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The effect of essential oils of *Tagetes minuta* and *Tithonia diversifolia* on on-host behaviour of the brown ear tick *Rhipicephalus appendiculatus*

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Abstract

On–host behaviour of *Rhipicephalus appendiculatus* was studied in the field in Bungoma County in Kenya to evaluate the putative repellent effects of essential oils of *Tagetes minuta* and *Tithonia diversifolia* at its predilection feeding site. Oils of both plants caused a disruption of orientation, movement and attachment behaviour of ticks. More ticks dropped off in the treatments with the two essential oils than with the control.

Treating the ear pinna with the essential oil of *T. minuta* caused the highest percentage of ticks to drop off the host body. No tick reached the ear pinna treated with the essential oil of *T. minuta* and up to 30% of ticks (from the forehead release site) reached the ear base. When the ear pinna was treated with the essential oil of *T. diversifolia*, one tick reached the ear pinna and up to 40% of ticks (from the dewlap release site) reached the ear base. The results show that *T. minuta* repels ticks more strongly than *T. diversifolia*. However, both essential oils offer possibilities for exploitation of potentially effective and environmentally acceptable tools for on–host tick control.

Key words: *cattle, feeding site, Kenya, on-host orientation behavior*

Introduction

Ticks and tick–borne diseases (TBDs) are a major constraint to the development of livestock industry throughout the tropics. Worldwide, an estimated 600 million cattle are exposed to anaplasmosis and babesiosis, whereas 200 million cattle are exposed to theileriosis, all being economically important TBDs. In eastern, central and southern Africa, East Coast fever (ECF), caused by *Theileria parva* (Theiler 1904) Bettencourt, Franca & Borges, 1907 and transmitted by the vector, *Rhipicephalus appendiculatus* Neumann, 1901, is considered to be the most significant tick–borne disease of cattle and a costly disease in Africa (Norval et al 1991; Taylor et al 2016). The parasite infects cattle as well as African buffalo (Cape buffalo), *Syncerus caffer*, Sparrman 1779, large antelopes and the vector is also found on other small animals such as hares, dogs and warthogs in 12 countries of the region (Mukhebi et al 1992; Walker et al *2005*). It is estimated that about 28 million cattle in the region (eastern, central and southern Africa) are at risk of ECF and the disease kills at least 1 million cattle per year with more socio-economic losses being felt by small-scale resource-poor livestock farmers (Minjauw and McLeod 2003; Gachohi et al 2012; Mans et al 2015; Perry 2016; Nene et al 2016).

For many years, the control of ticks and the diseases they transmit has been largely through the application of acaricides as dips, dust, and pour–ons, using ear tags, sprays or systemic acaricides. The repeated application of acaricides prevents transmission of the parasites and this method has been used very successfully throughout the sub–Saharan Africa, in conjunction with control of animal movement, quarantine and slaughter of infested animals (Lawrence 1991). However, the development of resistance in ticks to successive acaricide compounds has been a major problem (Norval et al 1992). This problem has been compounded by the increasing costs of acaricides, unregulated cattle movement, civil unrest, poor management and inadequate maintenance of dips (abuse of acaricides and dips, under dilution of acaricides, use of counter fake acaricides), especially at the communal level. The devastating extent of recurring prolonged droughts in some parts of Africa has made many dip–tanks non–operational, due to lack of sufficient water to maintain them. Another complication associated with the use of acaricides is that they are environmental pollutants and have been found to contaminate milk and meat (Mitchell 1996).

Tick control by the use of repellents is considered an alternative strategy (Muthuswami and Nisha 2006). Some tick– and insect–repellents are available and widely used for protection by humans (Frances and Wirtz 2005). The use of topical repellents provides an effective prophylactic measure against biting arthropod vectors and arthropod–borne diseases at an individual level (Gupta and Rutledge 1994; Hoch et al 1995; Nentwig 2003), especially in areas where suppression of arthropod vectors is not practical or feasible. Examples of such repellent materials, include, but are not limited to N,N–diethyl–3–methylbenzamide (DEET), p–menthane–3,8–diol (PMD), permethrin, allethrin, piperonyl butoxide, lemongrass oil, citronella oil, eucalyptus oil, camphor, geranium oil, ethyl hexanediol, ethyl butylacetylaminopropionate and hydroxyethyl–isobutyl–piperidine. Repellents commonly available to consumers contain the active ingredients DEET, a few repellents contain permethrin, while none or very few repellents contain botanical essential oils (New York State Department of Health (2737/04) 2004). The development of botanical essential oils as arthropod repellents has been on the increase in the recent decade, in order to replace DEET due to its reported environmental pollution and toxicity in human population using it (Waka et al 2004; *Seo* et al 2005; Jaenson et al 2006; Kegley et al 2007; *Walschaerts* et al 2007).

In the livestock industry, repellents are less commonly used than in human health, although traditional livestock owners may use a range of ethnobotanical products to protect their animals from tick bites (Martin et al 2001; Guarrera et al 2004; Van de Putte 2005; Mathias 2005; Passalacqua et al 2006; Bond 2007). Commercial repellent products containing botanical (plant–based) oils, such those of geranium, cedar, lemon grass, soy and or citronella have been available. There is limited information, however, on the effectiveness of botanical oils individually and when combined with other ingredients. Available information indicates that, compared to the effectiveness of DEET or permethrin, botanical essential oils generally do not provide the same duration of protection (Consumers Union 1993; 2000). The efficacy of these natural products is poorly understood, whilst commercial products are rare. There is need therefore to focus research on the development of effective and safe livestock tick repellents that can be incorporated in the existing Integrated Tick Management (ITM) for livestock tick control programmes (Mount et al 1999).

The life cycle of an ixodid tick often has a total duration of 6 years and host attachment may constitute less than 2% (1½ months) of this time. Ticks spend most of their life cycle away from their hosts, hiding either in the soil and vegetation or in the nests of their hosts. However, ticks select habitats with good opportunities to encounter a host for feeding. Host–seeking and–recognition are two most important and challenging activities in the life cycle of ticks. Many ticks, in particular certain*Hyalomma*, *Amblyomma*, *Ornithodoros* and *Dermacentor* species (Sonenshine 1991; Anon 2008), seek their hosts by hunting, whereas others use an ambushing strategy, e.g., the larvae of *Boophilus* spp and most ixodid ticks (Sonenshine 1993; Eckert et al 2005). Other tick species respond to volatile host emanations, vibrations, visual cues, radiant heat and touch with questing behaviour, e.g., *R. appendiculatus* (Speybroeck et al 2003). However, little is known on how different host specificities are encoded in the odours. Questing ticks climb up the stems of grass or perch on the edges of leaves on the ground in an erect posture, while the first pair of legs is waved in the direction of the stimuli of the host passing by. Subsequently, the ticks climb and grab onto the potential host body using their front–leg claws. Once on the host, gustatory and olfactory cues seem to aid the ticks in deciding whether it will remain on the host. By ‘push’ and ‘push–pull’ action modes of the host’s volatiles, ticks may be guided to particular feeding sites (Wanzala et al 2004). Some tick species prefer feeding sites where they are out of reach of attacks by the grooming behaviour of the hosts. On artificial substrates, ticks orientate towards certain host skin extracts (Akinyi 1991; Sika 1996), but the chemical nature of the directing cues is not yet known. However, gas chromatography–coupled electrophysiology recordings using different vertebrate odours has shown that lactone, methylsalicylate, carbon dioxide, sulfide, benzaldehyde, 2–hydroxybenzaldehyde, aliphatic aldehydes, 2,6–dichlorphenol, nitrophenol, pentanoic acid, 2–methylpropanoic acid, butanoic acid, ammonia, and 3–pentanone, may be involved in host identification by the ticks (Anon 2008). Masking these natural volatiles and diverting ticks away from their prospective hosts may be an attractive strategy to incorporate into the existing ITM for livestock tick control and management (Olwoch et al 2008).

Some tick species exhibit specialization in selecting their feeding site on their hosts. Such specificity may serve to maximize survival and reproduction of the species (Chilton et al 1992). Adult *R. appendiculatus* have a marked preference for feeding mainly inside the ears of bovids, whereas their immatures show less selectivity, and are found feeding on many parts of the host body in considerable numbers (Walker 1974). A host–derived odour–based ‘push’ and ‘push–pull’ pair of stimuli has been suggested to be responsible for the orientation behaviour of adult *R. appendiculatus* to its preferred feeding site (Sika 1996; Wanzala et al 2004).

In this study, we investigated the on–host behaviour of unfed adults of the brown ear tick *R. appendiculatus* and assessed the effect of the application of essential oils of *Tagetes minuta* L. (Family: Asteraceae) and *Tithonia diversifolia* (Hemsl.) A. Gray (Family: Asteraceae) on this behaviour in natural field sites in Bungoma County, western Kenya.

Material and methods

The study site

The experiments were conducted under outdoor conditions at the study site in Bungoma County, western Kenya, which lies between latitude 0^{o} 25’N and 0^{o}53’N and longitude 34^{o}21’E and 35^{o}04’E (Wanzala et al 2012). The altitude ranges from 1200–3500 m above sea level. The mean annual temperature ranges from 21 to 22 ^{o}C with variations in mean maximum and minimum temperature ranging from 25 to 32 ^{o}C and 11 to 13 ^{o}C, respectively. Rainfall in the district has a bimodal pattern and varies on average from 1 000–2 000 mm annually. The major rainy season is from March to July, while the minor one starts in August and continues into October.

Experimental ticks

The tick species used (*Rhipicephalus appendiculatus*) was obtained from colonies reared in the insectary at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. Rearing conditions and handling were as described previously (Bailey 1960; Irvin and Brocklesby 1970). The newly hatched and 24 h–starved adult *R. appendiculatus* were transported in glass vials buried in moist sand from ICIPE, Nairobi to Bungoma (a distance of about 500 km), and used within 48 h after hatching.

Behavioural studies

Observation of on-host behaviour was done using indigenous zebu steers (body weight 150–230 kg), bought from livestock farmers near and or within the study site (Figure 1). The animals had not been exposed to acaricides or other chemicals. The animals were held in a crutch facility built at Mwibale village, Bungoma County, Kenya (Wanzala et al 2012). The responses of ticks and their navigation patterns were monitored starting from six different body locations on zebu steers, representing varying distance by the observer (Wycliffe Wanzala) from preferred feeding sites and possible areas of attachment by the ticks from their questing positions on the vegetation (Browning 1976) (Figure 1). One tick at a time of mixed age and sex was placed at one of six sites on the host animal and observed for up to 14 h, between 07:00 h and 24:00 hours (Figure 1). All observations were made during dry weather with day temperatures in the range of 24–28 ^{o}C and relative humidity ranging between 60–85%. Each observation was replicated 20 times, each time using a naïve tick. The eventual pheromone trail left by each tick (Sonenshine 2006) was removed by wiping the animal with 99% alcohol and leaving it to evaporate before a new tick was placed on the animal.

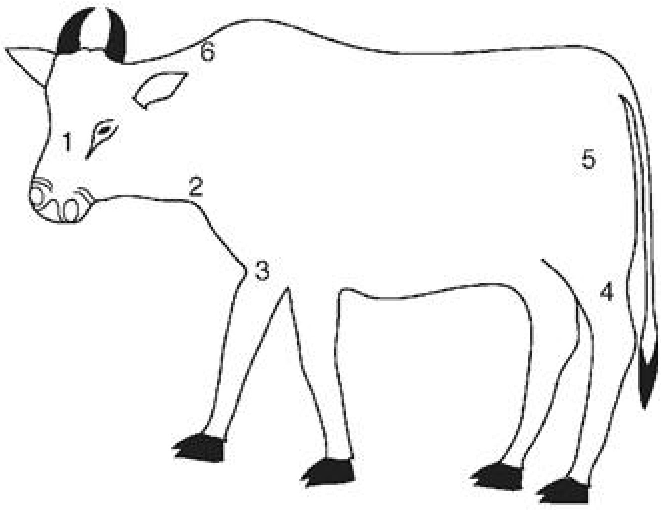

Figure 1. Sites on the host animal (bovid) used for the study of on-host navigation behaviour of *R. appendiculatus* toward the predilection feeding site (the ear) in the presence of essential oils. Marked site 1 = Forehead, 2 = Dewlap, 3 = Foreleg, 4 = Rear leg, 5 = Escutcheon and 6 = Shoulder.

Dispensing of essential oil volatiles

Essential oil of *T. minuta* and *T. diversifolia* had been obtained by the hydrodistillation method using a Clevenger type distillation apparatus (Sereshti and Samadi 2007). Essential oils were diluted to 10% in odourless vaseline petroleum jelly (BP–USP 100% Grade) (Unilever, Kenya)–skin protectant. This carrier material contains no colours, fragrances or irritants. One ml of the stock 10% formulation of essential oils was placed in a 5 cm^3–Eppendorf tube, which was attached by a string to a plastic ear tag on the inner side of the ear pinna. The Eppendorf tube was left open so that there was continuous release of either essential oil volatiles or petroleum jelly. The implication was that at all times a dose of 100 ml of pure essential oil in 900 ml of vaseline petroleum jelly was exposed to the ticks on the inner side of the ear of the animal. The odour from the applied essential oils was presumed to gradually contaminate the ear and its surrounding area. Both ears were simultaneously treated and ticks were released from previously marked sites (Wanzala et al., 2004) (Figure 1). The 20 ticks were released one at a time and monitored until the final destination was determined.

Legal use of experimental animals in the field

All procedures requiring the use of experimental animals in the field were approved by Bungoma County Veterinary Officer (CVO) of Bungoma County, western Kenya. The importance, seriousness and risk–free nature of the project were further explained to the Bukusu community in western Kenya by the CVO and agricultural extension officers working within the study area. The field experiments were performed in compliance with guidelines published by Kenya Veterinary Association and Kenya Laboratory Animal Technician Association, regarding the ethical use and handling of laboratory and farm animals in the field (KVA and KLATA 1989). Informed consent was obtained from the livestock farmer volunteer from whom we rented field experimental plots.

Statistics

Data were entered in Excel database structure and then entered into a Statistical Products and Service Solutions (SPSS version 15 for Windows) database for analysis. Data for on–host tick behaviour were analysed by one–way analysis of variance (ANOVA) and univariate analysis using the general linear model (GLM) procedure for SPSS.

The mean differences were compared and separated using Student–Newman–Keuls test at $\alpha = 0.05$ (Sokal and Rohlf 1995).

Differences between responses of the ticks under different treatments and at different release points (locations) on the host body were analysed using the Kruskal Wallis H–test (Kruskal and Wallis 1952). Any significant results obtained by the Kruskal Wallis H–test, between and within groups of release points and treatments, were analysed using Wilcoxon–Mann–Whitney U–test with Bonferroni correction (Wilcoxon 1945; Mann and Whitney 1947; Bergmann et al 2000).

Results

The stereotyped sequence of on–host orientation behaviours of *R. appendiculatus* revealed a set of sequential activities (particularly in the control experiment). These activities ranged from an inactive stationary/scanning phase to the onset of erratic movements that became increasingly rapid and directional and finally to arrestment at the predilection feeding site. Although variations occurred in the duration of each set of orientation behaviours for every tick observed, the stereotyped sequence of orientation behaviours consistently occurred, irrespective of type of treatment, release point on the hosts' body and age, sex and body size of candidate ticks. As observed previously in a controlled environment (Wanzala et al 2004), these stereotyped responses comprised runs and strides of varying intensities, alternating with stops, and occasionally, walk–away and back–up movements. At the predilection feeding site, ticks behaved differently, each taking time to start the attachment process by mouth insertion into the host integument before imbibing blood much later.

The results of oriented responses and behavioural movements of *R. appendiculatus* to the ear treated with the essential oils of *T. minuta* and *T. diversifolia* demonstrated a disruptive effect (Tables 1 and 2).

Both release points and treatments (the essential oils of *T. minuta* and *T. diversifolia* and petroleum jelly) had a significant effect on the responses of ticks on the host while searching for a predilection feeding site. For all the release points, the number of ticks reaching the ear base [H (2) = 6.086, p = 0.048], ear pinna [H (2) = 14.500, p = 0.001] and those that dropped off [H (2) = 8.97, p = 0.011], were significantly affected by treatment. No tick reached the ear pinna treated with the essential oil of *T. minuta*, while only one tick reached the ear pinna treated with the essential oil of *T. diversifolia*. More ticks dropped off in the treatment with the two essential oils than in the control, with the ear pinna treated with the essential oil of *T. minuta* showing the highest number of ticks dropping off from various release points (Table 1). More ticks from the various release points reached the base of the ear when the essential oil of *T. diversifolia* was applied on the ear pinna than in the case of the treatment with the essential oil of *T. minuta* (U = 4.5, r = –0.630, p = 0.029). The number of ticks from various release points reaching the ear base in the control treatment was significantly different from the treatment with *T. minuta* (U = 5.50, r = –0.585, p = 0.043) but not from the treatment with *T. diversifolia* (U = 17.5, r = –0.024, p = 0.934). The number of ticks reaching the ear pinna was significantly higher in the controls than in the treatment with either *T. minuta* (U = 0.00, r = –0.890, p = 0.002) or *T. diversifolia* (U = 0.500, r = –0.842, p = 0.004). More ticks were affected by the treatment with *T. minuta* and *T. diversifolia* than the treatment with the petroleum jelly (Table 1). However, whereas the treatment with *T. minuta* and *T. diversifolia* equally prevented ticks from getting attached at the ear pinna (U = 15.00, r = –0.289, p = 0.317), the treatment with the essential oil of *T. diversifolia* did not prevent the ticks from getting attached at the ear base like its counterpart.

The number of ticks that was attached in the neighbourhood of release points (NRP) [H (2) = 2.589, *p* = 0.274] and that representing the non–respondent ticks once placed at the release points [H (2) = 0.834, *p* = 0.659], were not affected by the treatment with petroleum jelly and the essential oils of *T. minuta* and *T. diversifolia*.

The site of release of the ticks on the host did affect their directional behaviour in searching for a predilection feeding site ($p<0.05$). The number of ticks reaching the ear base from release point 1 was the highest and significantly different from all other release points ($p<0.05$). However, the number of ticks reaching the predilection feeding site from release point 1 did not significantly differ from that arriving from release point 2 ($p>0.05$). The site of release of ticks on the host animal significantly affected the non-responding ticks [H (5) = 11.533, *p* = 0.042]. For example, the location of release sites 3 and 4 may not have allowed the ticks placed at these sites to receive sufficient amounts of host–derived attractants or repellents, or applied essential oils of the two plants. The number of ticks not responding from release site 4 was the highest and significantly different from all other release sites ($p<0.05$) (Mann–Whitney U–test) (Table 1).

The site of release of the ticks, the essential oil treatment and the interaction of these two factors had a significant effect on the behavioural responses of tick vectors on the host ($p<0.05$) (Table 2). Response times of ticks released at forehead, escutcheon, shoulder and dewlap were short and not significantly different (P<0.05) for all the treatments except for dewlap in the control. Ticks took significantly longer to respond when released at fore– and rear legs ($p<0.05$). For a specific release point, the three treatments had the same significant effect on response time except for the foreleg and dewlap release points (Table 2). At the foreleg, ticks responded in a significantly shorter time to the treatment with *T. diversifolia* than in the controls. At the dewlap, ticks took a significantly shorter time to respond to the treatment with *T. minuta* and *T. diversifolia* than in the controls; with the response time to the treatment with *T. minuta* being the shortest.

The initial walking speed of ticks at forehead, fore– and rear legs was not significantly different for all the treatments. At the dewlap, escutcheon and shoulder, the initial speed was highest in the treatment with the essential oil of *T. minuta* and lowest in the control except at the escutcheon release point. These differences are statistically significant except for the treatment with *T. diversifolia* and *T. minuta* at the shoulder release site (Table 2).

The time between release of ticks at the various locations and their arrival at the predilection feeding site was not significantly different for all the treatments except for rear leg and escutcheon release points. For both the rear leg and escutcheon release points, ticks took significantly much longer time to reach the predilection feeding site in the control treatment than in the treatment with the two essential oils (Table 2).

Generally, it was observed that the time it took ticks to reach the ear pinna and base and become attached was much longer than the time to drop off or attach in the vicinity of the release points. The effect of this was observed to be greater in the treatments of the two essential oils than in the control. However, these differences were not statistically significant (Table 2).

Table 1. On–host behaviour of *Rhipicephalus appendiculatus* released at various locations on zebu steers resulting from treatment of the predilection feeding sites with either petroleum jelly (Control) or the essential oil of either *T. minuta* (Tm) or *T. diversifolia* (Td) (n = 20)

Site of tick release	Treatment	No. of ticks attached near and or at the ear base	No. of ticks attached at the ear pinna	No. of ticks attached in the NRP[1]	No. of ticks that dropped off	Percentage of non-responding ticks[2]
	Control	10	7	2	1	0
1. Forehead	Tm	6	0	4	10	0
	Td	6	1	1	11	5
	Control	6	7	2	0	25
2. Dewlap	Tm	2	0	6	12	0
	Td	8	0	6	6	0
	Control	4	3	9	3	5

3. Foreleg	Tm	1	0	7	5	35
	Td	5	0	4	5	45
	Control	2	1	6	0	55
4. Rear leg	Tm	0	0	4	4	60
	Td	4	0	4	0	60
	Control	5	9	4	0	10
5. Escutcheon	Tm	2	0	11	7	0
	Td	5	0	12	3	0
	Control	5	10	3	1	5
6. Shoulder	Tm	1	0	8	11	0
	Td	4	0	4	12	0

[1] NRP–Neighborhood of Release Points (body locations).

[2] The term 'responding' refers to the percentage of the total number of ticks at different release points that initiated any movements within and or away

Table 2. On–host walking behaviour of *Rhipicephalus appendiculatus* to the predilection feeding site; ticks were released at six different locations on zebu cattle (n = 20)

Site of tick release	Mean response[1] time (h) (± SE)	Mean initial walking speed[2] (cm/min) (± SE)	Mean time taken to reach the ear pinna[3] (h) (± SE)	Number of ticks reaching the ear pinna
(a) Animals treated with petroleum jelly (control experiment)				
1. Forehead	0.01 ± 0.01e	2.29 ± 0.22ab	1.76 ± 0.44c	7
2. Dewlap	0.53 ± 0.28ab	1.12 ± 0.06cd	3.06 ± 0.34bc	7
3. Foreleg	0.90 ± 0.29a	0.73 ± 0.04d	3.40 ± 0.67b	3
4. Rear leg	0.92 ± 0.44a	0.70 ± 0.05d	8.60	1
5. Escutcheon	0.05 ± 0.03d	2.10 ± 0.21b	5.38 ± 0.21a	9
6. Shoulder	0.09 ± 0.03d	1.55 ± 0.21bc	3.53 ± 0.42b	10
(b) Animals treated with essential oil of *Tagetes minuta*				
1. Forehead	0.02 ± 0.01e	2.36 ± 0.34ab	-	0
2. Dewlap	0.13 ± 0.03cd	2.53 ± 0.31a	-	0
3. Foreleg	0.71 ± 0.12ab	0.95 ± 0.17cd	-	0
4. Rear leg	0.66 ± 0.05ab	0.66 ± 0.08d	-	0
5. Escutcheon	0.06 ± 0.01d	2.98 ± 0.31a	-	0
6. Shoulder	0.02 ± 0.01e	2.71 ± 0.31a	-	0
(c) Animals treated with essential oil of *Tithonia diversifolia*				
1. Forehead	0.04 ± 0.01d	2.01 ± 0.17b	12.84	1
2. Dewlap	0.37 ± 0.06c	1.62 ± 0.19bc		0
3. Foreleg	0.51±0.19ab	0.69±0.10d	-	0
4. Rear leg	0.98±0.28a	0.84±0.06d	-	0
5. Escutcheon	0.08±0.03d	1.93±0.11bc	-	0
6. Shoulder	0.08±0.02d	2.16±0.17ab	-	0

For a given column, means followed by the same letter are not significantly different from one another at p = 0.05 (Student-Newman-Keuls test).

[1] Time taken by ticks from placement on the host animal to initial movement within and or away from the release points.

[2] The walking speed of the ticks from time they initiated their first movement within and or away from the release points until they stopped.

[3] Time taken by ticks from placement on a given release point to the arrival at the predilection feeding site on the host animal.
- Indicates that no tick from the corresponding site of release on the host body reached the predilection feeding site, the ear pinna .

Discussion

In this study, we evaluated the on-host effects of essential oils of *T. minuta* and *T. diversifolia* plants from Bungoma District, western Kenya against newly hatched and 24 h–starved adult *R. appendiculatus* under field conditions. This is one of the few studies in which the essential oils of putative repellent plants are evaluated in the presence of host–derived attractive and repellent stimuli (Sika 1996; Dautel et al 1999; Wanzala et al 2004).

The formulated essential oil of *T. minuta* changed its colour gradually from yellow to light–yellow and then to pale white–yellowish, a colour that was close to the carrier material. This change in colour of the formulation may reflect the escape of some of the volatile compounds that constituted the essential oil composition or a biochemical change of oil components. The gradual change of the colour supported the fact that the many compounds previously identified by capillary Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC–MS) (Singh et al 2003; Singh et al 2006; Anon 2006; Moghaddam et al 2007; Adekunle et al 2007), have different volatility properties and may behave differently under the same conditions. This may undoubtedly affect the repellent activity of the essential oil, particularly its efficacy and residual activity, thus causing it to take longer to cause significant effects (Cloyd and Chiasson 2007). In our studies, compounds that first escaped from the formulation could probably be the ones causing the ticks to drop off at a high rate and thereafter, the low repellency of the remnant compounds allowed ticks to reach the predilection feeding site but after a much longer time. The effect of this was greater in the treatments than in the control thus demonstrating a behavioural effect of both the essential oils. This poses great challenges to the essential oil application in the field, but improvements in efficacy and residual activity may be realized with appropriate stable formulations (Thavara et al 2007). The essential oil of *T. diversifolia* could be behaving in a similar manner only that a colour change was not seen, as the formulation is colourless.

On-host observations of the ticks in our field site showed the typical sequence of behavioural patterns reported previously (Sika 1996; Wanzala et al 2004). In fact, the tick response pattern in the control experiment almost followed the pattern of on-host responses of ticks observed in Wanzala et al (2004). Although the responses of ticks at all release points was the same as that obtained in Wanzala et al (2004), the number of ticks successfully reaching the predilection feeding site was relatively low, implying that the essential oils applied had a significant repellent effect. This effect was further manifested in the high number of ticks losing their way and dropping off in the essential oil–treatments, while another sizeable percentage of ticks did not respond at all. Few ticks in the control dropped off. Although most ticks responded at all release points, the orientation and appropriate navigation toward locating and attaching at the predilection feeding site was undermined by repellent effects of the essential oils. This also meant that the previously suggested mediation of specific attractive and repellent host–derived stimuli in the process of orientation and navigation toward predilection feeding sites (Wanzala et al 2004) was masked by the volatiles of the essential oils, as initially hypothesized.

With the disruptive effect of the two essential oils, the stereotyped sequence of orientation behaviours of *R. appendiculatus* on the host animal observed in the control experiment and described in Wanzala et al (2004), was not evident. This implied that the percentage of ticks reaching the predilection feeding site and exhibiting the unique complex set of behaviours, did so against the repelling forces of essential oils of *T. minuta* and *T. diversifolia*. Most notably missing in these stereotyped sequences of orientation behaviours were the second and third phases, involving random and directional movements, respectively. In the two essential oil treatments, ticks were observed randomly navigating on the host body and seeming to have accidentally arrived at the predilection feeding site, as they behaved in a manner that suggested that they were not affected by a feeding–site–specific cue.

The critical key variables of this study, the reaction time, initial speed and time between release and arrival at the predilection feeding site, were evaluated. However, other variables, such as the percentage of ticks at different sites that initiated movement (respondents), the percentage of ticks reaching feeding sites and the percentage of ticks that attached in the neighbourhood of release points, the percentage of ticks that dropped off after losing the way, and that which did not respond at all, were also evaluated. By comparing the percentage of ticks responding and those reaching the predilection feeding site for all six release points, the results showed relatively low rates of successful orientation, location and attachment of the ticks to their predilection feeding site the ear pinna, with the highest numbers coming from the controls followed by the animals treated with *T. diversifolia* essential oil and then *T. minuta* essential oil, in that order. This suggests the masking effect of natural tick attractant stimuli that guide ticks to a predilection feeding site by the two essential oils, which again differ in the way they mask. This supports our hypothesis that intercepting the tick movement toward a predilection feeding site with repellent essential oils of *T. minuta* and *T. diversifolia* may provide a prophylactic mechanism to protect animals from tick bites and must be considered as a management strategy. This control strategy works at individual level and reduces tick–host contact and, subsequently, reduces the chances for the transmission of *Theileria parva* parasite that causes the East Coast fever (ECF) in the hosts (Muthuswami and Nisha 2006). It is possible that in the presence of repellent essential oils, even attached ticks may not sustain the attachment for the period of time (between 24 and 72 h) that is sufficient to allow an effective transmission of the tick–borne pathogen (*T. parva*) to the host (Ochanda et al 1988; Konnai et al 2007).

That the essential oil of *T. minuta* has a higher repellent effect than the essential oil *T. diversifolia*, is manifested in the fact that treatment by the former causes the highest percentage of ticks to drop off the host from various body locations and that none of the ticks got attached to the ear pinna, the predilection feeding site. A considerable number of ticks in the control became attached to the ear pinna and just a few dropped off, whereas only one tick got attached to the ear pinna of the animals treated with the essential oil of *T. diversifolia*, where a considerable percentage of ticks dropped off the host body. Although both essential oils showed significant effects on *R. appendiculatus* locating their preferred feeding site, the essential oil of *T. diversifolia* did not prevent the ticks from becoming attached to the ear base, like its counterpart.

The data further support results obtained previously in Wanzala et al (2004) regarding the responses of ticks deposited on body locations further away (escutcheon, upper rear legs and forelegs) and close to (forehead and shoulder) predilection feeding sites (Wanzala et al 2004). For instance, although release site 5 (escutcheon) was relatively furthest removed from the predilection feeding site, the ticks oriented responses and behavioural navigations from this point were similar to those of ticks navigating from body locations closer to the ear pinna. For these two regions on the host animals, there was no significant difference in the mean reaction time, initial speed and time taken to reach the predilection feeding site. These observations imply the previously-noted operation of both avoidance (closer to none predilection feeding sites) and attraction (closer to the predilection feeding sites) responses of the ticks (Sika 1996; Wanzala et al 2004). However, the influence of the essential oil can not be ruled out. Also the fact that more ticks responded in the treatments than in the controls, may have been due to the repellent effects of the essential oils. For instance, from release points of the host animals treated with the essential oils of *T. minuta*, the ticks' mean reaction time was lower and initial speed higher than either those from the control animals or the animals treated with the essential oil of *T. diversifolia*. Therefore, it is possible that these essential oils can be used to confuse the ticks while on the host, and adversely affect their feeding habits through which they transmit the etiologic agents of tick–borne diseases (Wanzala et al 2017).

Conclusions

- From the results presented in the study, we showed that both essential oils had repellent effects on adult *R. appendiculatus* and that the essential oil of *T. minuta* repels ticks more than the essential oil of *T. diversifolia*.

- Both essential oils may offer potential for incorporation into integrated tick control and management (ITCM), particularly following the laboratory and field studies of individual constituent compounds and selected blends. Together with the essential oils from plants such as wild basil, *Ocimum suave* Willd., molasses grass, *Melinis minutiflora* Beauv., neem, *Azadirachta indica* Adr. Juss. and African spiderflower, *Gynandropsis gynandra* (L.) Briq. (Mwangi et al 1995a, b; Ndung'u et al 1995; Malonza et al 1992; Waka et al 2004; Garboui et al 2006), they offer possibilities of exploitation of this potential in effective and environmentally acceptable methods of tick control mechanisms.

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References

Adekunle O K, Acharya R and Singh B 2007 Toxicity of pure compounds isolated from *Tagetes minuta* L. oil to *Meloidogyne incognita*. Australasian Plant Disease Notes 2: 101–104.

Akinyi S 1991 Studies on pheromones and other semiochemicals of the African brown ear tick *Rhipicephalus appendiculatus*. MSc. thesis, Zoology Department, Faculty of Science, Kenyatta University, Nairobi, Kenya.

Anonymous 2006 Chemical composition of the essential oil of *Tagetes minuta* L. from Zambia. Fragrance Oils and Extracts, Culinary and Hospitality Industry (ed. by Lawrence, M.B.) Publications Services, India.

Anonymous 2008 Ticks–Encyclopedic Reference of Parasitology. http://parasitology informatik.uni–wuerzburg.de/login/n/h/1453.html, as retrieved on 11th November, 2008.

Bailey K P 1960 Notes on the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. Bulletin of Epizootic Disease of Africa 8: 33–43.

Bergmann R, Ludbrook J and Spooren W P J M 2000 Different Outcomes of the Wilcoxon-Mann-Whitney Test from Different Statistics Packages. The American Statistician 54: 72–77.

Bond B A 2007 Herbal Tick Repellent for Dogs. Care2 Green Living Newsletters. http://www.care2.com/greenliving/tick-repellant.html , as retrieved on 23 February 2008.

Browning T O 1976 The aggregation of questing ticks, *Rhipicephalus pulchellus*, on grass stems, with observations on *Rhipicephalus appendicuiatus*. Physiological Entomology 1: 107–114.

Chilton N B, Andrews R H and Bull C M 1992 Interspecific differences in the movements of female ticks on reptiles . International Journal of Parasitology 22: 239–242.

Cloyd A R and Chiasson H 2007 Activity of an essential oil derived from *Chenopodium ambrosioides* on greenhouse insect pests. Journal of Economic Entomology 100: 459–466.

Consumers Union 1993 Bug off! How to repel biting insects. Consumer Reports. July 1993 version., pp. 451–454.

Consumers Union 2000 Buzz off! Which repellents work best? This year, the answer may be especially important. Consumer Reports. June, 2000, pp. 14–17.

Dautel H, Kahl O, Siems K, Oppenrieder M, Müller-Kuhrt L and Hilker M 1999 A novel test system for detection of tick repellents. Entomologia Experimentalis et Applicata 91: 431–441.

Eckert J, Friedhoff K T, Zahner H and Deplazes P 2005 Lehrbuch der Parasitologie für die Tiermedizin. Enke Verlag in MVS Medizinverlag Stuttgart., pp. 343–363.

Frances P S and Wirtz A R 2005 Repellents: past, present, and future. Journal of American Mosquito Control Association 21: 1–3.

Gachohi J, Skilton R, Hansen F, Ngumi P and Kitala P 2012 Epidemiology of East Coast fever (*Theileria parva* infection) in Kenya: past, present and the future. Parasites and Vectors 5: 194.

Garboui S S, Jaenson T G and Pålsson K 2006 Repellency of MyggA (R) Natural spray (para–menthane–3,8–diol) and RB86 (neem oil) against the tick *Ixodes ricinus* (Acari: Ixodidae) in the field in east–central Sweden. Experimental and Applied Acarology 40: 271–277.

Guarrera M P, Forti G and Marignoli S 2004 Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy). Journal of Ethnopharmacology 96: 429–444.

Gupta R K and Rutledge L C 1994 Role of repellents in vector control and disease prevention. American Journal of Tropical Medicine and Hygiene 50: 82–86.

Hoch A L, Gupta R K and Weyandt T B 1995 Laboratory evaluation of a new repellent camouflage face paint. Journal of American Mosquito Control Association 11: 172–175.

Irvin A D and Brocklesby D W 1970 Rearing and maintaining *Rhipicephalus appendiculatus* in the laboratory. Journal of the Institute of Animal Technicians. 1970; 21: 106–112

Jaenson T G T, Pålsson K and Karlson A 2006 Evaluation of extracts and oils of mosquito (Diptera: culicidae) repellent plants from Sweden and Guinea–Bissau. Journal of Medical Entomology 43: 113–119.

Kegley S, Hill B and Orme S 2007 PAN Pesticide Database, Pesticide Action Network, North America (San Francisco, CA. 2007). http://www.pesticideinfo.org, as retrieved on 3rd March, 2008.

Konnai S, Yamada S, Imamura S, Simuunza M, Chembensof M, Chota A, Nambota A, Ohashi K and Onuma M 2007 Attachment Duration Required for *Rhipicephalus appendiculatus* to Transmit *Theileria parva* to the Host. Vector–Borne and Zoonotic Diseases 7: 241–248.

Kruskal H W and Wallis A W 1952 Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association 47: 583–621.

Kenya Veterinary Association and Kenya Laboratory Animal Technician Association 1989 Guidelines for Care and Use of Laboratory Animals in Kenya. Kenya Veterinary Association (KVA) and Kenya Laboratory Animal Technician Association (KLATA), publication, Nairobi, Kenya.

Lawrence J A 1991 Retrospective observations on the geographical relationship between *Rhipicephalus appendiculaus* and East Coast fever in Southern Africa. Veterinary Record 128: 180–183.

Malonza M M, Dipeolu O O, Amolo A O and Hassan S M 1992 Laboratory and field observations on anti-tick properties of the plant *Gynandropsis gynandra* (L) Brig. Veterinary Parasitology 42: 123–136.

Mann H B and Whitney D R 1947 On a test of whether one of two random variables is stochastically larger than the other. Annals of Mathematical Statistics 18: 50–60.

Mans B J, Pienaar R and Latif A A 2015 A review of Theileria diagnostics and epidemiology. International Journal for Parasitology: Parasites and Wildlife **4** (1): 104–118.

Martin M, Mathias E and McCorkle M C 2001 Ethnoveterinary Medicine: An Annotated Bibliography of Community Animal Healthcare. Intermediate Technology Development Group Publishing, Practical Action, London, UK.

Mathias E 2005 Ethnoveterinary medicine: A resource for development. New Agriculturist on line: Reporting Agriculture for the 21st Century. http://www.new-agri.co.uk/00-6/focuson/focuson3.html , as retrieved on 23rd May, 2005.

Minjauw B and McLeod A 2003 Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.

Mitchell M 1996 Acaricide resistance–back to basics. Tropical Animal Health and Production 28: 53s–58s.

Moghaddam M, Omidbiagi R and Sefidkon F 2007 Chemical Composition of the Essential Oil of *Tagetes minuta* L. Journal of Essential oil Research. http://www. findarticles.com/p/articles/mi_qa4091/is_200701/ai_n17220474, as retrieved on 5th March, 2008.

Mount G A, Haile D G, Barnard D R, Daniels E 1999 Integrated management strategies for *Amblyomma americanum* (Acari : Ixodidae) in non–agricultural areas. Experimental and applied acarology 23: 827–839.

Mukhebi A W, Perry B D and Kruska R 1992 Estimated economics of theileriosis control in Africa. Preventive Veterinary Medicine 12: 73–85.

Muthuswami K and Nisha M 2006 N,N–Diethyl Phenylacetamide (DEPA): A safe and effective repellent for personal protective against hematophagous arthropods. Journal of Medical Entomology 43: 518–525.

Mwangi E N, Hassanali A, Essuman S, Nyandat E, Moreka L and Kimondo M 1995a Repellent and acaricidal properties of *Ocimum suave* against *Rhipicephalus appendiculatus* ticks. Experimental and Applied Acarology 19: 11–18

Mwangi E N, Essuman S, Kaaya G P, Nyandat E, Munyinyi D and Kimondo M 1995b Repellence of the tick *Rhipicephalus appendiculatus* by the grass *Melinis minutiflora.* Tropical Animal Health and Production 27: 211–216.

Ndung'u M W, Lwande W, Hassanali A, Moreka L and Chhabra C S 1995 *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellents. Entomologia Experimentalis et Applicata 76: 217–222.

Nene V , Kiara H, Lacasta A, Pelle R, Svitek N, Steinaa L 2016 The biology of Theileria parva and control of East Coast fever – Current status and future trends. Ticks and Tick-borne Diseases 7: 549–564.

Nentwig G 2003 Use of repellents as prophylactic agents. Parasitology Research 90: S40–S48.

New York State Department of Health (2737/04) 2004 Health Advisory: Tick and Insect Repellents. Information for a Healthy New York; New York State, USA. Website: http://www.health.state.ny.us, as retrieved on 26 June, 2006.

Norval I A R, Perry D B and Young S A 1992 The Epidemiology of Theileriosis in Africa. Academic Press, UK. 481 pp.

Norval R, Lawrence J, Young A, Perry B, Dolan T and Scott J 1991 *Theileria parva* : Influence of vector, parasite and host relationships on the epidemiology of theileriosis in southern Africa. Parasitology 102(3): 347-356.

Ochanda H, Young A S, Mutugi J J, Mumo J and Omwoyo P L 1988 The effect of temperature on the rate of transmission of*Theileria parva* infection to cattle by its tick vector *Rhipicephalus appendiculatus.* Parasitology 97: 239–245.

Olwoch J M, Reyers B, Engelbrecht F A and Erasmus B F 2008 Climate change and the tick-borne disease, Theileriosis (East Coast fever) in sub-Saharan Africa. Journal of Arid Environments 72 (2): 108–120.

Passalacqua G N, de Fine G and Guarrera M P 2006 Contribution to the knowledge of the veterinary science and of the ethnobotany in Calabria region (Southern Italy). Journal Ethnobiology and Ethnomedicine 2: 52.

Perry B D 2016 The control of East Coast fever of cattle by live parasite vaccination: A science-to-impact narrative. One Health 2: 103–114.

Seo J, Lee Y G, Kim S D, Cha C J, Ahn J H and Hur H G 2005 *Biodegradation of the Insecticide N,N* –Diethylm Toluamide by Fungi: Identification and Toxicity of Metabolites. Archives of Environmental Contamination and Toxicology 48: 323–328.

Sereshti H 1 and Samadi S 2007 Comparison of hydrodistillation-headspace liquid phase microextraction techniques with hydrodistillation in determination of essential oils in *Artemisia Haussknechtii* Boiss. JUST 33: 7–17.

Sika K F N 1996 Behavioural responses of *Rhipicephalus appendiculatus* Neumann 1901 and *R. evertsi* Neumann 1889 (Acari: Ixodidae) to host and non–host semiochemicals. PhD thesis, Zoology Department, Kenyatta University, Nairobi, Kenya.

Singh S, Singh V, Kiran Babu G D, Kaul V K, Ahuja P S 2006 Techno economic feasibility of wild marigold (*Tagetes minuta* L.) oil production in Himachal Pradesh. Journal of Non–timber Forest Products 13: 267–271.

Singh V, Singh B and Kaul V K 2003 Domestication of wild marigold (*Tagetes minuta* L.) as a potential economic crop in western Himalaya and north Indian Plains Economic Botany 57: 535–544.

Sokal R R and Rohlf F J 1995 Biometry. The Principles and Practices of Statistics in Biological Research. 3rd edn. Freeman and Company, New York, USA.

Sonenshine D E 1991 Biology of Ticks, Vol. 1. Oxford University Press, New York, USA.

Sonenshine D E 1993 Biology of Ticks, Vol. 2. Oxford University Press, New York, USA.

Sonenshine D E 2006 Tick pheromones and their use in tick control. Annual Review of Entomology 51: 557–580.

Speybroeck N, Madder M, Brandt J, Chungu H, Van Den Bossche P, Mbao V and Berkvens D 2003 Questing activity of *Rhipicephalus appendiculatus* (Acari: Ixodidae) nymphs: a random process?**Physiological Entomology 28: 356–361.**

Thavara U, Tawatsin A, Bhakdeenuan P, Wongsinkongman P, Boonruad T, Bansiddhi J, Chavalittumrong P, Komalamisra N, Siriyasatien P and Mulla M S 2007 Repellent activity of essential oils against cockroaches (Dictyoptera: Blattidae, blattellidae, and blaberidae) in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health 38: 663–673.

Taylor M A, Coop R L and Wall R L 2016 *Rhipicephalus appendiculatus* (brown ear tick). Veterinary Parasitology, 4th edn., John Wiley & Sons. pp. 249.ISBN 9780470671627.

Van de Putte A J 2005 Toxic, ichthyotoxic, insect-repellent, and bee-attractant plants used in the Serra do açor (Portugal). Journal of Ethnobiology 25: 228–239.

Waka M, Hopkins J R and Curtis C 2004 Ethnobotanical survey and testing of plants traditionally used against hematophagous insects in Eritrea. Journal of Ethnopharmacology 95: 95– 101.

Walker B J 1974 The Ixodid Ticks of Kenya: A review of Present Knowledge of their Hosts and Distribution. Commonwealth Institute of Entomology, The Eastern Press Limited, London, UK.

Walker B J, Keirans, E J and Horak G I 2005 Accounts of individual species occurring in the Afrotropical region. *Rhipicephalus appendiculatus* Neumann 1901. The Genus: *Rhipicephalus* (Acari, Ixodidae): A Guide to the Brown Ticks of the World. Cambridge University Press. pp. 59–71.ISBN 9781316583746.

Walschaerts M, Muller A, Auger J, Bujan L, Guerin J F, Lannou D L, Clavert A, Spira A, Jouannet P and Thonneau P 2007 Environmental, occupational and familial risks for testicular cancer: a hospital –based case– control study. International Journal of Andrology 30: 222 – 229.

Wanzala W, Sika K F N, Gule S and Hassanali A 2004 Attractive and repellent host odours guide ticks to their respective feeding sites. Chemoecology14: 229–232.

Wanzala W, Takken W, Mukabana R W and Hassanali A 2017 Essential oils of indigenous plants protect livestock from infestations with *Rhipicephalus appendiculatus* and other tick species in herds grazing in natural pastures in western Kenya. Journal of Pest Science, pp. 1-10 DOI: 10.1007/s10340-017-0853-0 http://link.springer.com/article/10.1007/s10340-017-0853-0.

Wanzala W, Takken W, Pala A O, Mukabana R W and Hassanali A 2012 Ethnoknowledge of Bukusu community on livestock tick prevention and control in Bungoma district, western Kenya. Journal of Ethnopharmacology 140 (2): 298-324.

Wilcoxon F 1945 Individual comparisons by ranking methods. Biometrics Bulletin 1: 80–83.

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