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Repellent activity of catmint, *Nepeta cataria*, and iridoid nepetalactone isomers against Afro-tropical mosquitoes, ixodid ticks and red poultry mites

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ABSTRACT

The repellent activity of the essential oil of the catmint plant, *Nepeta cataria* (Lamiaceae), and the main iridoid compounds (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone, was assessed against (i) major Afro-tropical pathogen vector mosquitoes, i.e. the malaria mosquito, *Anopheles gambiae* s.s. and the Southern house mosquito, *Culex quinquefasciatus*, using a World Health Organisation (WHO)-approved topical application bioassay (ii) the brown ear tick, *Rhipicephalus appendiculatus*, using a climbing repellency assay, and (iii) the red poultry mite, *Dermanyssus gallinae*, using field trapping experiments. Gas chromatography (GC) and coupled GC–mass spectrometry (GC–MS) analysis of two *N. cataria* chemotypes (A and B) used in the repellency assays showed that (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone were present in different proportions, with one of the oils (from chemotype A) being dominated by the (4a*S*,7*S*,7a*R*) isomer (91.95% by GC), and the other oil (from chemotype B) containing the two (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers in 16.98% and 69.83% (by GC), respectively. The sesquiterpene hydrocarbon (*E*)-(1*R*,9*S*)-caryophyllene was identified as the only other major component in the oils (8.05% and 13.19% by GC, respectively). Using the topical application bioassay, the oils showed high repellent activity (chemotype A RD_{50} = 0.081 mg cm^{-2} and chemotype B RD_{50} = 0.091 mg cm^{-2}) for *An. gambiae* comparable with the synthetic repellent DEET (RD_{50} = 0.12 mg cm^{-2}), whilst for *Cx. quinquefasciatus*, lower repellent activity was recorded (chemotype A RD_{50} = 0.34 mg cm^{-2} and chemotype B RD_{50} = 0.074 mg cm^{-2}). Further repellency testing against *An. gambiae* using the purified (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone isomers revealed overall lower repellent activity, compared to the chemotype A and B oils. Testing of binary mixtures of the (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers across a range of ratios, but all at the same overall dose (0.1 mg), revealed not only a synergistic effect between the two, but also a surprising ratio-dependent effect, with lower activity for the pure isomers and equivalent or near-equivalent mixtures, but higher activity for non-equivalent ratios. Furthermore, a binary mixture of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers, in a ratio equivalent to that found in chemotype B oil, was less repellent than the oil itself, when tested at two doses equivalent to 0.1 and 0.01 mg chemotype B oil. The three-component blend including (*E*)-(1*R*,9*S*)-caryophyllene at the level found in chemotype B oil had the same activity as chemotype B oil. In a tick climbing repellency assay using *R. appendiculatus*, the oils showed high repellent activity comparable with data for other repellent essential oils (chemotype A RD_{50} = 0.005 mg and chemotype B RD_{50} = 0.0012 mg). In field trapping assays with *D. gallinae*, addition of the chemotype A and B oils, and a combination of the two, to traps pre-conditioned with *D. gallinae*, all resulted in a significant reduction of *D. gallinae* trap capture. In summary, these data suggest that although the nepetalactone isomers have the potential to be used in human and livestock protection against major pathogen vectors, intact, i.e. unfractionated, *Nepeta* spp. oils offer potentially greater protection, due to the presence of both nepetalactone isomers and other components such as (*E*)-(1*R*,9*S*)-caryophyllene.

1. Introduction

Vector-borne infectious diseases affecting livestock and human health contribute directly to food insecurity, poverty and mortality on a global scale (FAO, 2005; WHO, 2000). Therefore, the availability of a range of tools for control of pathogen vectors, predomi-

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nantly invertebrate arthropods (mosquitoes, ticks and mites), is of the utmost importance. Arthropods rely predominantly upon olfactory perception of volatile, host-derived semiochemicals (behaviour-modifying chemicals) during host location (Logan and Birkett, 2007), and, therefore, whilst the potential for deploying broad-spectrum neurotoxins, i.e. pesticides, in vector control has been reduced in recent years, the nervous system still remains a major target for intervention, through behaviour modification. Thus, protection against arthropod biting and blood-sucking behaviour through the development of topically-applied and spatial repellents has received much attention in recent years (Debboun et al., 2006), with development being based upon three hypotheses that relate to the evolution of repellency: (i) the avoidance of strong plant cues from botanicals, (ii) the avoidance of closely related, but taxonomically different, unsuitable hosts and (iii) the avoidance of highly resistant individuals within a host species complex (Pickett et al., 2010).

A number of repellents have been identified from botanical sources, based on prior ethnobotanical use, thus exploiting the natural ability of such compounds to interfere with host location. These include citronella oil, *Eucalyptus* spp. oils containing *p*-menthane-3,8-diol and eucamalol, and gum resins from members of the Burseraceae, which have been investigated for use as repellents for human or livestock protection (Nishimura and Satoh, 1999; Peterson and Coats, 2001; Birkett et al., 2008). However, most of the active components from botanical sources are highly volatile and can be readily lost, therefore losing efficacy (Lindsay et al., 1996; Chou et al., 1997). This loss of activity has resulted in such materials being overlooked in favour of synthetic repellents such as DEET, KBR3023 and IR3535 that were developed through structure–activity relationship studies (Debboun et al., 2006). Botanically-derived repellents have been discovered which may avoid problems with loss of activity, and which may overcome the negative impact of synthetic repellents. These include isoprenoids from *Callicarpa* spp. (Verbenaceae) (Cantrell et al., 2005; Carroll et al., 2007) and the isoprenoid-derived compound isolongifolenone (Zhang et al., 2009). Much attention has focused on the catmint plant, *Nepeta cataria* (Lamiaceae), and its major essential oil components, i.e. iridoid nepetalactones, of which the (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers predominate. Repellent activity has been reported against mosquitoes (Chauhan et al., 2005; Bernier et al., 2005; Schultz et al., 2006). Hydrogenated *N. cataria* oil enriched in dihydronepetalactone isomers has been reported to be effective against mosquitoes and blackflies (Spero et al., 2008). The activity of *N. cataria* oil against *Aedes*, *Anopheles* and *Culex* spp. mosquitoes in North Africa, Australia and the Far East has been reported (Amer and Mehlhorn, 2006; Webb and Russell, 2007; Polsomboon et al., 2008). However, repellent activity against either sub-Saharan populations of Afro-tropical mosquito species or global populations of acarine pests, i.e. ixodid ticks and mites, has not been reported.

Based on existing ethnobotanical principles, the repellent activity of essential oils from several plant families, including members of the Lamiaceae family, has been assessed against sub-Saharan populations of Afro-tropical mosquito species and ixodid ticks (Omolo et al., 2004; Odalo et al., 2005; Mwangi et al., 1995; Ndungu et al., 1995; Lwande et al., 1999). Thus, the aim of this study was to assess the repellent activity of oils from two chemotypes of *N. cataria*, i.e. another member of the Lamiaceae, enriched in (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone isomers (Birkett and Pickett, 2003) against the same target species, using a WHO-approved topical application assay (WHO, 1996) and a tick-climbing repellency bioassay (Wanzala et al., 2004). The study also investigated the repellent activity of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers versus another acarine pest, the red poultry mite, *Dermanyssus gallinae*, in order to determine their potential for use as general acarine repellents.

2. Results

2.1. Nepeta cataria oil composition

Coupled GC–MS analysis of the oils revealed the presence of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone isomers, respectively, with one of the oils (chemotype A) being dominated by the (4a*S*,7*S*,7a*R*) isomer (91.95% by GC), and the other oil (chemotype B) containing both (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers (16.98% and 69.83% by GC, respectively) (Fig. 1). The sesquiterpene hydrocarbon (*E*)-(1*R*,9*S*)-caryophyllene was also identified in chemotype A and B oils (8.05% and 13.19%, respectively) (Fig. 1).

2.2. Repellency assays with essential oil samples

Using a WHO-approved topical application bioassay, the *N. cataria* oils showed high repellent activity (chemotype A RD_{50} = 0.081 mg cm^{-2} and chemotype B RD_{50} = 0.091 mg cm^{-2}) for *Anopheles gambiae* comparable with the synthetic repellent DEET (RD_{50} = 0.12 mg cm^{-2}) (Fig. 2), whilst for *Culex quinquefasciatus*, lower repellent activity was recorded (chemotype A RD_{50} = 0.34 mg cm^{-2} and chemotype B RD_{50} = 0.074 mg cm^{-2}) (Fig. 3). In a tick climbing repellency assay using *Rhipicephalus appendiculatus*, the oils showed high repellent activity (chemotype A RD_{50} = 0.005 mg and chemotype B RD_{50} = 0.0012 mg) (Fig. 4).

2.3. Repellent activity of individual compounds and synthetic blends of essential oil components

Repellency testing against *An. gambiae* using purified (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone isomers revealed overall lower repellent activity compared to the chemotype A and B oils (Fig. 5). Testing of binary mixtures of the (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers across a range of ratios, but all at the same overall dose (0.1 mg), revealed not only synergistic activity for mixtures, but also a ratio-dependent effect, with lower activity for pure isomers and equivalent or near-equivalent mixtures (Fig. 6). Furthermore, a binary mixture of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers, in a ratio equivalent to that found in chemotype B oil, was less repellent than the oil itself, when tested at two doses equivalent to 0.1 and 0.01 mg chemotype B oil (Fig. 7). Addition of (*E*)-(1*R*,9*S*)-caryo-

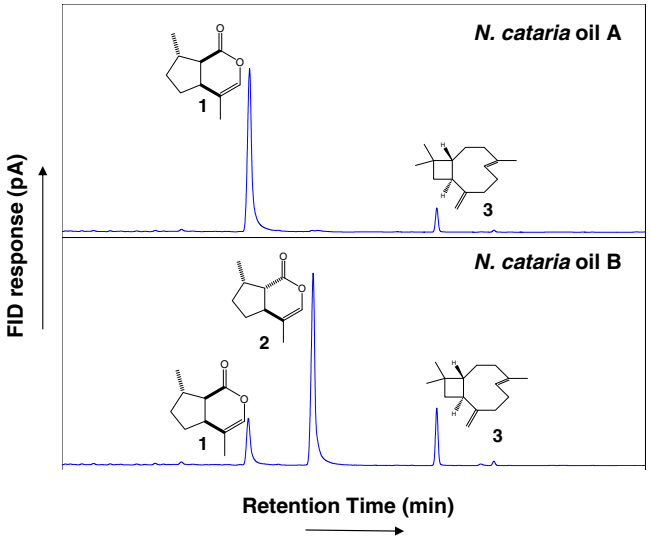

Fig. 1. Coupled GC–MS analysis of *Nepeta cataria* chemotype A and B oils obtained by steam distillation of wet-harvested materials. **1** = (4a*S*,7*S*,7a*R*)-nepetalactone; **2** = (4a*S*,7*S*,7a*S*)-nepetalactone; **3** = (*E*)-(1*R*,9*S*)-caryophyllene.

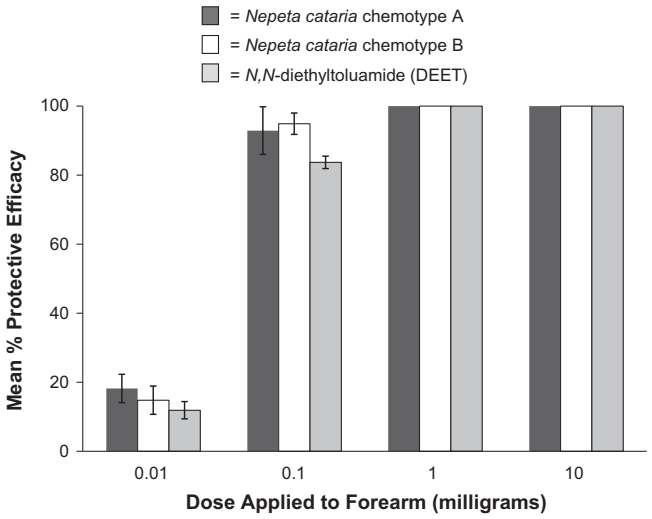

Fig. 2. Mean (±SE) percentage protective efficacy provided by *Nepeta cataria* chemotype A and B oils, and *N*,*N*-diethyltoluamide (DEET) against *Anopheles gambiae* s.s. mosquitoes, using a WHO-approved topical application bioassay.

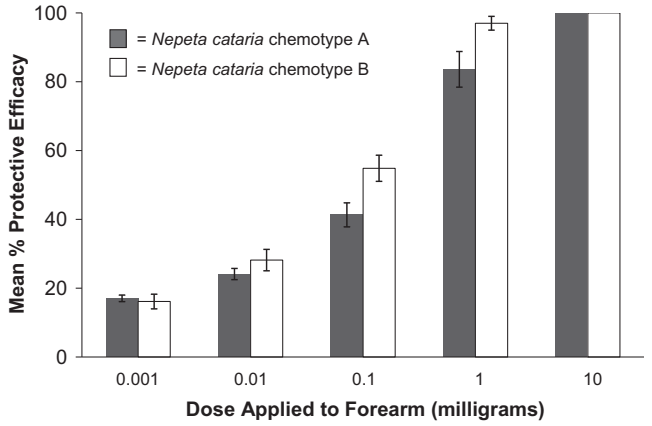

Fig. 3. Mean (±SE) percentage protective efficacy provided by *Nepeta cataria* chemotype A and B oils against *Culex quinquefasciatus* mosquitoes, using a WHO-approved topical application bioassay.

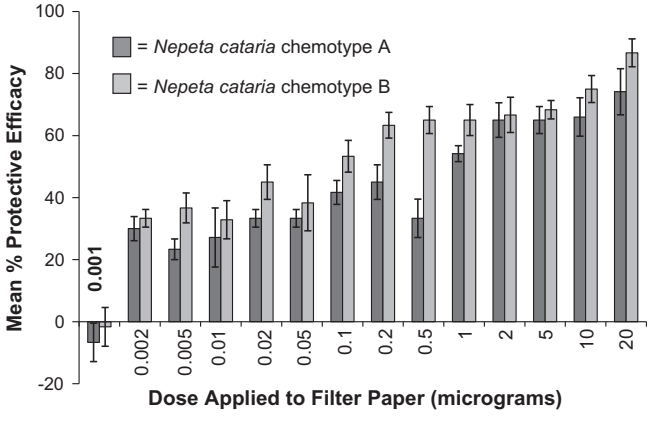

Fig. 4. Mean (±SE) percentage repellency provided by *Nepeta cataria* chemotype A and B oils, against *Rhipicephalus appendiculatus* ticks, using a climbing repellency bioassay.

phyllene to the mixture at the level found in chemotype B oil, and testing of the three-component blend, showed that this blend had the same activity as chemotype B oil (Fig. 7).

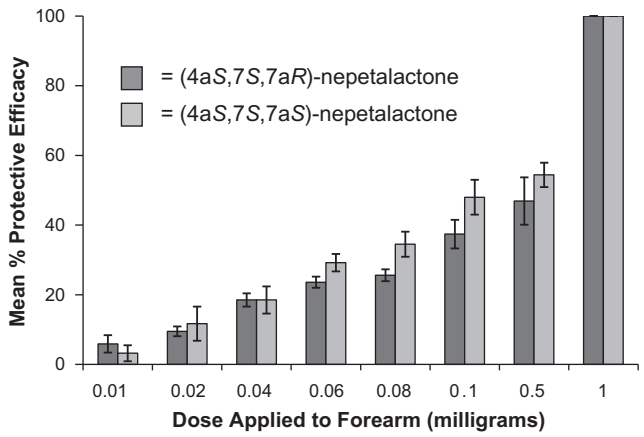

Fig. 5. Mean (±SE) percentage protective efficacy provided by pure (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone isomers against *Anopheles gambiae* s.s. mosquitoes, using a WHO-approved topical application bioassay.

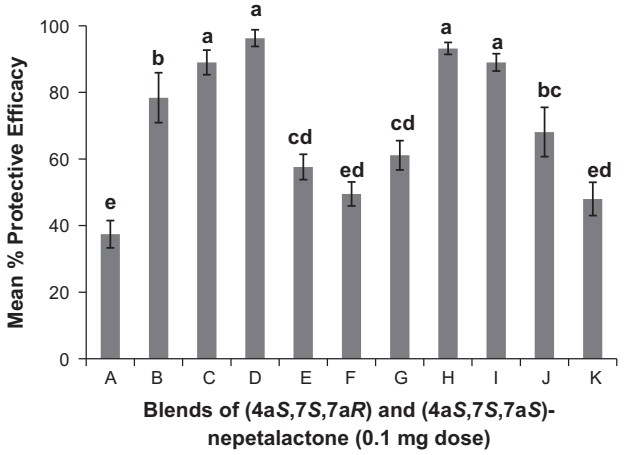

Fig. 6. Mean (±SE) percentage protective efficacy provided by pure (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone isomers and binary mixtures against *Anopheles gambiae* s.s. mosquitoes, using a WHO-approved topical application bioassay. Letters represent different blends of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*) isomers, respectively: A (1:0), B (4:1), C (3:1), D (3:2), E (5:4), F (1:1), G (4:5), H (2:3), I (1:3), J (1:4) and K (0:1). Means with same letter are not significantly different (Proc GLM, LSD test, $P < 0.05$).

In field trapping assays with *Dermanyssus. gallinae*, pre-conditioning of traps with *D. gallinae* significantly increased the level of trap capture (Fig. 8; treatment (i) versus (ii) ($P < 0.05$)). Addition of the chemotype A and B oils, and a combination of the two, to traps pre-conditioned with *D. gallinae*, all resulted in a significant reduction of *D. gallinae* trap capture (Fig. 8; treatment (ii) versus (iii) ($P < 0.05$), (iv) ($P < 0.05$) and (v) ($P < 0.01$)). No significant difference was found between treatments (iii), (iv) and (v).

3. Discussion

The results in this study confirm that the essential oils obtained from two chemotypes of the catmint, *N. cataria* (Birkett and Pickett, 2003), are significantly repellent towards sub-Saharan populations of *An. gambiae* and *Cx. quinquefasciatus* mosquitoes, in line with mosquito repellency studies conducted in other global regions. The data suggest that pure (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone have lower activity compared to oils, when tested individually and in a binary mixture at a ratio found in the oil, and that full

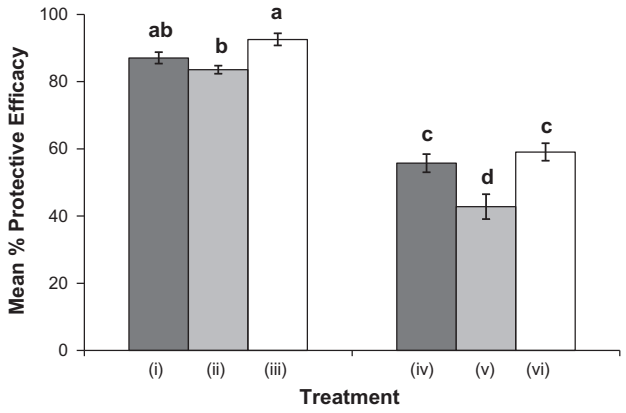

Fig. 7. Mean (±SE) percentage protective efficacy provided by *Nepeta cataria* chemotype B oil, pure (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone and (*E*)-(1*R*,9*S*)-caryophyllene against *Anopheles gambiae* s.s. mosquitoes, using a WHO-approved topical application bioassay. Treatment (i) chemotype B oil (0.1%), (ii) (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone at equivalent levels found in chemotype B oil at one dose (0.1 mg dose), (iii) (4a*S*,7*S*,7a*R*), (4a*S*,7*S*,7a*S*)-nepetalactone and (*E*)-(1*R*,9*S*)-caryophyllene at equivalent levels found in chemotype B oil (0.1 mg dose), (iv) chemotype B oil (0.01 mg dose), (v) (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone at equivalent levels found in chemotype B oil (0.01 mg dose), (vi) (4a*S*,7*S*,7a*R*), (4a*S*,7*S*,7a*S*)-nepetalactone and (*E*)-(1*R*,9*S*)-caryophyllene at equivalent levels found in chemotype B oil (0.01 mg dose). Means with same letter are not significantly different (Proc GLM, LSD test, $P < 0.05$).

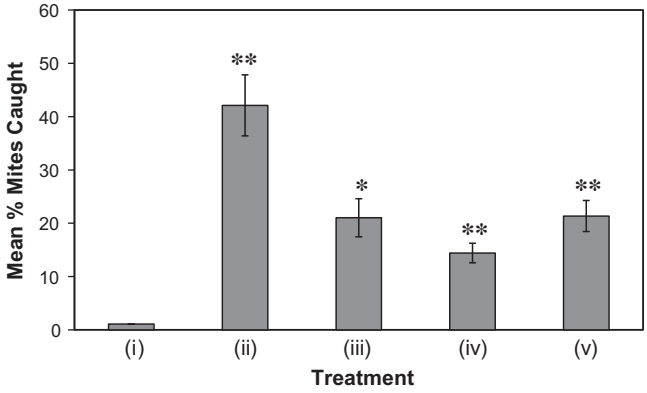

Fig. 8. Mean (±SE) percentage trap catches of *Dermanyssus gallinae* mites in a chicken stable ($n = 5$). (i) Clean trap; (ii) mite-conditioned trap; (iii) mite-conditioned trap + *Nepeta cataria* chemotype A oil (5 mg); (iv) mite-conditioned trap + *Nepeta cataria* chemotype B oil (5 mg); (v) mite-conditioned trap + *Nepeta cataria* chemotype A and B oils (5 mg each). Data were analysed by log transformation (log($x + 1$), Zar, 1974) of the means for each treatment, and comparison of treatment (ii) with treatments (i), (iii), (iv) and (v), using a pair-wise Students *t*-test ($* = P < 0.05$; $** = P < 0.01$).

repellent activity is observed when the sesquiterpene hydrocarbon (*E*)-(1*R*,9*S*)-caryophyllene is included in a synthetic blend with the nepetalactone isomers. Furthermore, the results in this study show a potent repellent effect of *N. cataria* oils against the brown ear tick, *R. appendiculatus*. To our knowledge, this is the first published report of *N. cataria* repellency against a tick species, thus extending the range of pest arthropods that *N. cataria* can be potentially used against to provide protection.

N. cataria oil has been reported as a potent repellent for the northern house mosquito, *Cx pipiens*, comparing favourably with DEET (Schultz et al., 2006), but studies elsewhere have shown that the purified (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactones are less effective than DEET (Chauhan et al., 2005). Our data are in line with these previously reported observations. The presence of (*E*)-(1*R*,9*S*)-caryophyllene in *N. cataria* floral volatiles was reported (Schultz et al., 2004), but no investigation of the role of this component in contributing towards repellency of *N. cataria* oil was reported. Therefore, our study is the first to demonstrate that (*E*)-(1*R*,9*S*)-caryophyllene contributes towards the overall repellency of *N. cataria* oil. Furthermore, the data highlight the fact that mixtures of compounds contribute to greater repellent activity versus *An. gambiae*, as demonstrated previously with essential oils collected from Kenyan plant species (Omolo et al., 2004).

Previous work on the seasonal occurrence of nepetalactone isomer content in *N. cataria* reported variation in the ratio of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone over the course of several weeks, but no clear relationship between ratios and cockroach repellent activity was found (Schultz et al., 2004). The repellency data with oils from *N. cataria* chemotypes A and B in this study reveal differences in repellent activity towards both *An. gambiae* and *Cx. quinquefasciatus*, possibly arising as a consequence of differences in the ratios of nepetalactone isomers between the two oils, as revealed by GC–MS analysis. This hypothesis was supported by the testing of binary mixtures across a range of ratios at a single dose (0.1% mg). Mixtures of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone were consistently more repellent than the isomers when tested alone, apart from equivalent or near-equivalent mixtures, for which repellent activity was surprisingly lower. This suggests that the observed synergistic activity of the nepetalactone isomers is composition dependent.

There is a growing amount of evidence to support the hypothesis that host location by phytophagous insects involves the olfactory perception of ratios of ubiquitous plant compounds (Bruce et al., 2005), as exemplified by the identification of host plant compounds for the black bean aphid, *Aphis fabae* (Webster et al., 2008). It is hypothesised that in conveying a strong plant cue, botanicals interfere with host location by biting and blood-sucking arthropods (Pickett et al., 2010), thus rationalising the development of botanical repellents. The ecological basis for avoidance of *N. cataria*, including specific mixtures of nepetalactone isomers, by mosquitoes is unclear, and further work is required to provide a greater understanding of *N. cataria* and nepetalactone repellency at the neurophysiological and behavioural level.

The use of botanically-derived materials (oils and resins) for the control of African tick pests has been studied elsewhere (Birkett et al., 2008; Mwangi et al., 1995; Ndungu et al., 1995; Lwande et al., 1999). As described above, to our knowledge, this study is the first to report a repellent effect for *N. cataria* against ticks, with activity comparable with other essential oils (Lwande et al., 1999). The gum resin from *Commiphora holtziana* (Burseraceae), known as gum haggar, has been shown to be repellent for the red poultry mite, *D. gallinae*, which is another major acarine pest, affecting chicken production systems (Birkett et al., 2008). The ability of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone to interfere with *D. gallinae* behaviour in this study demonstrates the potential for using *N. cataria* oils and compounds as general purpose acarine repellents, especially in organic production systems, where the use of broad-spectrum toxicants, i.e. acaricides, is prohibited.

In summary, the results of this study reinforce the potential for using *N. cataria*-derived materials in protection against Afro-tropical mosquito pathogen vectors, but also demonstrate their potential to be used to protect against ixodid ticks, and other acarine pests. Furthermore, *Nepeta* spp. essential oils appear to offer greater protection compared to pure nepetalactone isomers, due to the presence of minor components, e.g. (*E*)-(1*R*,9*S*)-caryophyllene, that contribute to their overall activity. However, it should be noted that variation in the activity of oils from different chemotypes suggests that the screening and selection of specific varieties is crucial for developing product(s) with optimal activity.

4. Experimental

4.1. Nepeta cataria extraction and chemicals

The essential oils from flowering harvested *N. cataria* (chemotypes A and B) were prepared by steam distillation using cyclohexane as the co-solvent as described previously (Botanix Ltd., Paddock Wood, Kent) (Birkett and Pickett, 2003). Authentic samples of (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone (1.5 g; >99% purity by GC) were obtained by liquid chromatographic isolation from *N. cataria* A and B oils over silica (40–60 mesh, Sigma–Aldrich, Gillingham, UK), using hexane and diethyl ether (1:9) as eluant, and their identities confirmed by comparison of ^{1}H and ^{13}C NMR data with literature values (Dawson et al., 1996). (*E*)-(1*R*,9*S*)-Caryophyllene (>99% by GC) was purchased from Sigma–Aldrich (Gillingham, UK).

4.2. GC–MS analysis

GC–MS analysis of oils collected from *N. cataria* chemotypes A and B was performed using a fused silica capillary column (50 m × 0.32 mm i.d., film thickness 0.52 μm, DB-1, J&W Scientific), attached to a cool on-column injector, which was directly coupled to a magnetic sector mass spectrometer (Autospec Ultima, Fisons Instruments, Manchester, UK). Ionization was by electron impact (70 eV, source temperature 250 °C). Helium was the carrier gas. The oven temperature was maintained at 30 °C for 5 min, and then programmed at 5 °C/min to 250 °C. Tentative identifications were made by comparison of spectra with mass spectral databases (NIST, 2005), and confirmed by peak enhancement on GC using authentic compounds (Pickett, 1990).

4.3. Mosquitoes, ticks and mites

Mosquitoes used in the this study were obtained from laboratory colonies reared at ICIPE Duduville campus, Nairobi, Kenya, according to a WHO protocol (1996). *Anopheles gambiae* s.s. (Ifakara stain) was cultured in 1998 from specimens originally obtained from Njage, 70 km from Ifakara, south-east from Tanzania. A colony of *Culex quinquefasciatus* (Nairobi strain) was established in 2006 from field collections of larvae from typical culicine pools. Larvae were reared in plastic trays (39 × 28 × 14 cm) at a density of ~500 larvae in 3 l of distilled water at 32–36 °C and fed daily with TetraMin®. The adults were kept in cubic cages (30 × 30 × 30 cm) in separate rooms maintained at 26–28 °C, 70–80% humidity and photoperiod of 12:12 (L:H), and were provided with 6% glucose solution to feed on ad libitum. The females were fed on human blood from a volunteer's forearm thrice a week. Approval for feeding mosquitoes on human volunteers was obtained from the Kenya National Ethical Review Board (protocol number KEMRI/RES/7/3/1). Adult ticks of *Rhipicephalus appendiculatus* were obtained from a colony established at ICIPE, Nairobi, since 2002, which had originated from a culture obtained from International Livestock Research Institute (ILRI), Nairobi, Kenya. The ticks were reared under conditions and management initially developed by Bailey (1960) and then optimised by Irvine and Brocklesby (1970). Red poultry mites, *Dermanyssus gallinae*, were collected from poultry sheds at Lovsta Research Station, Swedish University of Agricultural Sciences, Uppsala, using cardboard traps (Nordenfors and Chirico, 2001), and used directly for field experiments.

4.4. Mosquito repellency assay

Assays were performed with 5–7 day old female *An. gambiae* or *Cx. quinquefasciatus* that had been starved for 18 h, but previously fed on 6% glucose solution. Six human volunteers were selected from those who showed mild or no allergic reaction to mosquito bites or oils/chemicals. They had no contact with lotions, perfumes, oils or perfumed soaps on the day of the experiment. Participation of human volunteers in the repellency assays was part of the approval obtained from the Kenya National Ethical Review Board, protocol number KEMRI/RES/7/3/1. Assays were carried out in aluminium-frame cages (50 × 50 × 50 cm) with cotton stockinet sleeves on the front (WHO, 1996) in a 7 × 5 × 3 m room at 30–32 °C and relative humidity of 65–80%, with 50 female *An. gambiae* or *Cx. quinquefasciatus* starved for 18 h in each cage. Test solutions (0.5 ml) were dispensed on one of the forearms of a volunteer from the wrist to the elbow. The rest of the hand was covered with a glove. Acetone (0.5 ml, HPLC grade) was dispensed on the other forearm to serve as a control. The control and treated arms were interchanged regularly to eliminate bias. The control arm was introduced into the cage 3 min after releasing mosquitoes (to allow the mosquitoes to settle) and kept there for 3 min. The number of insects that landed on that arm during the test duration was recorded. The treated arm was then introduced into the cage for the same period of time and the number of landing insects recorded. The different concentrations of each test sample were tested sequentially starting with the lowest dose. Repellency data from six replicates, expressed as protective efficacy (PE) at each dose, were calculated using the formula, PE = (% control mean − % test mean)/% control mean. The data were transformed and subjected to ANOVA. Means were ranked using the Student–Newman–Kuels (SNK) test.

The following materials were tested: (i) *N. cataria* chemotype A and B oils, between 0.01 and 10 mg versus *An. gambiae*, and 0.001 and 10 mg versus *Cx. quinquefasciatus*; (ii) (4a*S*,7*S*,7a*R*) and (4a*S*,7*S*,7a*S*)-nepetalactone, tested individually between 0.01 and 1 mg versus *An. gambiae*; (iii) binary mixtures of (4a*S*,7*S*,7a*R*)-nepetalactone + (4a*S*,7*S*,7a*S*)-nepetalactone in the following ratios: 1:0, 4:1, 3:1, 3:2, 5:4, 1:1, 4:5, 2:3, 1:3, 1:4 and 0:1, all at a 0.1 mg dose versus *An. gambiae*; (iv) *N. cataria* chemotype B oil at 0.1 and 0.01 mg, (4a*S*,7*S*,7a*R*)-nepetalactone + (4a*S*,7*S*,7a*S*)-nepetalactone in a ratio and dose equivalent to chemotype B oil (at 0.1 and 0.01 mg) and (4a*S*,7*S*,7a*R*)-nepetalactone + (4a*S*,7*S*,7a*S*)-nepetalactone + (*E*)-(1*R*,9*S*)-caryophyllene in a ratio and dose equivalent to chemotype B oil (at 0.1 and 0.01 mg). RD_{50} values for the chemotype A and B oils were obtained by probit analysis using the PE values obtained from replicated experiments (Omolo et al., 2004).

4.5. Tick climbing assay

A dual choice tick repellency climbing assay (Wanzala et al., 2004) that exploits the behaviour of *R. appendiculatus* to climb up grass stems to await potential passing hosts (Browning, 1976; Chiera, 1985) was used. *N. cataria* chemotype A and B oils were diluted serially from 1000 parts per million (ppm) to 0.05 ppm using methylene chloride (GC grade). An aliquot of each dose (20 μl) was applied onto a filter paper strip, with an equivalent volume of methylene chloride being added to control filter paper strips. Five mixed adult *R. appendiculatus* were introduced at the base of a climbing assay set-up (Wanzala et al., 2004) and their movements observed. Observations were made over a 1 h period at 15, 30, 45 and 60 min. Twelve replicates for each dose were carried out. The repellency was calculated using the formula: (number of ticks in control arm − number of ticks in treated arm/total responding ticks) × 100. Dose–response data were subjected to probit analysis using the % repellency values obtained from replicated experiments (Wanzala et al., 2004).

4.6. Chicken mite field experiment

Cardboard traps (Nordenfors and Chirico, 2001) were used to assess the impact of different treatments on catches of red poultry mites, *D. gallinae*, in a chicken stable (Fig. 8). Five treatments (i)–(v) were assessed. Treatment (i) comprised clean traps, which were used as a passive control, whereas treatment (ii) comprised traps which had been subject to mite conditioning by placing them overnight together with 500 freshly caught mites. The conditioning reinforces the mite arrestment behaviour and significantly increases trap catches (Fig. 8). Treatments (iii) and (iv) comprised mite-conditioned traps with *N. cataria* chemotype A and B oils added, respectively (5 mg per trap). Treatment (v) comprised a mite-conditioned trap with both oils added (5 mg of each). The traps were randomly distributed in the chicken stable, left for a period of 24 h, then collected and placed in a freezer to permit a convenient counting of individuals caught. Data were analysed by log transformation ($\log(x+1)$, Zar, 1974) of the means for each treatment, and comparison of treatment (ii) with treatments (i), (iii), (iv) and (v) using a pair-wise Student's-*t*-test. The experiment was replicated five times.

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