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Original article

The repellent efficacy of eleven essential oils against adult *Dermacentor reticulatus* ticks

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ABSTRACT

Dermacentor reticulatus ticks are among the most important arthropod vectors of zoonotic disease agents in Europe. Eleven essential oils, namely basil (*Ocimum basilicum*), bergamot (*Citrus bergamia*), clove bud (*Syzygium aromaticum*), citronella (*Cymbopogon winterianus*), creeping thyme (*Thymus serpyllum*), lavender (*Lavandula angustifolia*), lemon-scented gum (*Corymbia citriodora*), marjoram (*Origanum majorana*), peppermint (*Mentha piperita*), spearmint (*M. spicata*), and red thyme (*Thymus vulgaris*) were tested for repellency against adult *D. reticulatus* ticks at concentrations of 1 and 3%. Clove bud, creeping thyme and red thyme essential oils were the most efficient – repelling 83, 82 and 68% of ticks when diluted to 3%, respectively. The mixture of creeping thyme and citronella containing 1.5% of each showed higher repellency (91%) than individual essential oils at the concentration of 3%.

1. Introduction

The ornate dog tick, *Dermacentor reticulatus* is a three-host tick that occurs in temperate climate zones, from south-western England and France to the Yenisei river basin in Siberia (Siuda, 1993). The distribution of *D. reticulatus* within its geographic range is highly focal. Rapidly changing patterns in the spatial distribution of *D. reticulatus* were observed in the last decade in Europe (Sréter et al., 2005; Dautel et al., 2006; Bullová et al., 2009; Gray et al., 2009; Široký et al., 2011; Paulauskas et al., 2015; Mierzejewska et al., 2016). Climate change, availability of more fallow land and increasing abundance of large animal hosts such as deer are thought to be responsible for the spread and increasing incidence throughout Europe of diseases caused by *D. reticulatus* borne pathogens (Gray et al., 2009; Mierzejewska et al., 2015).

Dermacentor reticulatus may harbour several disease agents, e.g. rickettsiae (*Rickettsia slovaca, R. raoultii, R. sibirica), Anaplasma phagocytophilum*, *A. marginale*, *Francisella tularensis, Coxiella burnetii,* protozoa (*Babesia canis, B. caballi* and *B. microti*), and TBEV (Bonnet et al., 2012; Spitalska et al., 2012; Karbowiak et al., 2014; Jongejan et al., 2015). Human infection occurs via bites by the adult tick stage as nymphs are nidicolous and only rarely found host-seeking from vegetation (Siuda, 1993; Foldvári et al., 2013).

Avoiding tick-infested habitats, thorough examination after visiting areas where ticks may occur, as well as the use of personal repellents are means to decrease the risk of tick bites and tick-transmitted diseases. Several synthetic repellent substances are on the market, of those DEET is the most widely used as an active ingredient in the majority of commercially available tick repellents (Bissinger and Roe, 2010). Concerns about possible adverse health and environmental effects of synthetic products have caused increased demands for "natural" repellents (Abdel-Rahman et al., 2001; Del Fabbro and Nazzi, 2013; Ellse and Wall, 2014). A growing body of evidence suggests that products of plant origin e. g. essential oils and plant extracts may play a role in an integrated approach to control ticks and mites (Ellse and Wall, 2014; Sparagano et al., 2016). Essential oils could also represent an alternative tick-bite prevention method for people who, for various reasons object using synthetic repellents (Dietrich et al., 2006; Shapiro, 2012). Many essential oils are known to be safe at low doses (diluted to 0.05–4%) (Price and Price, 2007). On the other hand, high concentrations of some essential oils and undiluted essential oils were reported to cause rash or skin irritation in sensitive individuals and adverse skin reactions in animal dermal toxicity studies (Tisserand and Young, 2013). Therefore, essential oils able to repel ticks at low doses would be preferable when daily use is considered.

The aims of the present study were to assess the influence of low concentrations of readily available essential oils on *D. reticulatus* adults, in order to estimate if they may decrease the risk of tick bite and therefore could be used as an alternative to commercial repellents in the prevention of *Dermacentor*-borne diseases.

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Table 1
Essential oils tested for repellent properties against *Dermacentor reticulatus* adults.

Essential oil	Plant species	Plant family	Country of origin	Main constituents
basil	*Ocimum basilicum*	Lamiaceae	Comoros	methyl chavicol, methyl eugenol, cineole, linalool, eugenol
bergamot	*Citrus bergamia*	Rutaceae	Italy	limonene, linalool, linalyl acetate
clove bud	*Syzygium aromaticum*	Myrtaceae	Sri Lanka	eugenol, caryophyllenes, eugenyl acetate
citronella (Java)	*Cymbopogon winterianus*	Poaceae	Java	citronellal, geraniol, citronellol, geranyl acetate, limonene
creeping thyme	*Thymus serpyllum*	Lamiaceae	France	geraniol, linalool, thymol
lavender	*Lavandula angustifolia*	Lamiaceae	France	linalool, linalyl acetate, limonene, caryophyllene
lemon scented gum	*Corymbia citriodora*	Myrtaceae	China	citronellal, citronellol, geraniol, linalool, pinenes, limonene
marjoram	*Origanum majorana*	Lamiaceae	Egypt	terpineol, thujanol, linalool
peppermint	*Mentha piperita*	Lamiaceae	USA	menthol, menthone, cineole
spearmint	*Mentha spicata*	Lamiaceae	USA	carvone, limonene, myrcene
red thyme	*Thymus vulgaris*	Lamiaceae	Spain	thymol, linalool, caryophyllene, p-cymene

2. Materials and methods

2.1. Ticks and essential oils

Adult *D. reticulatus* were collected by flagging the vegetation in localities along the Danube river during the Spring (March–April) and Autumn (September–October) months from 2014 to 2016. Ticks were stored in glass vials with cotton cloth covering the opening, at an ambient temperature (22 °C), with a 12 h/12 h light/dark photoperiod regime and 90% relative humidity. They were used in experiments 2–3 weeks after collection. All experiments were conducted in ventilated room at laboratory temperature. Only ticks responding to human breath by vigorously walking prior to the experiment were used. Human breath was also used to induce host-seeking behaviour in laboratory bioassays. Essential oils (Table 1) were purchased from Nobilis Tilia (Krásná Lípa, Czech Republic) and stored at 4 °C. Dilutions of essential oils in 40% ethanol were kept for up to 2 months at 4 °C, and energetically shaken for at least 2 min before pipetting onto the filter paper in experimental designs.

2.2. Petri dish assay

Ticks were tested in this assay as described by Del Fabbro and Nazzi (2008) with slight modifications. Briefly, two concentric circles (lines A and B; 2.5 cm distant from each other) were drawn on the Petri dish lid. A circular, 0.5 cm wide filter paper strip was placed outside line B. One hundred μL of control (40% ethanol) or essential oil diluted to 1% was applied on the filter paper, and let evaporate for 10 min. Afterwards, each Petri dish was placed on the moist circular filter paper inside a larger Petri dish.

D. reticulatus adults were tested individually in a paired design, with each tick tested first on the control dish and subsequently on the dish with essential oil. Up to ten ticks were used for one run of each experiment. Ticks were observed until they crossed the line B, and time spent between lines A and B was recorded. Table 2 lists the number of ticks analysed for each essential oil.

2.3. Climbing bioassay

The upper 5 cm of a glass container (19 × 9 × 9.5 cm) were lined up with filter paper. One mL of diluted essential oil (1 or 3%) or control (40% ethanol) was applied on the uppermost 1.5 cm, and let evaporate for the designed time period (10 min or 2 h) outside the laboratory. Afterwards, the container was moved inside a larger open vessel (40 × 32 × 11 cm) with the bottom covered with distilled water to prevent ticks from escaping (Fig. 1). Thirty ticks (15 females and 15 males) for each assay were placed at the bottom of the experimental arena, and their behaviour was constantly observed during two hours. Ticks that stepped on the treated area with their whole body and did not immediately fall off were considered as not repelled, and removed from the system. Time from the beginning of the experiment until crossing the line between the untreated and treated areas was recorded. Each experiment was repeated three times and compared to controls previously carried out on other ticks collected on the same date. Three experimental designs were conducted: A) 1% essential oils were applied 10 min prior to the experiment; B) 3% essential oils were applied 10 min before the experiment; C) 3% essential oils were applied 2 h before the experiment. Additionally, two mixtures of essential oils (creeping thyme and citronella containing 1.5% of each; and clove bud and red thyme containing 1.5% of each) were also tested in experimental designs B and C, and their effect on ticks compared to those of individual essential oil at 3% concentration.

2.4. Statistical analysis

Non-parametric methods were applied to evaluate the results of the Petri Dish assay: Wilcoxon signed rank test was used to assess the repellent potential of essential oils; and Mann-Whitney *U* test was used to assess if essential oils had same influence on females and males, and to compare essential oils.

The repellent effect of essential oils on ticks in the climbing bioassay was evaluated as repellency 30 min after the beginning of the experiment, as well as an increase in the median time ticks spent in the untreated area of the experimental arena compared to control ticks. Repellency percentages were calculated according to the Henderson equation/Modified Abott's formula: $100 - [(\text{Mean number of ticks not repelled})/(\text{Mean number of ticks not repelled on controls})] \times 100$. If ticks were present on untreated surfaces of the experimental arena at the end of the experiment (after two hours), 120 min was their recorded time for the purpose of calculating the median, first and third quartiles. Kaplan-Meier estimates and log rank test were also used to assess and compare the efficacy of essential oils (Therneau, 2015).

3. Results

3.1. Petri dish assay

The numbers of ticks tested were 614 and 723 for the Spring and Autumn populations, respectively. Results are summarized in Table 2. The time spent between lines A and B ranged from 3 s, minimum observed on a control Petri dish (PD), to 6.75 min spent by one female tick on PD with 1% clove bud oil (control time for this tick was 12 s). Statistically significant differences between the time spent on a control PD and a treatment PD with 1% essential oil were observed for seven essential oils (Table 2), ranked by their efficacy: creeping thyme > red thyme > clove bud, citronella > marjoram, basil, lemon-scented gum. The median time spent on a control PD was 8 s, while the median time spent on a treatment PD ranged from 13 to 36 s. The median difference between the time individual ticks spent on PD with essential oil and time they spent on a control PD ranged from 4 to 24 s (Table 2).

Lavender, bergamot, peppermint and spearmint essential oils seemed to have some minor repellent properties against *D. reticulatus*