (75.70%), linalool (9.0%), *p*-cymene (4.33%) and thymol (1.9%) as the major constituents (Supplementary material, Table S1). Baser and co-workers reported that *O. onites* EO has two chemotypes: carvacrol-type and linalool-type and they concluded that climatic conditions and altitude are important factors in determining the chemistry and diversity of *O. onites* (Baser et al. 1993). As the carvacrol content is high (75.70%) in our sample (Table S1), the current *O. onites* sample is considered as carvacrol-type. In the recent study, Baranauskaitė et al. (2016) reported that carvacrol content found depended on the oregano species and the extraction methods.
Ticks (Am. americanum) exposed to higher concentrations of O. onites EO and some of its constituents behaved similarly to those exposed to higher concentrations of DEET; they dropped off the test strip or would not cross into the treated area (i.e. they were repelled). The estimated proportion repelled with a 95% confidence interval for each compound-concentration combination is given in Figure S1 (Supplementary material). The constituent p-cymene, tested at a high concentration, showed little repellent activity. Carvacrol, tested only at 0.075 mg/cm², appears to be about as effective as O. onites EO at that concentration. Thymol appears to be the most repellent constituent tested; its repellent activity was high and differed from the EtOH control even at the lowest concentration tested, 0.075 mg/cm². Since enantiomers can have different biological activities, we isolated linalool from O. onites EO and was it found to be (−)-linalool by optical rotation; it was not active at the concentration tested.

O. onites EO showed good repellent activity against Ae. aegypti with a minimum effective dosage (MED) of 0.011 ± 0.000 mg/cm² compared to the reference control DEET (MED = 0.007 ± 0.003 mg/cm²) when tested using human volunteers in a cloth patch assay. The major compound carvacrol was the best repellent of all tested compounds. The second most abundant compound, (−)-linalool had mild repellency. The third major compound, p-cymene, having the same structural skeleton as carvacrol and thymol but without the hydroxy group, was not repellent at the highest tested dose of 1.5 mg/cm². Thymol, the fourth major compound in the oil, had a repellency 2.3-fold lower than carvacrol (Supplementary material, Table S2). We previously investigated the repellency of some monoterpenes and sesquiterpenes (Tabanca et al. 2016). Of the three cyclic diolefins, terpinolene demonstrated good repellency, however α-terpinene and γ-terpinene were not active at the highest tested concentration. This finding implies that the location of double bonds may play an important role in repellent activity. The tertiary alcohols, terpinen-4-ol and α-terpineol, which have the hydroxyl group, located in different positions, showed better activity than bicyclic compounds 1,8-cineole and (−)-β-pinene. Among the sesquiterpenes, α-humulene, β-caryophyllene, and caryophyllene oxide did not repel Ae. aegypti at the highest tested concentration. The position and number of double bonds, location of the hydroxyl group, and presence of an epoxide apparently contribute to repellent activity. However, additional different functional groups and structures from monoterpenes and sesquiterpenes should be tested to better define their repellency as a chemical class. This study further suggests investigating the repellency of (±)-linalool. Minor components may also contribute to the activity of the essential oil against Ae. aegypti.

Similar concentrations of O. onites EO and DEET were comparable in effectiveness in repelling Am. americanum and Ae. aegypti. Among the four major constituents (carvacrol, thymol, (−)-linalool and p-cymene) of the O. onites EO, carvacrol and thymol were strongly repellent to Ae. aegypti and Am. americanum. The compound p-cymene showed virtually no repellent activity, as was also reported by Weldon et al. (2011), who tested this compound in a similar bioassay against Am. americanum and Ae. aegypti. Weldon and coauthors (2011) did find some variable repellent activity of linalool against Am. americanum. In our tests with Am. americanum, (−)-linalool did not differ from the controls in repellency, but was a weak repellent against Ae. aegypti. These results demonstrate that repellent activity can vary with arthropod species, as well as compound structure. Terpinen-4-ol, α-terpineol, and carvone, present in low amounts (0.7, 0.8 and 0.2, respectively) in the O. onites EO, were reported by Weldon et al. (2011) to repel Am. americanum. It is unclear whether such low concentrations
of terpinen-4-ol, α-terpineol and carvone alone or in combination, exerted any repellent responses in the ticks, particularly with carvacrol and thymol in such abundance.

Carvacrol and thymol have been implicated as skin allergens and sensitisers (Xu et al. 2006), which may limit their potential as topically applied arthropod repellents. Although we found that low concentrations of carvacrol and thymol repelled ticks, the allergenic and sensitisation issues must be considered in any development of the carvacrol type *O. onites* as a repellent product to be used on skin. Repellents can be used in ways other than dermally, such as on clothing (Schreck et al. 1982). Jordan et al. (2012) field-tested carvacrol applied to cloth coveralls as a tick repellent. Carvacrol repelled all *Ixodes scapularis* Say adults the first day of testing and gradually declined in efficacy over succeeding days; against *Am. americanum* adults, carvacrol was not effective (Jordan et al. 2012). Adult *Am. americanum* are much larger and faster than the nymphs used in our bioassays. Among eight essential oils tested against *Am. americanum* nymphs, Meng et al. (2016) found that *O. vulgare* EO was the most repellent although not quite as effective as DEET. We found the essential oil of *O. onites* similar in efficacy to deet. Coskun et al. (2008) showed that continued exposure of the brown dog tick, *Rhipicephalus sanguineus* Latreille, to dilutions of *O. onites* EO was toxic to this species. Coskun et al. (2008) also found that carvacrol, the main constituent of *O. onites* EO, killed *Rh. turanicus*. In field trials, Dolan et al. (2009) found that carvacrol suppressed numbers of *Ix. scapularis*, which is principal vector of the Lyme disease pathogen in the U.S.A, in addition to suppressing *Am. americanum*. Thus, carvacrol-rich *O. onites* EO, carvacrol and possibly thymol appear to have potential for use to protect humans and domestic animals against mosquitoes and ticks.

3. Conclusion

*O. onites* EO was repellent to both *Ae. aegypti* and *Am. americanum*. Carvacrol and thymol were strongly repellent to *Ae. aegypti* and *Am. americanum*. Since carvacrol and thymol have been implicated as skin sensitisers, the allergenic and sensitisation issues must be considered in any development of the carvacrol type *O. onites* EO as a repellent product to be used on skin.

Supplementary material

Experimental details related to this article are available online, alongside Tables S1–S2 and Figure S1.

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