REVIEW ARTICLE

The use of essential oils in veterinary ectoparasite control: a review

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Abstract. There is a growing body of evidence indicating the potential value of essential oils as control agents against a range of arthropod ectoparasites, particularly lice, mites and ticks. Toxicity has been demonstrated following immersion and physical contact with treated surfaces, as well as after exposure to the vapour of these oils; the last of these factors implies that there is a neurotoxic, rather than simply a mechanical, pathway in their mode of action. However, the volatile nature of essential oils suggests that their residual activity is likely to be short-lived. A possible advantage of essential oils over conventional ectoparasite treatments may refer to their reported ovicidal efficacy, although it is unclear whether this results from neurotoxicity or mechanical suffocation. There are many difficulties in comparing the findings of existing studies of essential oil toxicity. One major issue is the wide variation among batches in the relative concentrations of oil constituents. A second issue concerns the fact that many experimental designs make it difficult to confirm that the effect seen is attributable to the oil; in many cases inappropriate controls mean that the effects of the excipient on mortality cannot be distinguished. Hence, it is important that an excipient-only control is always included in these bioassays. Furthermore, in direct contact assays, when attempting to identify the toxicity pathway of the essential oil tested, it is important to include a hydrophobic control. Without this, it is impossible to distinguish simple mechanical effects from neurological or other cellular toxicity. The use of essential oils in the control of veterinary ectoparasites is an area which holds considerable potential for the future and research into their use is still at an early stage. More extensive field trials, the standardization of components, the standardization of extraction, the standardization of good experimental design, mammalian toxicology profiling and excipient development, as well as further investigation into the residual activities and shelf-lives of these oils are all required to allow the full realization of their potential.

Key words. Botanical products, ectoparasites, essential oils, flies, lice, mange, mites, myiasis, pediculosis, ticks.

Introduction

The control of ectoparasites of veterinary importance using synthetic neurotoxic insecticides has been progressively undermined by the development of insecticide resistance. This is seen particularly clearly in the control of lice (Johnson et al., 1992; James et al., 1993; Levot, 1995; Ellse et al., 2012), mites (Beugnet et al., 1997) and ticks (Foil et al., 2004). In addition, restrictions on the use of some insecticides, such as organochlorines, organophosphates and pyrethroids, because of their effects on human health (Kolaczinski & Curtis, 2004) and the environment (Ramwell et al., 2009), combined with a growing interest in organic farming practices, have led to an increase in research into the development of alternative approaches to ectoparasite management, amongst which botanical alternatives, such as essential oils, are currently receiving particular attention.

Essential oils are blends of approximately 20–80 different plant metabolites which are usually extracted from plants through steam distillation (Bakkalai et al., 2008). These
metabolites are volatile molecules of low molecular weight. Essential oils usually contain two or three major terpene or terpenoid components, which constitute up to 30% of the oil (Bakkalai et al., 2008). The insecticidal or acaricidal efficacy of many essential oils has been well documented in a variety of pests. This efficacy is often attributed to the oil’s major component(s); however, there is also evidence that the various oil components may work in synergy (Yang et al., 2003). This may occur because some oil components aid cellular accumulation and absorption of other toxic components (Cal, 2006). Nevertheless, the mode of action of many essential oils or their components is largely unknown, although there is evidence of a toxic effect on the insect nervous system. For example, terpinen-4-ol, 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; Lopez & Pascual-Villalobos, 2010). Alternatively, 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 (Burgess, 2009) or suffocation.

A great deal of the recent research into the efficacy of essential oils as agents for the control of arthropod ectoparasites of veterinary importance has been carried out using such widely different extraction procedures, formulations, application methods and approaches to toxicity measurement that it can be difficult to replicate or compare studies. The experimental designs used in a large number of cases are not ideal, particularly in relation to the inclusion of appropriate control groups, and undermine the conclusions drawn. This review therefore aims to present a comparative synopsis of current research, against which the relative efficacies of these compounds are assessed and good experimental practice is highlighted. For ease of reference, the common and systematic names of the plants from which the essential oils are derived are shown in Table 1.

### Mites

**Poultry red mite.** The poultry red mite, *Dermanyssus gallinae* (De Geer) (Mesostigmata: Dermanyssidae), has been the subject of extensive acaricide assays using a wide range of essential oils (Kim et al., 2004; George et al., 2009a, 2010a, 2010b). At a concentration of 0.35 mg/cm\(^2\) of filter paper, 50 of 56 essential oils tested resulted in 100% mortality after 24 h of contact with impregnated filter papers in a closed chamber and 12 of the tested oils produced 100% mite mortality at concentrations as low as 0.07 mg/cm\(^2\) (Kim et al., 2004). A second study by the same authors suggested that the contact activity of some essential oils, when exposure was measured in a closed chamber, was comparable with that of commercially available acaricides (Kim et al., 2004). These effects of exposure to the vapour from essential oils suggested that the insecticidal efficacy could be attributed to the volatile components of the oils (Kim et al., 2004, 2007; George et al., 2009a). Placing mesh-ended cylinders containing *D. gallinae* into sealed vials containing filter papers impregnated with 0.28 mg/cm\(^2\) of essential oil resulted in 100% mortality of mites after 24 h of exposure to the essential oils of cade (*Juniperus oxycedrus*), clove (*Eugenia caryophyllata*), coriander (*Coriandrum sativum*), horseradish (*Armoracia rusticana*) and mustard (*Brassica juncea*) (Kim et al., 2004). Subsequent similar experiments with *D. gallinae* supported these findings (Kim et al., 2007).

The acaricidal efficacy of essential oils appears to be linked to the vapour pressure to which the mites are exposed, presumably because this affects the concentration of volatile

### Table 1. Common and systematic names of plant essential oils investigated in the papers included in this review.

<table>
<thead>
<tr>
<th>Common name</th>
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<tr>
<td>Allspice</td>
<td>Pimenta dioica</td>
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<td>Basil</td>
<td>Ocimum basilicum</td>
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<td>Bay</td>
<td>Laurus nobilis</td>
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<td>Bog myrtle</td>
<td>Myrica gale</td>
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<td>Cade</td>
<td>Juniperus oxycedrus</td>
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<td>Camphor</td>
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<td>Carnation</td>
<td>Dianthus caryophyllus</td>
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<td>Catnip</td>
<td>Nepeta cataria</td>
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<td>Chamomile</td>
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<td>Cinnamon</td>
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<td>Citronella</td>
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<td>Clove</td>
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<td>Coriander</td>
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<td>Cumin</td>
<td>Cuminum cyminum</td>
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<td>Eucalyptus (blue gum)</td>
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<td>Garlic</td>
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<td>Geranium</td>
<td>Pelargonium roseum</td>
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<td>Horsemint</td>
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<td>Horseradish</td>
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<td>Lavender</td>
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<td>Lemon eucalyptus</td>
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<td>Manuka</td>
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<td>Thyme</td>
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<td>Ylang ylang</td>
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<td>Lippia triplinervis</td>
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<td>Rhododendron tomentosum</td>
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components. For example, a study in which mites were allowed contact with oils in open or closed chambers showed that even the most effective of the three oils tested, essential oil of manuka (Leptospermum scoparium), resulted in mortality of just 30% in contact assays carried out in open chambers compared with >80% in contact assays conducted in closed chambers at the same concentration (George et al., 2009a). Similarly, >90% mortality was observed after 24 h of exposure to the vapour of essential oil of thyme (Thymus vulgaris) in closed chambers, whereas vapour assays conducted in open chambers using the same concentration resulted in only approximately 10% mortality (George et al., 2009a).

The highly volatile nature of essential oils may be responsible for the relatively short persistence of the acaricidal efficacy of some oils. Indeed, contact exposure to 0.21 mg/cm² of essential oil of lavender (Lavandula angustifolia) in a closed chamber resulted in >70% mortality if the oil solution was applied to the filter paper 3 min prior to exposure, whereas mortality dropped significantly to approximately 11% if the filter paper had been left in a fume cupboard for 24 h prior to the introduction of the mites (George et al., 2008). However, despite the decrease in acaricidal efficacy, olfactory experiments indicate that the volatile nature of essential oil of thyme may be sufficient to repel D. gallinae for up to 13 days (George et al., 2009b).

Another limitation of essential oils identified in bioassays against D. gallinae is the variance between experiments in the toxicity of the same essential oil (Kim et al., 2004; George et al., 2010a), even when the oils are supplied by the same source (George et al., 2010a). In addition, essential oils produced from different varieties of the same plant species showed marked differences in toxicity against D. gallinae in experiments using essential oils of lavender (George et al., 2010a) and cinnamon (Cinnamomum zeylanicum) (Nag et al., 2011). Indeed, gas chromatography–mass spectrometry (GC-MS) found that the major constituents of two different strains of essential oil of catnip (Nepeta cataria) differed greatly from one another in that one strain was comprised of 92% R-enantiomer nepetalactone and 8% caryophyllene, whereas the other was comprised of 17% R-enantiomer nepetalactone, 70% S-enantiomer nepetalactone and 13% caryophyllene (Birckett et al., 2011). This inconsistency in oil composition among batches is an inherent problem in experimental work and commercial applications. Such differences are important because the precise composition of an essential oil may determine its acaricidal efficacy. Individual constituents of cinnamon and cassia oils have been shown to have acaricidal efficacy against D. gallinae (Na et al., 2011). Closed-chamber vapour assays of 34 compounds derived from Cinnamomum spp. and Cassia spp. showed that α-methyl-E-cinnamaldehyde and E-cinnamaldehyde had acaricidal efficacy comparable with that of the acaricide dichlorvos with 50% lethal doses (LD₅₀) of 0.45 µg/cm², 0.66 µg/cm³ and 0.3 µg/cm³, respectively, which were significantly better than the LD₅₀ of 11.79 µg/cm³ attributed to the most effective whole cinnamon essential oil (technical grade) (Na et al., 2011).

The chemical compositions of the various essential oils may affect their mechanisms of action, which may explain differences in the ovicidal efficacy of some oils against D. gallinae (George et al., 2010b). Whereas the essential oils of cinnamon, garlic (Allium sativum) and pennyroyal (Mentha pulegium) were found to have significant ovicidal activity at concentrations equal to the 50% lethal concentration (LC₅₀) for adult D. gallinae, essential oils of clove, thyme, cade and manuka had no effect on egg hatch at their corresponding adult LC₅₀ levels (George et al., 2010b). This result may reflect differences in the modes of action of the oils. However, an alternative explanation may lie in the experimental design, as the study used LC₅₀ concentrations for adults which had been determined previously, and this meant that some of the oils which appeared to be non-ovicidal (e.g. essential oil of thyme) were used at concentrations at least five times lower than those of the effective oils (George et al., 2010a).

As yet, no major in vivo tests of the acaricidal efficacy of essential oils against D. gallinae appear to have been published. A repellence experiment, in which red mite traps which had been treated with two different strains of catnip essential oil were placed into hen houses, found a significant reduction in the number of mites caught in the essential oil-treated trap compared with the control trap (Birckett et al., 2011). An in vitro study looking at the impact of environmental factors in hen houses, such as temperature, humidity and dust levels, suggested that some essential oils may be particularly appropriate for further investigation in the field (George et al., 2010a). However, a study of the impact of spraying pennyroyal and thyme essential oils in hen houses suggested there were a range of negative impacts associated with the presence of high concentrations of pennyroyal essential oil: two chickens died after chronic exposure to treated hen houses, and reductions in both chicken weight gain and egg production were observed (George et al., 2010c). By contrast, thyme essential oil did not have any negative effects on the birds used (George et al., 2010).

Psoroptes spp. Early in vitro studies found that lavender essential oil and many of its constituents were effective against Psoroptes cuniculi (Hering) (Sarcoptiformes: Psoroptidae) (Perrucci et al., 1994, 1996). These studies used contact and vapour exposure assays to show that there was a structural basis to the acaricidal properties of essential oil derivatives (Perrucci et al., 1995). In the latter study, monoterpen hydrocarbons, limonene and γ-terpinene were found to be ineffective against mites in open-chamber contact assays and closed-chamber vapour assays at concentrations up to 1% (v/v) (Perrucci et al., 1995), whereas the phenols, thymol and eugenol were extremely effective at killing mites, even at concentrations of 0.125%, in open-chamber contact assays and closed-chamber vapour assays. Both linear (linalool, geraniol and nerol) and cyclic (terpinen-4-ol and menthol) terpene alcohols were highly effective at concentrations of 0.125%, with mortality rates of >97% in open-chamber contact assays; however, mortality in closed-chamber vapour assays varied widely (Perrucci et al., 1995). The structure of the components of essential oils has also been used to explain marked differences in the efficacy of the oils of two Laurus spp. against P. cuniculi in vitro (Macchioni et al., 2006). Experiments using GC-MS found that essential oil of bay (Laurus nobilis), which
provided relatively poor acaricidal effects with a 10% (v/v) concentration resulting in mortality of only 73% after 24 h of contact exposure, is comprised mainly of 1,8-cineole (39.2%), a monoterpenoid. By contrast, Laurus novocanariensis had greater acaricidal effects, with a >5% (v/v) concentration resulting in 100% mite mortality; its major constituents were α-pinene (10.4%), 1,8-cineole (9.6%) and β-selinene (7.2%), which are sesquiterpenes. However, neither oil was effective at the lower concentration of 3% (Macchioni et al., 2006).

In vivo treatment of Psoroptes sp. infections with the essential oil components linalool (Perrucci et al., 1997) and trans-cinnamic acid (Wall & Bates, 2011) resulted in high mite mortality. Rabbits that had been experimentally infected with ear mites were cleared of infestation when treated twice weekly for 3 weeks with a 5% linalool, saline and petroleum oil solution (Perrucci et al., 1997). The decrease in mite abundance was comparable with that provided by conventional insecticide treatments (Perrucci et al., 1997). These positive results suggest that a similar solution may serve as the basis of future scab control programmes. However, some of the animals treated with higher concentrations of linalool (10%) had a short-lived reddening of the ear (Perrucci et al., 1997). Seven of eight experimentally infected sheep treated with 10% trans-cinnamic acid were completely cured of scab and the eighth sheep exhibited a significant reduction in mite abundance and scab size; this protection was retained for 56 days post-application and no adverse effects of treatment were reported (Wall & Bates, 2011).

Whole essential oils of cinnamon leaf and clove have been shown to have high levels of acaricidal efficacy against P. cuniculi in rabbits at concentrations of 2.5% (Fichi et al., 2007a, 2007b). After application, levels of excoriation, scabbing and mite abundance decreased to show no significant differences with those achieved by the positive control, Acacerulen® (Teknofarma SpA, Turin, Italy) (25% pyrethrins) in either essential oil treatment group (Fichi et al., 2007a, 2007b). An irritant effect of the essential oil of cinnamon leaf was evident, with reddening of the pinna and more regular ear scratching (Fichi et al., 2007b). No irritant effect was observed in the clove oil-treated group, which the authors attributed to the second most abundant component of clove essential oil, β-caryophyllene (24.9%) (Fichi et al., 2007a). This sesquiterpene, which itself showed no acaricidal efficacy in vitro, may have a local anaesthetic effect (Ghelardini et al., 2001; Fichi et al., 2007a).

Sarcoptes scabiei. Contact assays against Sarcoptes scabiei (De Geer) (Sarcoptiformes: Sarcoptidae) found that the essential oil of clove and derivatives of its major constituent, eugenol, caused significant mortality at concentrations as low as 1.5%, whereas essential oils of ylang ylang (Cananga odorata) and nutmeg (Myristica fragrans) had limited effects (Pasay et al., 2010). Notably, mortality appeared to differ between permethrin-resistant and -susceptible populations of S. scabiei, with resistant mites having a higher 50% lethal toxicity (LT50); however, the sample size was not sufficient to support a statistical confirmation of this trend (Pasay et al., 2010). In vivo trials in which 1% emulsions of seven different essential oils were applied to groups of six S. scabiei var. suis-infested pigs showed that the essential oils of tea tree (Melaleuca alternifolia), pennyroyal and citronella (Cymbopogon nardus) all caused a reduction in mite numbers to <7% of the original infection 4 weeks post-treatment; essential oil of tea tree was the most effective, with only 1.45% of original infection present by the end of the trial (Magi et al., 2006).

Otodectes cynotis. In vitro contact assays using the dog ear mite, Otodectes cynotis (Hering) (Astigmata: Psoropticidae), found that the essential oil constituent, geraniol, killed all mites within 1 h at concentrations of ≥5% (v/v), whereas limonene, p-cymene and α-pinene were less effective, taking in excess of 19 h to achieve 100% mortality at concentrations of 10% (Traina et al., 2005).

Ticks

There has been extensive research in the last decade into the repellent and acaricidal effects of many essential oils against ticks. The majority of these studies have focused on species of Rhipicephalus andIxodes (both: Ixodidae: Ixodidae) ticks, largely in vitro. However, data on the effects of essential oils as tick treatments or repellents in vivo are very limited. One of the few studies in the latter category used a soap containing 0.03 μL/g of essential oil of Ageratum houstonianum against ticks biting goats. However, the concentration actually applied to the goat is unclear from the methodology presented. Eight days after goats were treated there was a 95% reduction in the numbers of biting ticks compared with a 23% reduction in the soap-only control (Pamo et al., 2005). Whether this was the result of mortality, irritation leading to detachment or the action of the soap/oil as a repellent that deterred the attachment of further questing ticks is unclear.

Immersion and contact assays. Mortality in Rhipicephalus microplus (Canestrini) after exposure to essential oils has been particularly widely examined in vitro. Typically, in vitro trials involve the temporary immersion of adults or mesh bags containing newly hatched larvae into various solutions of essential oil before mortality is assessed over subsequent days.

The potency of essential oils against tick larvae varied considerably among plant species. The results of a larval immersion test using three essential oils derived from plants of the Lamiaceae family [thyme, horsemint (Mentha longifolia) and Dorysteochas hasata] showed 100% mortality in R. microplus larvae 24 h after a 5-min submersion in 0.1% solutions of each oil (Koc et al., 2012). Cumin (Cuminum cyminum) and allspice (Pimenta dioica) essential oils were also effective against the same tick species in larval immersion tests at concentrations of 1.25% and 2.5%, respectively. However, basil (Ocimum basilicum) essential oil had no larvicidal properties, even at concentrations of 20% (Martinez-Velazquez et al., 2011). Mortality was also high in Rhipicephalus sanguineus (La Freile)
and *R. microplus* larvae immersed in hexane-extracted *Calea serrata* essential oil diluted in ethanol. However, mortality of 100% was not achieved until a concentration of 3.25% was used (Ribeiro *et al.*, 2008). In many cases it is difficult to compare the efficacies of essential oils between studies because inappropriate controls mean that the effects of the excipients used in each test cannot be distinguished. In addition, oil extraction techniques, which may also affect subsequent efficacy, vary widely. For example, methanol and hexane extractions from the same plant resulted in differences in *R. microplus* larval mortality (Ribeiro *et al.*, 2007). Steam distillation may yield an essential oil with different properties again. These differences in efficacy may be attributed to the concentration of the oil’s major component or the relative concentrations of oil constituents and their interactions. Analysis of the larvicidal efficacy of the oil of *Hesperozygis ringens* and its major component, pulegone, indicate that the oil as a whole has a superior efficacy (Ribeiro *et al.*, 2010), which may reflect the synergism of the oil’s components.

Essential oil toxicity is also dependent upon arthropod susceptibility. Whereas two species of tick, *R. sanguineus* and *R. microplus*, displayed no significant difference in mortality in response to the oil of *C. serrata* (Ribeiro *et al.*, 2008), different genera of ticks have shown marked differences in mortality in response to the same essential oil (Gomes *et al.*, 2012). Rates of mortality in larvae of *Dermacentor nitens* (Neumann) (Ixodida: Ixodidae) and *R. microplus* indicated LC50 values of 11.13 μL/mL and 5.59 μL/mL, respectively, after a 5-min immersion in essential oil of *Lippia sidoides* diluted in water and 2% Tween® 80 (Gomes *et al.*, 2012).

Comparisons of the efficacy of these oils are hampered by differences in the excipients used. For example, although the control mortality of excitients such as ethanol (Ribeiro *et al.*, 2008, 2010) or a trichloroethylene/olive oil mixture (Martinez-Velazquez *et al.*, 2011) may be acceptably low, it cannot be assumed that essential oils will interact in the same manner in such disparate media. In addition, in some cases the units used to record the concentrations are not intuitive and therefore it is extremely difficult to compare the efficacy of oils tested in one study with that of oils tested in another. For example, the use of mg/mL as a unit of measurement is surprising in a context in which the diluted product is a liquid and the specific weight is not stated (Ribeiro *et al.*, 2007; Lage *et al.*, 2013). Using this unit of measurement because it refers to the weight of the plant prior to oil extraction is also problematic as different plants and even sections of plants contain vastly different concentrations of essential oil.

The susceptibility of adult *Rhipicephalus* spp. to essential oils appears generally to be substantially lower than that of the larvae of this genus (Ribeiro *et al.*, 2007, 2008, 2010). No significant mortality was observed after the immersion of adult *Rhipicephalus* in essential oils of *Hypericum polyanthemum* (Ribeiro *et al.*, 2007), *C. serrata* (Ribeiro *et al.*, 2008) or *H. ringens* (Ribeiro *et al.*, 2010), although larvae immersed in the same oils experienced high levels of mortality. However, engorged adults of *Rhipicephalus* spp. immersed in 5% essential oil of geranium (*Pelargonium roseum*) (Pirali-Kheirabadi *et al.*, 2009) and 0.8% essential oil of oregano (*Origanum bilgeri*) (Koc *et al.*, 2013) were found to have mortalities of 79.2% after 24 h and 83.3% after 48 h, respectively. Neither of the oils gave 100% adult mortality at the concentrations tested and many had very poor acaricidal efficacy. Essential oil of eucalyptus (*Eucalyptus globulus*) killed just 37.5% of adult ticks at 6 days after immersion in a 5% solution, and some oils, including those of *H. polyanthemum* (Ribeiro *et al.*, 2007), *C. serrata* (Ribeiro *et al.*, 2008) and *Lippia triplinervis* (Lage *et al.*, 2013), have been shown to have no adult acaricidal effects at all.

A significant decrease in the egg mass laid by female *Rhipicephalus* was observed after immersion in the essential oils of *L. triplinervis* (Lage *et al.*, 2013), geranium (*Origanum onites*) had an LC50 of 2.34% (v/v) after 24 h against engorged *Rhipicephalus turanicus* (Pomerantsev) (Coskun *et al.*, 2008). Further studies in this area may be of considerable importance to allow the range of potential impacts of essential oils on ticks to be assessed.

Acaricidal vapour assays. As with mites, there is evidence that the acaricidal efficacy of essential oils against ticks may in part be attributable to their volatile components (Iori *et al.*, 2005; Cetin *et al.*, 2009, 2010). Exposure to closed vapour chambers containing the essential oils of tea tree (*Iori et al.*, 2005), oregano (*Origanum minutiflorum*) (Cetin *et al.*, 2009) and savoury (*Satureja thymbra*) (Cetin *et al.*, 2010) demonstrated acaridical efficacy against *Ixodes ricinus* (Linnaeus), *R. turanicus* and *Hyalomma marginatum* (Koch) (Ixodida: Ixodidae), respectively. The toxicity of the vapour phase of oregano (*O. minutiflorum*) essential oil against engorged *R. turanicus* was particularly high, with 95% mortality after 120 min of exposure to 5 μL/L oil (Cetin *et al.*, 2009). This effect was dependent on exposure time, with shorter exposure times resulting in lower mortality, even at higher concentrations (Cetin *et al.*, 2009). High mortality was also seen in unfed adult *H. marginatum* (Cetin *et al.*, 2010) and nymphal *I. ricinus* (Iori *et al.*, 2005) exposed to essential oil of savoury. The standardization of exposure time is undoubtedly an important factor in any attempt to compare levels of essential oil toxicity against ticks. For example, a contact study on the effect of exposure time on mortality in *I. ricinus* nymphs found that >4.5 h of contact time was required to achieve 100% mortality in nymphs placed on filter papers impregnated with 0.18 mL/cm² of the oil of lemon eucalyptus (*Corymbia citriodora*) (Elmhalli *et al.*, 2009).

Tick repellence assays. Studies have shown that essential oil of *Gynandropsis gynandra* (Lwande *et al.*, 1999) and two
the use of wormwood as an acaricide would be limited by its feeding (Jaenson et al., 2005). From a practical perspective, the effect may have been attributable to the presence of the trace element (0.1%), nerolidol, which was extremely effective, with 0.001 μL causing 98.3% repellency (Lwande et al., 1999). Two different strains of essential oil of catnip were shown, through GC-MS analysis, to have marked differences in chemical composition which were associated with significant differences in effect: 50% repellency dosages (RD50) were 0.05 mg and 0.0012 mg, respectively (Birkett et al., 2011).

Repellency of I. ricinus nymphs has also been shown in response to essential oils of Rhododendron tomentosum (Jaenson et al., 2005) and carnation (Dianthus caryophyllus) (Tunón et al., 2006). A suspension of 10% carnation essential oil repelled 100% of ticks at 4 h after treatment, which was comparable with the effects exerted in positive control groups using N,N-diethyl-meta-toluamide (DEET) and mandelic acid diethylamide (DEM) (Tunón et al., 2006). This efficacy dropped over time, but the suspension still gave a repellence of 91% at 8 h after treatment. In this study, variation in the effects of various oil constituents was evident; suggesting that some oil components may have better longevity than others (Tunón et al., 2006).

In a feeding repellence assay, 95% of I. ricinus were deterred from feeding when a concentration of 10% of essential oil of R. tomentosum was introduced, but essential oil of bog myrtle (Myrica gale) showed a less marked degree of repellency of 59% at the same concentration and essential oil of wormwood (Artemisia absinthium) had no effect on tick feeding (Jaenson et al., 2005). From a practical perspective, the use of wormwood as an acaricide would be limited by its high mammalian toxicity.

Flies

Musca domestica. In larvicidal tests in vitro against the housefly, Musca domestica L. (Diptera: Muscidae), in which early-stage larvae were submerged for 1 min in acetone and essential oil extracts at concentrations of 100–300 p.p.m. (0.01–0.03%), the most effective essential oil, peppermint (Mentha piperita), was found to have an LC50 of 104 p.p.m. (Morey & Khandagle, 2012). Peppermint essential oil also demonstrated a repellent effect when newly emerged adults were placed into cages containing two conical flask traps, of which one contained 1% essential oil in milk and the other contained only milk; 96.8% of the flies were found in the milk-only trap (Morey & Khandagle, 2012). In the same study, peppermint essential oil also deterred oviposition (Morey & Khandagle, 2012). Similarly, essential oils of both peppermint and eucalyptus were shown to be effective repellents at doses of approximately 70 μg/cm², with repellencies of 86% and 76%, respectively (Kumar et al., 2011). These oils also demonstrated high larvicidal efficacy: LC30 values were 5 μg/cm² for peppermint essential oil and 7 μg/cm² for eucalyptus essential oil. Mortality of 100% was achieved when pupae were exposed to 10% formulations of both oils (Kumar et al., 2011). However, the same authors found that essential oil of peppermint was effective at repelling M. domestica on cattle in vivo only at concentrations of 100%; 10% formulations applied to cattle had no effect (Kumar et al., 2011). On barn surfaces, a 10% peppermint oil formulation was effective at deterring M. domestica from landing (Kumar et al., 2011). However, the control used in the in vivo studies was water, whereas the excipient for the peppermint formulation contained 45% xylene, 3% butane, castor oil ethoxylate and nonylphenol. No excipient-only control was used and hence the contribution of the excipient to any repellent effect cannot be determined. This finding of relatively poor efficacy of peppermint oil as a deterrent in the field does not agree with earlier observations made in water buffalo (Khater et al., 2010). Numbers of three species of nuisance fly, Stomoxys calcitrans (Linnaeus) (Diptera: Muscidae), M. domestica and Hippobosca equina (Linnaeus) (Diptera: Hippobosidae), were found to decrease significantly on cattle treated for lice infestations with essential oils of camphor (Cinnamomum camphora), peppermint and champomile (Matricaria chamomilla) until 6 days post-treatment; however, this experiment used only an untreated control and thus did not account for any possible repellent effects of a hydrophobic solution (Khater et al., 2009).

Haematophagous flies. A study of the repellent effects of essential oils of M. gale against the biting midge Culicoides impunctatus (Goetghheuer) (Diptera: Ceratopogonidae) used a behavioural assay in which solutions of bog myrtle essential oil and its constituents were diluted until the ratio of control: test landings equalled 1 (Stuart & Stuart, 1998). The essential oil of bog myrtle was diluted 512 times to reach this point. Notably, the various oil components had a wide range of efficacies: terpinen-4-ol was the most repellent and required to be diluted 2048 times for repellence to cease (Stuart & Stuart, 1998). High mortality was also observed and was attributed to the highly volatile fraction.

Three essential oils and their major constituents were tested in vitro and in vivo against S. calcitrans (Zhu et al., 2012). In a repellency test, adult flies were starved for 48 h prior to being introduced into cages containing citrated bovine blood-soaked sanitary towels, the membranes of which had been impregnated with diluted essential oil. After 4 h flies were squashed to ascertain whether or not they had taken a bloodmeal. Essential oil of catnip was found to be a highly effective feeding and oviposition deterrent at 6.7%; the component nepetalactones were also 100% effective as deterrents at this concentration (Zhu et al., 2012). In vivo, 250 mL of 15% and 30% catnip essential oil in TritonX (3%) and water caused a significant decrease in the number of flies landing on cattle legs for up to 6 h (> 95% reduction). However, the repellence of the 15% solution was fairly short-lived; no significant difference
between this solution and the control were found at 8 h (Zhu et al., 2012). Interestingly, a mineral oil control also demonstrated a significant repellent effect in the field and in the feeding repellence assay (Zhu et al., 2012). This highlights the need to include non-essential (e.g. mineral) oil controls in toxicity and repellence assays in order to account for the effect of the hydrophobic nature of essential oils on ectoparasite survival and behaviour.

**Myiasis and carrion flies.** Studies have suggested that essential oils may provide effective prevention and control of the myiasis-causing fly *Lucilia cuprina* (Weidemann) (Diptera: Calliphoridae) (Callander & James, 2012) and the necrophagous *Synthesiomyia nudiseta* (Van De Wulp) (Diptera: Muscidae) (Khalaf et al., 2009). An extensive *in vitro* study found essential oil of tea tree to have significant repellent effects against adults and larvae, as well as larvicidal and ovicidal activity against *L. cuprina* (Callander & James, 2012). No oviposition was observed when gravid female *L. cuprina* were given oviposition media treated with 3% (v/v) tea tree essential oil. The mean ± standard deviation number of egg masses in the excipient-only control, which consisted of a 3% aqueous emulsion of ethoxylated castor oil and ethoxylated oleic acid, was 20 ± 1.56 (Callander & James, 2012). Furthermore, in choice experiments, female *L. cuprina* placed in cages with two oviposition sites, of which one was treated with 3% tea tree essential oil and the other with excipient, did not lay any eggs on the tea tree oil-treated media for 44 days, indicating that essential oil of tea tree has a substantial period of residual activity in treated wool (Callander & James, 2012). Second- and third-stage larvae were also repelled from feeding media treated with tea tree essential oil (Callander & James, 2012). However, there was marked variance in life stage susceptibility; the oil was highly toxic to *L. cuprina* eggs and first-stage larvae at 1% (v/v) concentrations, but failed to prevent the emergence of adults from third-stage larvae immersed even in very high concentrations of oil (Callander & James, 2012).

In *S. nudiseta*, the introduction of the essential oils of *Cupressus macrocarpa* and *Alpinia officinarum* into larval feeding media showed the oils to have larval LC50 values of 1.11% and 2.37%, respectively, both of which were statistically lower than that of the excipient, acetone (Khalaf et al., 2009). In addition, at the larval LC50 concentrations, 85% of surviving larvae failed to pupate and 91% of those pupae failed to emerge, whereas in the excipient-only control, 90% of larvae pupated and 87% of pupae emerged (Khalaf et al., 2009). The oil’s toxicity may be attributed to histopathology observed in the fat bodies and salivary glands of the exposed larvae.

**Lice**

A wide range of *in vitro* and *in vivo* studies have examined the effects of various essential oils against lice of veterinary importance. The majority of research has concentrated on chewing lice of the genus *Bovicola* (Phthiraptera: Trichodectidae) in sheep (James & Callander, 2012a, 2012b) and donkeys (Ellse et al., 2013) and only one study has investigated the effect of essential oils on the sucking louse, *Haematopinus tuberculatus* (Burmeister) (Phthiraptera: Haematopinidae) (Khatet et al., 2009).

*In vitro* investigation into the efficacy of essential oil of tea tree against *Bovicola ovis* (Schrank) suggested that it has high insecticidal efficacy in closed-chamber contact and vapour assays; however, its residual activity may be short-lived (James & Callander, 2012a). Assays showed that exposure to a 1% concentration of essential oil of tea tree caused 100% mortality of *B. ovis* adults and eggs in contact with dipped wool, and mortality was also high in adults exposed to tea tree essential oil vapour (James & Callander, 2012a). The efficacy of a 1% concentration of essential oil of tea tree as a sheep dip or jet product against *B. ovis* was confirmed in the field (James & Callander, 2012b). Sheep dipped in a 1% solution of essential oil of tea tree had no clinical pediculosis for 20 weeks after treatment. Jetting fully fleeced sheep with the same formulation resulted in a 94% reduction in louse numbers, which did not increase again for 12 weeks. However, both of these *in vivo* trials used untreated animals as controls and therefore some mortality may be attributed to the emulsifying agent used in the suspensions. In addition, the field efficacy of essential oil of tea tree may not be as great if the animals were subject to repeated louse challenge. Indeed, only low levels of mortality were seen when lice were introduced to cotton fabric that had been treated with essential oil of tea tree 1 day previously (James & Callander, 2012a).

The essential oil of tea tree used in the experiments described by James & Callander (2012a, 2012b) complied with international standard ISO 4730 with respect to its major constituents. The *in vitro* comparison of the toxicity of its major component, terpinen-4-ol, against *B. ovis* found that it also resulted in similarly high rates of mortality in vapour assays at the range of concentrations specified by its ISO number (30–48%). The authors therefore attributed the mortality caused by essential oil of tea tree primarily to the presence of terpinen-4-ol. Contact assays using terpinen-4-ol against the donkey chewing louse, *Bovicola ocellatus* (Piaget), agreed with these findings; in this study, the 50% lethal time (LT50) of 3% terpinen-4-ol was much lower than that of 3% tea tree essential oil, at 16.3 min compared with 30.2 min, respectively (Talbert & Wall, 2012). Closed contact assays of six oils against *B. ocellatus* found the essential oils of lavender and tea tree to be the most toxic, with LC50 values of 0.76% and 0.98%, respectively (Talbert & Wall, 2012). The efficacy of the vapour phases of these oils was confirmed against *B. ocellatus*, which demonstrated approximately 80% mortality after 2 h of exposure to 5% solutions of tea tree or lavender essential oil (Ellse et al., 2013). In a limited *in vivo* study, spray application of 2 mL/kg of a 5% solution of lavender or 5% tea tree oil in water and emulsifier (2% Tween® 80) to groups of 10 donkeys with natural infestations of *B. ocellatus* resulted in a significant decline in louse numbers for 2 weeks after application (Ellse et al., 2013).

In the cattle sucking louse, *H. tuberculatus*, *in vitro* contact assays comparing a range of oils found essential oil of camphor to be the most toxic with an LC50 of 2.74% and a significant ovicidal effect on eggs immersed for 10 min...
Interestingly, the treated animals in the positive control (D-phenothrin) group also recovered their infection 10 days post-treatment. Observations in the laboratory and field and between Bovicola sp. and H. tuberculatus suggested that the citrus oil component limonene is toxic to the cat flea Ctenocephalides felis (Bouché) (Siphonaptera: Pulicidae) (Collart & Hink, 1986; Hink & Fee, 1986) and the essential oil constituents carvacrol and nootkatone have been shown to cause high flea mortality in vitro (Panella et al., 2005). However, essential oils may be of limited use against fleas as they may not have a residual on-host phase that is sufficiently sustained to prevent re-infestation from the environment.

Fleas

To the authors’ knowledge, no specific studies on the efficacy of whole essential oils against fleas have been reported in the primary scientific literature. However, it has been suggested that the citrus oil component limonene is toxic to the cat flea Ctenocephalides felis (Bouché) (Siphonaptera: Pulicidae) (Collart & Hink, 1986; Hink & Fee, 1986) and the essential oil constituents carvacrol and nootkatone have been shown to cause high flea mortality in vitro (Panella et al., 2005). However, essential oils may be of limited use against fleas as they may not have a residual on-host phase that is sufficiently sustained to prevent re-infestation from the environment.

Discussion

There is a growing body of evidence supporting the efficacy of essential oils as control agents against arthropod ectoparasites; their efficacy has not only been apparent following immersion and physical contact with treated surfaces, but also after exposure to the vapour of the oils. The latter implies that there is a neurotoxic rather than simply a mechanical pathway in their mode of action. This may be beneficial in ectoparasite control. However, the volatile nature of essential oils may be an obstacle to their use as ectoparasite treatments because their insecticidal or acaricidal efficacy is likely to be relatively short-lived. This has been demonstrated in studies in which essential oil preparations have been allowed to dry in open environments for hours prior to the introduction of the test arthropods. These studies in mites (George et al., 2008; Wall & Bates, 2011) and lice (James & Callander, 2012a) all reported extremely poor mortality rates in these treatment groups. These findings suggest that the application of essential oils may be more appropriate for the control of permanent parasites because the oils’ low levels of residual activity may not afford protection from continual environmental challenges by parasites with free-living stages. By contrast, studies have suggested that the residual repellence of facultative parasites by essential oils is longer-lived than their insecticidal or acaricidal efficacy, presumably because lower doses are required to achieve deterrence (Callander & James, 2012). A possible advantage of essential oils over conventional ectoparasite treatments, such as pyrethroids, may lie in their ovicidal efficacy. As highlighted here, significant egg mortality has been reported in mites (George et al., 2010b) and flies (Callander & James, 2012) treated with essential oils. It is unclear, however, whether this also results from neurotoxic rather than mechanical suffocation and the ovicidal mode of action warrants further more detailed investigation, particularly into the importance of oil components on ovicidal activity. Ovicidal effects may be particularly beneficial in the control of permanent parasites as they would reduce the need for multiple treatments to kill newly hatched nymphs and thereby decrease treatment costs.

There are many difficulties in comparing the studies of essential oil toxicity that have been undertaken to date. One major issue refers to the variation in the relative concentrations of oil constituents between batches. This can lead to dramatic differences in the oils’ efficacy against ectoparasites (George et al., 2009a; Birkett et al., 2011). This also makes the comparison of oils between studies difficult without GC-MS profiles of the specific batch of oil used. The widely used and commercially important oil, essential oil of tea tree, is the only oil for which set guidelines for constituent concentrations are outlined in an international standard. This ensures that essential oil of tea tree, complying with this standard, can be used to provide consistency in ectoparasite control studies (James & Callander, 2012b). For large-scale control of ectoparasites, the products that make up an essential oil would need to be of similar quantification in order to support the making of replicable comparisons. A second issue is that in many experimental designs it is difficult to confirm whether or not the effect seen is attributable to the oil at all; in many cases inappropriate controls mean that the effects of the excipient used in each test cannot be distinguished. It is important to always include an excipient-only control in the study design. Furthermore, when attempting to identify the mechanism of toxicity of the essential oil tested in a direct contact assay, it is important to include a non-essential oil (e.g. a mineral oil) as a control. Without this, it is impossible to distinguish simple mechanical effects, such as blockage of the spiracles and suffocation, from essential oil-specific neurotoxicity or other cellular toxicity. The circumstances in which water alone can serve as an informative control in any study design are likely to be rare.

In order to maximize essential oil efficacy, further study of the development of appropriate excipients for on-host application is required. Indeed, most of the in vivo trials reported here have simply used water and a small amount of emulsifier as the excipient. By contrast, James & Callander (2012b) used an ethoxylated castor oil, oleic acid and water mix for the application of tea tree oil solution to sheep. Altering the hydrophobicity of the excipient is clearly likely to be of value because it may facilitate the better penetration of the oil into the animal’s coat and may also aid retention of the essential oil and hence prolong its activity.

Given the poor public opinion of traditional chemical treatments, essential oil-based insecticides are likely to appeal
to many pet owners. In addition, the shortfalls of essential oils, such as a limited half-life, are likely to be less problematic in a pet care context than in livestock management. Companion animals are usually kept singly or in very small groups and thus re-infestation rates should be minimal and owners are often willing to reapply products. However, caution should always be taken in applying essential oils to animals, especially those oils which are less widely available; toxicity data are often missing and prolonged exposure to high concentrations of certain oils can have deleterious effects on the behaviour, health and welfare of the host (George et al., 2008). The human health implications of many more commonly used essential oils have been documented and some oil components have been found to produce irritant and potentially mutagenic effects (Tunón et al., 2006).

In conclusion, the use of essential oils in the control of veterinary ectoparasites is an exciting area which holds huge potential for the future. However, research into the use of essential oils as control agents is still at a preliminary stage. Extensive field trials, standardization of components, standardization of extraction techniques, standardization of experimental design, mammalian toxicology profiling and excipient development, as well as further investigation into the residual activities and length of shelf-life of these oils are all required before their potential can be fully explored.

References


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