Taylor & Francis
Taylor & Francis Group

Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

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

John F. Carroll, Betul Demirci, Matthew Kramer, Ulrich R. Bernier, Natasha M. Agramonte, K. Husnu Can Baser & Nurhayat Tabanca

To cite this article: John F. Carroll, Betul Demirci, Matthew Kramer, Ulrich R. Bernier, Natasha M. Agramonte, K. Husnu Can Baser & Nurhayat Tabanca (2017): Repellency of the Origanum onites L. essential oil and constituents to the lone star tick and yellow fever mosquito, Natural Product Research, DOI: 10.1080/14786419.2017.1280485

To link to this article: http://dx.doi.org/10.1080/14786419.2017.1280485

NATURAL PRODUCT RESEARCH, 2017
http://dx.doi.org/10.1080/14786419.2017.1280485

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

OPEN ACCESS

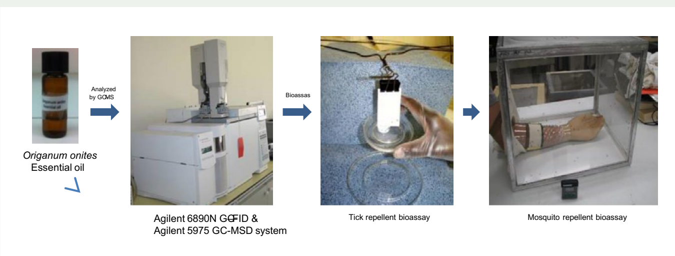
Repellency of the *Origanum onites* L. essential oil and constituents to the lone star tick and yellow fever mosquito

John F. Carroll[a], Betul Demirci[b], Matthew Kramer[c], Ulrich R. Bernier[d], Natasha M. Agramonte[d,e], K. Husnu Can Baser[f] and Nurhayat Tabanca[d,e†]

[a]USDA, ARS, Invasive Insects Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, Beltsville, MD, (retired) USA; [b]Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University, Eskisehir, Turkey; [c]USDA, ARS, Statistics Group, Beltsville Agricultural Research Center, Beltsville, MD, USA; [d]USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, USA; [e]Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA; [f]Faculty of Pharmacy, Department of Pharmacognosy, Near East University, Nicosia, N. Cyprus, Mersin 10, Turkey

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.



ARTICLE HISTORY
Received 27 May 2016
Accepted 24 December 2016

KEYWORDS
Origanum onites; thymol; carvacrol; (−)-linalool; terpinolene; α-humulene; natural repellency; *Amblyomma americanum*; *Aedes aegypti*

CONTACT Ulrich R. Bernier uli.bernier@ars.usda.gov; Nurhayat Tabanca nurhayat.tabanca@ars.usda.gov
† USDA-ARS, Subtropical Horticulture Research Station, 13601 Old Cutler Rd., Miami, FL 33158 USA

Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2017.1280485.

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/cm^2, 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/cm^2. 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/cm^2 compared to the reference control DEET (MED = 0.007 ± 0.003 mg/cm^2) 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/cm^2. 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, *Rhiphicephalus 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.

Acknowledgements

We thank James L. McCrary, Abdul Saboor Khan (USDA, ARS, IIB and BL, Beltsville, MD) and Greg Allen (USDA, ARS, CMAVE, Gainesville, FL) for performing the tick and mosquito bioassays.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported in part by the DWFP Research Program Grant funded by the U.S. Department of Defense through the Armed Forces Pest Management Board.

References

Armstrong PM, Brunet LR, Spielman A, Telford SR III. 2001. Risk of Lyme disease: perceptions of residents of a lone star tick-infested community. Bull World Health Organ. 79:916–925.

Baranauskaitė J, Jakštas V, Ivanauskas L, Kopustinskienė DM, Drakšienė G, Masteikova R, Bernatonienė J. 2016. Optimization of carvacrol, rosmarinic, oleanolic and ursolic acid extraction from oregano herbs (*Origanum onites* L., *Origanum vulgare* spp. *hirtum* and *Origanum vulgare* L.). Nat Prod Res. 30:672–674.

Baser KHC, Özek T, Tümen G, Sezik E. 1993. Composition of the essential oils of Turkish oregano species with commercial importance. J Essent Oil Res. 5:619–623.

Bernier UR, Gurman P, Clark G, Elman N. 2015. Functional micro-dispensers based on micro-electro-mechanical-systems (MEMS) integrated with fabrics as functional materials to protect humans from mosquito feeding. J Controlled Release. 220 (Part A):1–4.

CDC. 2002. Lyme disease. Department of Health and Human Services, Centers for Disease Control and Prevention, Ft. Collins, CO, 12pp.

Childs JE, Paddock CD. 2003. The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. Annu Rev Entomol. 48:307–337.

Cimmino A, Evidente M, Masi M, Ali A, Tabanca N, Khan IA, Evidente A. 2015. Papyracillic acid and its derivatives as biting deterrents against *Aedes aegypti* (Diptera: Culicidae): structure–activity relationships. Med Chem Res. 24: 3981–3989.

Coskun S, Girisgin O, Kürkcüoglu M, Malyer H, Girisgin AO, Kırımer N, Baser KH. 2008. Acaricidal efficacy of *Origanum onites* L. against *Rhipicephalus turanicus* (Ixodidae). Parasitol Res. 103:259–261.

Demirci B, Tsikolia M, Bernier UR, Agramonte NM, Alqasoumi SI, Al-Yahya MA, Al-Rehaily AJ, Yusufoglu HS, Demirci F, Başer KHC, et al. 2013. A new natural source: *Phoenix dactylifera* L. spathe essential oil: chemical composition and repellent activity against the yellow fever mosquito, *Aedes aegypti*. Acta Trop. 128:557–560.

Dolan MC, Jordan RA, Schulze TL, Schulze CJ, Manning MC, Ruffolo D, Schmidt JP, Piesman J, Karchesy JJ. 2009. Ability of two natural products, nootkatone and carvacrol, to suppress *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in a Lyme disease endemic area of New Jersey. J Econ Entomol. 102:235–2324.

Goddard J, Varela-Stokes AS. 2009. Role of lone star ticks, *Amblyomma americanum* (L.), in human and animal diseases. Vet Parasitol. 160:1–12.

Jordan RA, Schulze TL, Dolan MC. 2012. Efficacy of plant-derived and synthetic compounds on clothing as repellents against *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). J Med Entomol. 49:101–106.

Meng H, Li AY, Costa Junior LM, Castro-Arellano I, Liu J. 2016. Evaluation of DEET and eight essential oils for repellency against the lone star tick, *Amblyomma americanum* (Acari: Ixodidae). Exp Appl Acarol. 68:241–249.

Mixson TR, Campbell SR, Gill JS, Ginsberg HS, Reichard MV, Schulze TL, Dasch GA. 2006. Prevalence of *Ehrlichia*, *Borrelia*, and rickettsial agents in *Amblyomma americanum* (Acari: Ixodidae) collected from nine states. J Med Entomol. 43:1261–1268.

Rasmussen SA, Jameson DJ, Honein MA, Petersen LR. 2016. Zika virus and birth defects – reviewing the evidence for causality. N Engl J Med. 374:1981–1987. doi:10.1056/NEJMsr/604338.

Schreck CE, Mount GA, Carlson DA. 1982. Wear and wash persistence of permethrin used as a clothing treatment for personal protection against the lone star tick (Acari: Ixodidae). J Med Entomol. 19:143–146.

Stafford, KC III. 2007. Tick management handbook. New Haven, CT: Connecticut Agric Exp Stn Bull No 1010; p. 78.

Stromdahl EY, Hickling GJ. 2012. Beyond Lyme: aetiology of tick-borne diseases with emphasis on the south-eastern United States. Zoonoses Public Health. 59:48–64.

Tabanca N, Bernier UR, Agramonte NM, Tsikolia M, Bloomquist JR. 2016. Discovery of repellents from natural products. Curr Org Chem. 20:2690–2702.

Waladde SM, Rice MJ. 1982. The sensory basis of tick feeding behavior. In: Obenchain FD, Galun R, editors. Physiology of ticks. New York: Pergamon; p. 71–118.

Weldon PJ, Carroll JF, Kramer M, Bedoukian, Coleman RE, Bernier UR. 2011. Anointing chemicals and hematophagous arthropods: responses by ticks and mosquitoes to *Citrus* (Rutaceae) peel exudates and monoterpene components. J Chem Ecol. 37:348–359.
Xu H, Delling M, Jun JC, Clapham DE. 2006. Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci. 9:628–635.