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Preventing tick attachment to dogs using essential oils

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ABSTRACT

Preventing tick bites using repellents could make a valuable contribution to an integrated tick management programme for dogs. Here, the ability of a range of essential oils or active ingredients of commercially available repellents, to abolish the orientation and taxis of the tick *Ixodes ricinus* towards sebum extracted from dog hair was examined in laboratory bioassays. Substantial differences between oils were observed, but turmeric oil was both able to prevent a climbing response by ticks and had a longer residual activity than other oils. A blanket-drag field assay was then used to compare the attachment of ticks to blankets impregnated with one of: turmeric oil, DEET (positive control), orange-oil or excipient only (negative controls). In total, 899 ticks were counted, with an average of 23.3 (SD ± 21.3) ticks per blanket drag for excipient-only (n = 16), 26.9 (SD ± 28.6) for orange oil (n = 16), 2.6 (SD ± 2.0) for turmeric oil (n = 16) and 3.4 (SD ± 3.7) for DEET (n = 16). Finally, in a participatory *in vivo* trial, tick acquisition by 15 untreated control dogs was compared with 24 dogs sprayed with turmeric-oil and 16 dogs sprayed with orange oil (both 2.5% v/v diluted in water with a 1% coco glucoside excipient) before each walk in known tick infested areas. The percentage of dogs with ticks attached to the legs or belly of dogs sprayed with turmeric oil suspension (15% ± 19.4%) was significantly lower than that of ticks attached to the same areas of dogs sprayed with orange oil suspension (85% ± 19.4%) and unsprayed dogs (73% ± 26.2%) ($P < 0.05$). The data indicate that turmeric-oil may form a valuable component of a tick management programme for domestic dogs.

1. Introduction

Effective control and management of ticks may best be achieved using a multifaceted approach; combining the benefits of a range of methods is likely to increase probability that ticks and tick-borne pathogens are more effectively eliminated. With neurotoxic acaricides, efficacy and residual activity depend on the active ingredient and mode of application, with the range of topical, systemic or slow-release products currently available offering a mixture of advantages and disadvantages. This has encouraged the commercialisation of combinations of actives, offering different complementary properties, and a search for alternative methods of tick control, such as vaccines and biocontrol with parasitoids, predators and entomopathogenic fungi (Samish et al., 2008; Perez-Perez et al., 2010). As part of a tick management programme, avoidance and prevention of tick bites, using repellents, may also make a valuable contribution (Ellse and Wall, 2013; Lupi et al., 2013; Abdel-Ghaffar et al., 2015).

Two types of repellency are defined (Halos et al., 2012). The first, repellency sensu stricto, may be attributed to a compound producing an irritant effect through direct contact, which causes a tick to move away from the treated surface/animal or to fall off before attaching to the host. The latter, repellency sensu lato (or expellency), causes the inhibition of attachment or the detachment of already attached ticks. In the last decade, there has been extensive research into the repellent effects of many compounds against ticks. The majority of these studies have focused on *in vitro* studies of sensu stricto repellence (Pamo et al., 2005; Ribeiro et al., 2008; Cetin et al., 2010; Štefanidesová et al., 2017).

A variety of commercial tick repellents are available, including both synthetic and plant derived compounds (Nerio et al., 2010; Lupi et al., 2013; Rehman et al., 2014), including DEET (N,N-Diethyl-3-methylbenzamide), IR3535 (3-N-acetyl-N-butylamino-proprionic ethyl ester), icaridin (1-piperidine-carboxylic acid 2e2 hydroxyethyl-1-methylester), as well as a natural *Eucalyptus citriodora* derivative (*para*-menthane-3,8-diol) (Semmler et al., 2009; Abdel-Ghaffar et al., 2015; Benelli et al., 2016).

Plant-derived essential oils are blends of approximately 20–80 different metabolites which are usually extracted from plants via steam distillation (Bakkali et al., 2008). These metabolites are volatile molecules of low molecular weight and usually contain two or three major terpene or terpenoid components, which constitute up to 30% of the oil (Bakkali et al., 2008). There is a growing body of evidence indicating

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that they possess varying mechanisms of action against arthropods; they have been shown to inhibit feeding and the synthesis of chitin, decrease growth, development or reproduction, and affect behaviour including acting as repellents (Pazinato et al., 2016; Rosado-Aguilar et al., 2017). The efficacy of essential oils is often attributed to the oil's major component(s); however, there is also evidence that the various oil components may work in synergy (Nerio et al., 2010). This may occur because some oil components aid cellular accumulation and absorption of other toxic components (Cal, 2006).

The efficacy of essential oils against ticks has been demonstrated following immersion and physical contact with treated surfaces, as well as after exposure to the vapour of oils; the latter implies that there is a neurotoxic, rather than simply a mechanical pathway in their mode of action. Terpinen-4-ol, for example, a monoterpenoid found at high concentrations in tea tree oil, inhibits arthropod acetylcholinesterase, an enzyme essential for transmission of action potentials (Mills et al., 2004; López and Pascual-Villalobos, 2010). Additionally, the hydrophobic nature of the oils may simultaneously exert mechanical effects on the parasite such as by disrupting the cuticular waxes and blocking the spiracles, which leads to death by water stress or suffocation (Burgess, 2009).

The aims of the work presented here were to use an *in vitro* laboratory bioassay to screen essential oils for repellency in the tick *Ixodes ricinus*, to further test the most promising oils using a blanket-drag field assay and finally to investigate the efficacy of oils as natural tick repellent for dogs walked regularly in tick infested areas.

2. Materials and methods

2.1. Repellency bioassay

The oils used in this study were selected based on previous reports of biological activity. Essential oils from bog myrtle (*Myrica gale*), cajeput (*Melaleuca cajeputi*), geranium (*Pelargonium gravolens*), ginger (*Zingiber officinale*), grapefruit (*Citrus paradisi*), lavender (*Lavendula angustifolia*), niaouli (*Melaleuca viridiflora*), orange (*Citrus sinensis*), peppermint (*Mentha arvensis*), spearmint (*Mentha spicata*), thyme (*Thymus vulgaris*) and turmeric root (*Curcuma longa*) were used, as well as the carrier oils blackseed (*Nigella sativa*) and soya (*Soja hispida*). The oils were screened initially at a concentration 5% (v/v), following previously published studies (Ellse and Wall, 2013). All essential oils were obtained from one supplier (Naissance Trading & Innovation Co Ltd., Neath, United Kingdom) and had been extracted via steam distillation, with the exception of the citrus peel oils (orange and grapefruit), which had been cold pressed. Oils were stored at 4 °C to prevent thermo-degradation or oxidation. Ethanol (Sigma-Aldrich, ≥99.8%, Scientific Laboratory Supplies Ltd., Dorset, United Kingdom) was used to dilute the oils to varying concentrations. As an attractant, sebaceous secretions were extracted from hair clippings obtained from an English Springer Spaniel (Crooks and Randolph, 2006). Clumps of hair were chopped, placed in 50 ml of methanol (Sigma-Aldrich, 99.8%, Scientific Laboratory Supplies Ltd., Dorset, United Kingdom) and stirred continuously for 10 min. The beaker was then allowed to stand at room temperature (21 ± 1 °C) for 48 h and the remaining liquid strained from the hair using a sieve. The sebum suspension was divided into aliquots and stored at −20 °C until use. Ethanol only and a suspension of olive oil in ethanol, provided negative controls (Martinez-Velazquez et al., 2011). DEET (N,N-Diethyl-3-methylbenzamide) and PMD (*p*-menthane- 3,8-diol, Sigma-Aldrich, Scientific Laboratory Supplies Ltd., Dorset, United Kingdom, which is found in small quantities in the essential oil from the leaves of the *Eucalyptus citriodora* tree and used as an active ingredient in many insect repellents, provided positive controls.

Filter paper strips (8 × 1 cm) were suspended from one end by a cotton thread. Before each test, 10 μl of test compound was applied to the end of the filter paper strip from which it was suspended using a pipette; this amount was sufficient to impregnate the top 1 cm of the filter paper only. The ethanol was allowed to evaporate prior to testing (≈30 s). The strip was suspended vertically, 1 cm below the tip of a horizontal glass rod. 50 μl of the dog hair sebum attractant was applied to a circle of filter paper (15 mm diameter), which was then allowed to dry for 5 min in a fume cupboard. This was attached to the tip of the glass rod, using double sided tape, so that it was directly above the suspended vertical strip of filter paper (Fig. 1).

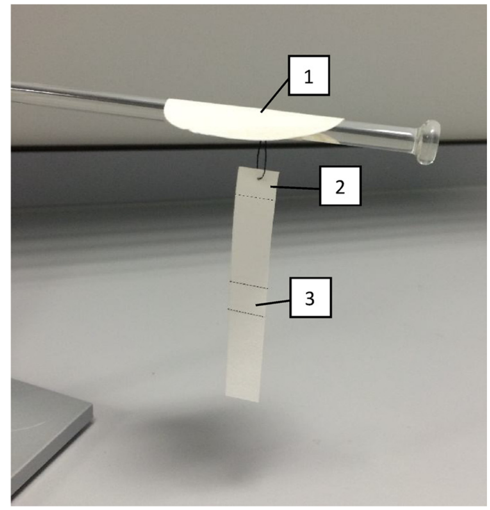

Fig. 1. The repellency bioassay apparatus showing 1) the attractant, 2) the treated-tip and 3) the starting point.

Nymphal *I. ricinus* were collected using a standardised blanket-dragging technique from vegetation at the edge of an area of woodland in south west England. After collection, nymphs were stored at 7 °C and were acclimated to room temperature (21 ± 1 °C) for 24 h prior to testing. Ticks were used within three days after collection and each tick was used only once. A fine paintbrush was used to transfer an individual tick, selected at random, onto the centre of the suspended vertical strip of filter paper and its behaviour was observed for 5 min, to record the movement of the tick on the filter paper. The number of ticks that reached the top of the filter paper strip or dropped off was recorded. Ten ticks were tested per oil and each filter paper strip was used only once. The attractant-treated filter paper was replaced with each new oil (every 10 ticks). The oils for which fewest ticks reached the tip and most dropped off – thyme, spearmint, ginger, geranium, turmeric, peppermint and lavender, were then restested at a concentration of 5%, alongside 20% DEET and 5% PMD after drying times of 1 and 4 h post-treatment to examine their residual activity. Turmeric was also retested at 2.5% and 1.25%.

2.2. Blanket-drag sampling

Following the results of the *in vitro* assay described above, turmeric oil was used as a repellent and orange oil was used as a hydrophobic negative control. Both oils were tested at 2.5% (v/v) in a 1% coco-glucoside excipient diluted in water. DEET (20% v/v) was used as a positive control and 1% coco-glucoside diluted in water as an excipient-only control. Each treatment type was applied to four 1 m^2 white cotton blankets (16 in total); treatments were placed in a pump-action spray bottle and each blanket was sprayed in a fume cupboard 100 times on each side, at one pump every 10 × 10 cm. Each pump delivered approximately 0.275 ml and the application rate delivered approximately 55 ml of treatment suspension per blanket. The blankets were then placed in individual airtight bags for transport to the field. Blankets were machine washed before being re-used and only blankets sprayed with the same treatment suspension were washed together.

The study site was at the edge of woodland within Ashton Court