LAVENDER, EUCALYPTUS, AND ORANGE ESSENTIAL OILS AS REPELLENTS AGAINST *IXODES RICINUS* FEMALES*

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This study evaluated the repellent effect of three essential oils against females of *Ixodes ricinus*, which is considered to be the main arthropod disease vector in Europe. The essential oils could be regarded as user- and environment-friendly alternatives to synthetic repellents. As a comparison sample, the most widely used synthetic repellent DEET was used. All the tested oils exhibited moderate to high initial repellency of 65–85% 5 min after application. The testing was terminated after 80 min, when lavender and eucalyptus repelled 45% and 15% of ticks, respectively. No effect of orange oil was observed after a 20-min mark. The effect of DEET was found to be high and stable (95–100%) throughout the experiment. This study thus revealed that the investigated oils are not as effective as DEET. On the other hand, especially lavender showed an interesting potential as an alternative repellent for outdoor activities of shorter duration.

repellency, alternative repellents, DEET, sheep tick, vector

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INTRODUCTION

In Europe, the sheep tick (*Ixodes ricinus* L.) is considered to be the main arthropod disease vector (Jaenson et al., 2006; Hartemink, Takken, 2016). The most common of such illnesses are Lyme disease, which infects 65 500 people annually in Europe (Rizzoli et al., 2011) and tick-borne encephalitis, infecting 5000–12 000 people annually in Europe (European Centre for Disease Prevention and Control, 2014). Moreover, rickettsial, babesia, bartonella and anaplasma syndromes transmitted by ticks were newly described in the past decade (Dietrich et al., 2010; Schorn et al., 2011; Sormunen et al., 2016; Szekeres et al., 2016). Effective vaccinations are not available for many of these infections, so using repellents is a desirable option for preventing tick-borne diseases (Del Fabbro, Nazzi, 2008).

Repellents are defined as substances that force arthropods to move away from a repellent's source, which may be applied directly to skin, clothing or shelter (Dautel et al., 2013). In the past five decades, many synthetic chemical repellents were developed to protect human health. DEET (N, N-diethyl-m-toluamid) is one of the most widely used synthetic repellents (Goodyer, Behrens, 1998) providing an adequate protection for most common situa-

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Table 1. Chromatographic profiles of tested essential oils

	Component	Percentage
Orange essential oil	limonene	18.5–24.0
	cineol	5.0–8.0
Eucalyptus essential oil	α-pinene	0.05–10
	β-pinene	0.05–1.5
	limonene	0.05–15.0
	1,8-cineol	> 60.0
Lavender essential oil	limonene	> 1.0
	cineol	> 2.5
	3-octanone	> 2.5
	camphor	> 1.2
	linalool	20.0–45.0
	linalyn acetate	25.0–46.0
	terpinen-4-ol	0.1–6.0
	lavandulyl acetate	> 0.2
	lavandulol	> 0.1
	α-terpineol	< 2.0

tions in concentrations 10–35% (Katz et al., 2008). The long duration of protection DEET provides is an unquestionable advantage, but there are still doubts regarding safety for human health (Aquino et al., 2004). These uncertainties are based on documented medical complications occurring after its application, poisonings after inhalation or eye contact (Goodyer, Behrens, 1998). Additionally, damage to the central nervous system has also been reported, particularly in children (Frances, 2007;Osimitz et al., 2010). Other known complications associated with DEET exposure include cardiovascular and dermatological reactions (Stajković, Milutinović, 2013). Additionally, Semmler et al. (2011) also point out that DEET is sticky, has an unpleasant odour, and dissolves plastic. Due to its long half-life, DEET has also been identified as a contaminant of aquatic water systems all over the world (Aquino et al., 2004; Costanzo et al., 2007). For these reasons, the development of alternative repellents without adverse effects is very important.

Essential oils can be considered as an alternative repellent (Meng et al., 2016). These substances are complex mixtures of volatile organic compounds produced by metabolism of a plant as secondary metabolites (Nerio et al., 2010; Yoon et al., 2011). Some have been reported as quite effective natural repellents against arthropod pests (e.g. Carroll et al., 2007; Zeringóta et al., 2013; Meng et al., 2016), including *I. ricinus* (e.g. Jaenson et al., 2006; Thorsell et al., 2006; El-Seedi et al., 2012). However, due to their high volatility, repellency of essential oils is generally regarded as short term in nature (Zhu et al., 2001) and thus they offer protection for shorter periods of time (Jaenson et al., 2006; Nerio et al., 2010).

Our study is focused on comparison of repellency effect and duration between three essential oils and synthetic repellent 10% DEET, when used as a time-stable control, against host-seeking *I. ricinus* females. It should be noted that not all essential oils are safe for human use, and several types have even been described as allergens or mutagens (Thorsell et al., 2006). Our primary intention was to test essential oils that have some repellency effect and which are also 'user-friendly' and do not endanger human health. We therefore selected eucalyptus oil, lavender oil, and orange oil and statistically compared the efficiency of these oils with the effectiveness of DEET.

MATERIAL AND METHODS

Adult females of *I. ricinus* were obtained from a commercial source (Insect Services, Berlin, Germany). Prior to analysis, all ticks were kept unfed for one week to acclimate in polypropylene tubes (with strips of filter paper inside) stored in a desiccator outfitted with a plastic cup with soaked cotton wool (to maintain high humidity) at room temperature. Essential oils were obtained from Hofigal (Bucharest, Romania), which also provided chromatographic profiles – see Table 1. The synthetic DEET was obtained from Vertellus (Herriard, UK). Prior to conducting the bioassays, all repellent samples were diluted in diethyl ether (Penta, Chrudim, Czech Republic) to a 10% test concentration.

To evaluate repellency, we used a novel bioassay based on methods previously described by Carroll et al. (2011), Kröber et al. (2013), and da Camara et al. (2015). The experimental and control bioassays consisted of two concentric circles (Ø19 cm and Ø15 cm) drawn on an A4-size sheet of cardboard. In the experimental arena, the 4 cm wide zone between these circles was treated evenly with 1 ml of the investigated repellent solution and the same area in the control arena was treated with diethyl ether only. The cardboard sheets were treated separately with each of the three essential oils (orange, eucalyptus, lavender) and DEET. Five ticks were first placed in the control arena (without repellent) to exclude inactive ticks. If they crossed the outer border of the circle in the control arena within 60 s, they were then released to the central untreated zone in the experimental area. Each repellent was tested in this manner using a total of 120 individual ticks.

To investigate the effect of time on repellency, bioassays were performed in six 15 min intervals from 5 min to 80 min after application. Each tick was used only once. Ticks are known to be attracted by many compounds, usually found on a host's skin or breath (Dautel et al., 2013) so, all tested ticks were activated to host-seeking mode by the observer's

Table 2. Repellency of essential oils (GLM model)

	χ^2 test	P
Repellent	257.252	< 0.001
Time	61.112	< 0.001
Repellent*time	30.185	< 0.001

breath (to simulate host stimuli) immediately after their entrance to both experimental and control arenas. The ticks were considered to be repelled only if they did not cross the repellent barrier. Based on the test results, the ticks were then divided into two respective groups: repelled/unrepelled (1/0).

Repellent effectiveness data were processed using a generalized linear model (GLM) with quasibinomial distribution. Time and type of repellent were designated as fixed factors. Quasibinomial distribution was used because of expected overdispersion in our dataset. GLM was followed by a *post-hoc* Tukey's test, which was performed in the *multcomp* package using the *glht* function. All statistical tests were run using R software (Version 3.1.2, 2014).

RESULTS

We detected a great variability in repellent effectiveness (Table 2). The Tukey's *post-hoc* test revealed differences in effectiveness between each of the tested repellents ($P < 0.001$ for all tests). The orange oil stopped only 10.8% of ticks, while eucalyptus stopped 39.2%, lavender 73.3%, and DEET 98.3%. Effectiveness generally decreased over time (Fig. 1), but this was true mainly for the natural repellents (orange, eucalyptus, lavender). Efficiency of DEET was not influenced by time (Table 2, Fig. 1).

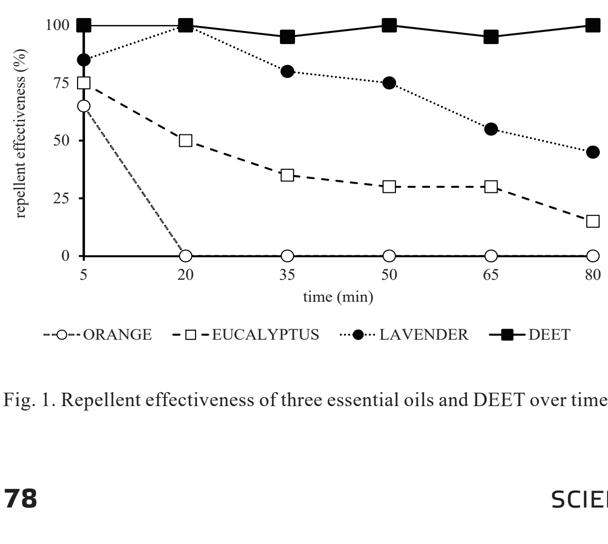

Fig. 1. Repellent effectiveness of three essential oils and DEET over time

DISCUSSION

The results obtained showed significant differences between the tested repellents as well as in the effect of time on repellency, particularly for the essential oils. All investigated samples showed high initial repellency. Five minutes after application, repellency ranged from 65 to 85%; however, the effectiveness of the investigated essential oils decreased after 80 min to a range of 0–45%.

The bioassay performed in this study also demonstrated that all the essential oils may be regarded as 'true repellents' and do not merely cover the host odour, as do some other oils according to a report by Jaenson et al. (2006). Sampled ticks apparently changed the direction of their movement immediately after first contact with treated areas.

A high initial repellency of essential oils against *I. ricinus* has been reported in previous studies by Jaenson et al. (2006) or Garboui et al.(2007). Additionally, the long-term effectiveness of oils against these ticks was demonstrated in a study by El-Seedi et al. (2012), who found no significant changes in repellency even after 24 h. That field test was performed, however, using treated flannel cloth, which has different evaporation characteristics as compared to cardboard, filter paper or human skin.

Some people (especially parents with children) do not spend long time in parks or other areas with natural tick incidence or maybe do not want to use synthetic repellents due to allergy or sensitivity. Therefore, we believe it is essential to understand fully the interaction between repellency and time. Lavender and eucalyptus oils were included into this study despite the results presented by Thorsell et al. (2006), who found no repellency from these two oils against ticks 4 h after application.

The strongest and most stable repellency effect was found for lavender oil. A rather high protective effect of this plant has previously been described against the cigarette beetle (*Lasioderma serricorne* Fabricius) (Hori, 2003) and spot clothing wax cicada (*Lycorma delicatula*) (Yoon et al., 2011). It also has been found to be quite effective against *I. ricinus* in high concentrations and shortly (15 min) after application (Kröber et al., 2013). On the other hand, it is apparent both from other previously published results (Thorsell et al., 2006; Semmler et al., 2011) and also from our data that lavender essential oil exhibits a significant protection period against *I. ricinus*. The aforementioned authors tested lavender oils on such surfaces as cardboard (this study), petri dishes (Thorsell et al., 2006), and bare skin (Semmler et al., 2011). We may expect a longer period of protection if lavender oil would be tested on textile materials.

Magano et al. (2011) tested the effectiveness of eucalyptus essential oil as a repellent against another tick species, *Hyalomma marginatum rufipes*, and demonstrated that the repellency of *Eucalyptus globoidea* (Blakely) extract compared favourably with that of the commercial arthropod repellent DEET. Moreover, Pirali-Kheirabadi et al. (2009) reported acaricidal effects of eucalyptus. Nevertheless, the repellency after 80 min determined in this study was only 15%.

To the best of our knowledge, this was the first evaluation of orange essential oil as a repellent against ticks. In comparison with established types of essential oils, it exhibited the fastest evaporation and its efficiency was the weakest from the onset of the experiment. We can conclude, therefore, that this type of oil shows no potential for future studies.

The protection periods offered by such essential oils as those from lemongrass (*Cymbopogon* Spreng), cloves (*Syzygium aromaticum* Merrill & Perry) (Thorsell et al., 2006), carnation flowers (*Dianthus caryophyllus* L.) (Tunón et al., 2006), and wormwood (*Artemisia absinthium* L.) (Jaenson et al., 2006) are comparable to that of 15% DEET (Thorsell et al., 2006; Tunón et al., 2006; Bissinger et al., 2016). On the other hand, it must be said that the duration of tick repellency among essential oils is connected with toxicity and various side effects (Thorsell et al., 2006). Thus, the potential user of essential oils as repellents faces a trade-off between longer duration of protection with greater health risks or safer but shorter protection. This trade-off suggests there to be two possible areas for future research on essential oil repellency: (*i*) eliminating health risks of essential oils providing strong and long protection, or (*ii*) enhancing the repelling efficacy of harmless oils. Such research should therefore focus mainly on the dosage or concentration of oils (Magano et al., 2011; Meng et al., 2016), synergistic effects (Hummelbrunner, Isman, 2001; Reegan et al., 2014), or a microencapsulation process (Boh, Knez, 2006; Faulde et al., 2012).

CONCLUSION

There are currently many essential oils with unknown repellency effects, and it is possible that a natural repellent based on essential oils both effective and harmless to the user may be discovered. At this time, lavender oil appears to satisfy the requirement for compromise between efficiency and health risk.

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