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ORIGINAL PAPER

Essential oils of indigenous plants protect livestock from infestations of *Rhipicephalus appendiculatus* and other tick species in herds grazing in natural pastures in western Kenya

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Abstract The effects of formulated essential oils of *Tagetes minuta* and *Tithonia diversifolia* on *Rhipicephalus appendiculatus* infesting livestock were evaluated in semi-field experiments. Forty-five zebu cattle naturally infested with ticks were randomly selected from 15 herds, three animals from each. Of the three animals within each herd, one was treated with 1 g of petroleum jelly (control), one with 1 g of essential oil of *T. minuta* and one with 1 g of essential oil of *T. diversifolia* on the inner side of ear pinna, the preferred feeding site of *R. appendiculatus*. Tick infestation on each treated host animal was monitored daily for 18 days by counting the number of ticks attached to the animals. Within 1–4 days post-treatment, the number of ticks on animals treated with essential oils was reduced by more than half the original population. By the 5th day post-treatment, more than 75 and 60% of *R. appendiculatus* and other tick species, respectively, became dislodged and dropped off. A stronger repellent effect was shown by the essential oil of *T. minuta* than the essential oil of *T. diversifolia*. Mean residual protection afforded by *T. minuta* was 12.5 days and for *T. diversifolia* 7.9 days. There was no significant difference in the effectiveness of essential oils between male and female *R. appendiculatus*. Both *T. minuta* and *T. diversifolia* essential oils affected several other less dominant but economically important tick species. Results suggest the potential for essential oil formulations in integrated pest management of ticks and associated tick-borne diseases among resource-limited livestock farmers in tropical Africa.

Keywords *Rhipicephalus appendiculatus* · Tick repellents · Plant essential oils · On-host field trials · Kenya · Cattle

Key message

- A semi-field trial was conducted to evaluate the repellency effect of the essential oils of *Tagetes minuta* and *Tithonia diversifolia* on *Rhipicephalus appendiculatus* infesting livestock in the natural habitat as putative repellents to control tick-borne diseases.
- By the 5th day post-treatment, more than 75% of *R. appendiculatus* and 60% of other tick species had dropped off.
- Results suggest the potential for essential oil formulations in integrated pest management of ticks and associated tick-borne diseases among resource-limited livestock farmers.

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Introduction

The socio-economic importance of livestock ticks has long been recognized worldwide, and therefore their control has been a priority for many countries in tropical and subtropical regions (D'Haese et al. 1999; Lodos et al. 2000; Rajput et al. 2006). The control of livestock ticks is mainly focused on the following approaches: chemical (Moyo and Masika 2008), genetical (Aiello and Mays 2003; Willadsen 2006), biological (Samish et al. 2004), immunological and cultural (Miller 2004). The limitations of these approaches in the programmes and strategies for control and management of livestock ticks are well known (Amoo 1992; Solomon and Kaaya 1996). The application of synthetic acaricides is still the main method for the control and management of livestock ticks and tick-borne disease worldwide, albeit affording only tick elimination from the host animal but no prevention of re-infestation (Wanzala 2009). Furthermore, the acaricides do not affect the larger population of free-living livestock ticks existing in the hosts' environment (vegetation), which provides a resource for tick re-infestation. The disadvantages of using these synthetic acaricides relate to acaricide resistance and environmental pollution (Norval et al. 1992; Aiello and Mays 2003). Therefore, there has been a worldwide search for alternative tick control methods that can be applied either alone or integrated with acaricides and or other tick control methods (Young et al. 1988; Kaaya 1992). Some of the alternative methods include the use of tick-resistant animals, behavioral manipulations of ticks using pheromones, quarantine techniques, habitat modification, anti-tick vaccines and biological control (Ghosh et al. 2007).

Few data are available in the literature on the use of plants and plant products that contain toxic or repellent compounds. However, the potential of some anti-tick plants in pastures and plant products as tick repellents or acaricides on hosts has been repeatedly demonstrated (e.g., Sutherst et al. 1982; Norval et al. 1983; Carroll et al. 1989; Miller et al. 1995). At the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, a participatory action research study to develop anti-tick pastures using molasses grass was investigated at its Nairobi location and in the Kuja area, western Kenya, in an effort to control *Amblyomma variegatum* Fabricius and *Rhipicephalus appendiculatus* Neumann, the vectors of cowdriosis and East Coast fever, respectively (Mwangi, et al. 1995b). Neem oil from *Azadirachta indica* A. Juss. was reported to inhibit larval and nymphal attachment and feeding, as well as to reduce fecundity, egg hatch, and molting in larvae and nymphs of *R. appendiculatus*, among others (ICIPE 1997). A 25% formulation of neem oil sprayed on de-ticked zebu cattle grazing on heavily infested pasture reduced infestations of tick larvae by 37–61%, nymphs by 24–65% and adults by 44–62% for 5 days. In in vivo assays, a 10% solution of the essential oil of *Ocimum suave* Willd. in paraffin oil was found to be effective against all immature *R. appendiculatus* and more than 70% of adults feeding on rabbits (Mwangi et al. 1995a). Three applications of the 20% concentration of the essential oil of *O. suave* reduced *R. appendiculatus* attachment by 68.8%. *Cleome hirta* (Klotzsch) Oliv. and *Gynandropsis gynandra* (L.) Briq. have been demonstrated to be possible tick-repellent plants in pastures (Dipeolu 1990; Malonza et al. 1992; Ndung'u et al. 1999). In another study on Rusinga Island, western Kenya, leaves of a local shrub, *Acalypha fruticosa* Forssk., were found to be attractive to *R. appendiculatus* under field and laboratory conditions, thus showing the potential for use as a trap plant in tick control strategies (Hassan et al. 1994).

Although achievement of the full potential of plants in livestock tick control poses challenging research and development problems, the plants' anti-tick properties so far manifested could make them suitable components of an integrated tick control strategy. Considerable research is needed, however, to select appropriate plants and plant products, establish their efficacy in the laboratory and field under different conditions, and devise efficient production strategies to allow for their practical use at local level. In this respect, the repellent action of the essential oils of *Tagetes minuta* and *Tithonia diversifolia* against the brown ear tick *R. appendiculatus* has been demonstrated in laboratory and semi-field studies. Cattle, treated with either oil, exhibited a significantly high drop-off rate of on-host ticks and a significantly reduced number of ticks climbing onto the host from the ground and vegetation. In the study described here, the effects of the essential oils of *T. minuta* and *T. diversifolia* on the infestation rate of adult *R. appendiculatus* ticks are evaluated on zebu cattle within herds of naturally grazing livestock in western Kenya.

Methods and materials

Geography of experimental field site

The study was carried out in Bungoma County, western Kenya, situated between latitude 00°28′ and 10°30′N and longitude 34°20′ and 35°15′E (Fig. 1). The County occupies an area of 3074 km^2 on the southern slopes of Mt. Elgon, with an altitude ranging from 1200 to 4000 m a.s.l. (Backes 1998). The predominant off-farm vegetation patterns are riverine forests, rocky forested hillsides, hedgerows, wooded grassland relicts, woodlands or colline forest relicts and tree groves, whereas the noticeably tree-rich on-

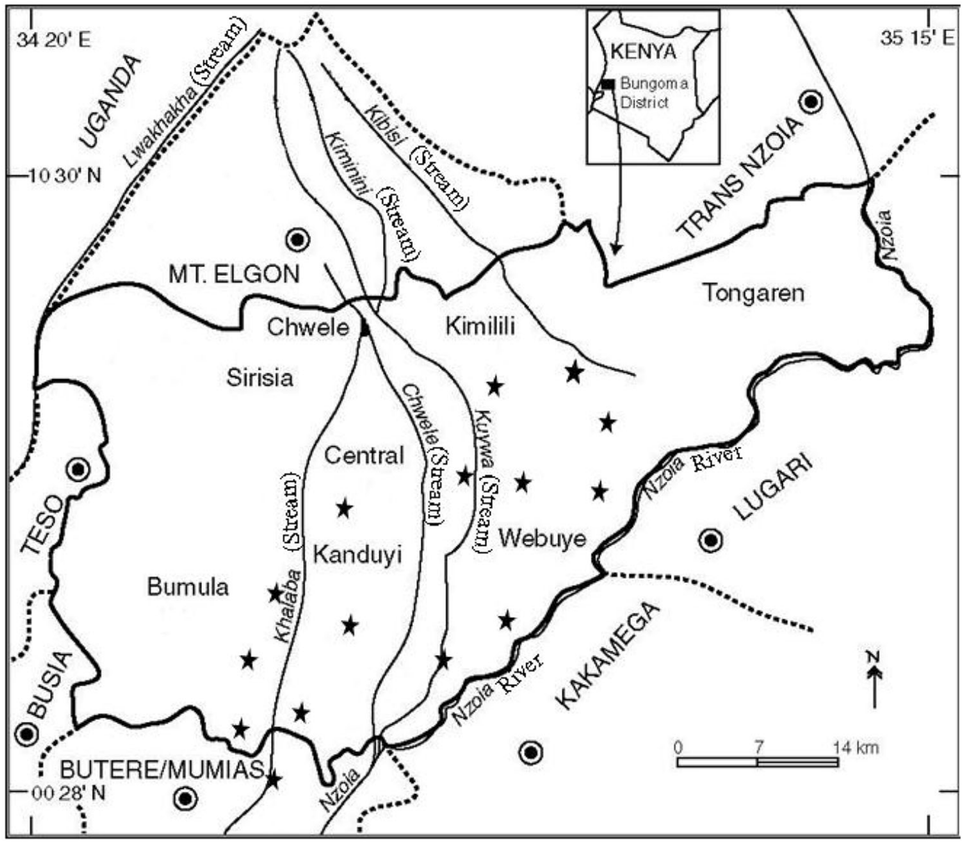

Fig. 1 The location of Bungoma District in western Kenya. The field experiments were located on 15 different livestock farms (indicated with *stars*)

farm management units are home gardens, homesteads, live fences, coffee and banana groves and annual cropping fields (Backes 2001). Average annual rainfall ranges from 1600 to 2000 mm, and mean annual temperatures in the southern areas are about 21–22 °C, whereas in the northern areas closer to the mountain they are in the lower range of 5–10 °C because of the altitude.

Identification of the site for field experiments within Bungoma District

The study site comprising 15 livestock herds was identified based on data from the Bungoma County Veterinary Office (CVO). The CVO receives reports from regulated and monitored local public markets within Bungoma County, where animals are traded following registration and examination of their health status (including livestock tick infestation). Additional limited reports regarding the livestock tick infestation situation in the County were received from individual livestock farmers (CVO, personal communication). A preliminary survey within this area confirmed the CVO's report that the site was more heavily infested with livestock ticks than any other area within the County.

Essential oils used in the study

The essential oils used were extracted from *T. minuta* and *T. diversifolia* as described by Wanzala et al. (2004). Petroleum jelly (BP-USP 100% Grade; Unilever, Kenya) was used in the control experiment and in the formulation of the essential oils. The essential oils were mixed with petroleum jelly as a 10% formulation, adopted from previous bioassay results in the laboratory and work at ICIPE (ICIPE 1998/99; Mwangi et al. 1995a). The essential oils were transported from Nairobi to Bungoma (a distance of about 500 km) in a coolbox and stored at −4 °C until applied on the selected hosts within a 12-h period.

Treatment of experimental animals and follow-up observations

In each selected herd, three animals were chosen for the experiment and marked with code numbers using a plastic tag and marker pen. One gram of petroleum jelly, containing the essential oil of either *T. minuta* or *T. diversifolia*, was applied to the inner side of the ear pinna of the animal and its behavioral effect compared with the control treatment of petroleum jelly only. The treated animals were monitored for their tick infestation each day for 18 days (a period exceeding re-infestation time), in collaboration with livestock farmers. We recorded *R. appendiculatus* ticks of both sexes as well as ticks of other species. The experimental animals in any given herd led their normal daily life except that they were not subjected to any forms of acaricide application within the study period.

Tick collection and identification

Ticks collected from each experimental animal were placed in 99% ethanol in glass vials, kept in a cool box and brought to the laboratory of ICIPE in Nairobi for identification. Tick samples were collected according to the predilection feeding sites as well as other places on the host as identified by the farmer. Voucher specimens were obtained from the Laboratory of Entomology, School of Biological Sciences, University of Nairobi, Kenya. Ticks were finally identified following the descriptions of Hoogstraal (1956a, b), Matthysse and Colbo (1987), Okello-Onen et al. (1999) and Walker et al. (2003). Some tick specimens could not be identified.

Data management and statistical analysis

Data collected were analyzed using Statistical Products and Service Solutions (SPSS v.15 for Windows). Prevention of tick re-infestation on hosts and reduction percentage of tick infestation on the animals due to treatment with each type of essential oil were analyzed using one-way analysis of variance (ANOVA) and univariate analysis of SPSS. Significant differences between tick counts on the animals following the three treatments, essential oils of either *T. minuta* or *T. diversifolia* and the control, were evaluated using the Kruskal–Wallis test (H) (Kruskal and Wallis 1952) at $\alpha = 0.05$ level of significance. Logarithmic transformation [Log_{10}(tick counts + 1)] (Zar 1996) was applied to the data and the transformed data subjected to a paired sample t test to evaluate a generally observed trend in the field that the number of male ticks was higher than that of female ticks on the animals at $\alpha = 0.05$ level of significance.

Legal use of experimental animals in the field

All procedures requiring the use of experimental animals in the field were approved by the County Veterinary Office (CVO) of Bungoma County, western Kenya. The importance of the project was further explained to the Bukusu community by the CVO and agricultural extension officers working within the study area through organized workshops and local sub-Chiefs' and Chiefs' meetings in the entire County. The field experiments were performed in compliance with guidelines published by the 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 volunteer livestock farmers whose animals and farms we used.

Results

Tick infestation on zebu cattle at the start of the experiment

Although experimental animals came from different farms and were randomly sampled, the results indicate that the initial tick infestation (i.e., the average numbers of adult male and female *R. appendiculatus* and other tick species on the hosts) were not significantly different between animals ($P > 0.05$) (Table 1). At the start of the experiment, more males than females of adult *R. appendiculatus* were found on the host [$t_{0.05}$ (44) = 4.325; $P < 0.05$].

Tick infestation of experimental animals after treatment

On control animals treated with petroleum jelly only, tick infestations increased on average by 60% during the 18-day experimental period due to natural infestations. Treatments with the essential oils of *T. minuta* and *T. diversifolia* resulted in the drop of tick infestation levels on host animals over time. This drop was significant in the treatment with the essential oil of *T. minuta* ($P < 0.05$) and not in the treatment with the essential oil of *T. diversifolia* ($P > 0.05$) (Fig. 2a, b). In the treatment with the essential oil of *T. minuta*, the tick infestation level dropper significantly up to 11–14 days post-treatment, after which it increases again due to tick re-infestation ($P < 0.05$). The Student Newman–Keuls post hoc criterion indicated that, for hosts treated with the essential oil of *T. minuta*, tick infestations of the first 1–2 days post-treatment and the last days 15–18 following re-infestation for males and the 1–3 days post-treatment and the last days 12–18 following re-infestation for females, are not significantly different ($P > 0.05$) (Fig. 2a, b). Between days 3 and 14 post-treatment, the mean tick infestation levels on hosts are significantly lower than the other days ($P < 0.05$) (Fig. 2a, b). For hosts treated with the essential oil of *T. diversifolia* and petroleum jelly (control), however, there were no significant differences of mean tick infestation levels between days on hosts ($P > 0.05$) for male and female *R. appendiculatus* and other tick species. There was a continuous and gradual drop in tick infestation up to day 8 on

Table 1 The mean (±SE) tick counts on day zero on hosts naturally infested with ticks and selected for different treatments before start of experiment (I = those hosts selected for treatment with petroleum jelly (as control), II = those hosts selected for treatment with 10% essential oil in petroleum jelly of *Tagetes minuta*, and III = those hosts selected for treatment with 10% essential oil in petroleum jelly of *Tithonia diversifolia*) ($n = 45$)

Tick species	Mean (±SE) tick counts[a]		
	I[b]	II	III
R. appendiculatus (♂)	32.27 ± 18.19 a	38.67 ± 18.47 a	24.73 ± 14.59 a
R. appendiculatus (♀)	31.33 ± 18.60 a	23.20 ± 12.92 a	24.13 ± 14.73 a
Other tick species	32.20 ± 21.07 a	38.67 ± 21.39 a	21.87 ± 11.09 a

[a] For values within a given row, means followed by the same alphabetical letter(s) are not significantly different from one another at $\alpha = 0.05$ (Students–Newman–Keuls *H* test)

[b] For explanation of the three different treatments (I, II, and III), see text

the hosts treated with the essential oil of *T. diversifolia* for both male and female *R. appendiculatus* and other tick species but this drop was not significant ($P > 0.05$) (Fig. 2a, b).

The percentage of reduction of tick infestation was higher in the treatment with essential oil of *T. minuta* than in that of *T. diversifolia*. However, the repellent effect of the two essential oils caused similar patterns of tick responses (Fig. 2a, b). In both essential oil treatments, there was a phase of reduction of tick infestation lasting 3–4 days with the essential oil of *T. minuta* and 6–7 days with the essential oil of *T. diversifolia,* and the effects of both essential oils reached an optimum between 3–5 and 6–7 days post-treatment, respectively (Fig. 2a, b). Tick re-infestation started between 11 and 12 days post-treatment in the hosts treated with the essential oil of *T. minuta,* whereas in the hosts treated with the essential oil of *T. diversifolia*, re-infestation started at 9 days post-treatment. More ticks re-infested hosts treated with the essential oil of *T. diversifolia* than those treated with the essential oil of *T. minuta* for male and female *R. appendiculatus* and other tick species. Moreover, there was a greater variation in tick infestation levels after re-infestation of the hosts treated with the essential oil of *T. diversifolia* than those treated with the essential oil of *T. minuta*. Tick infestation levels following re-infestation were maintained at a lower level than they were initially for the hosts treated with the essential oil of *T. minuta* compared to those treated with the essential oil of *T. diversifolia*. In all the control treatments, there was a moderate but gradual increase in tick infestation of the animals (Fig. 2a, b).

During the observation period, more males than females of adult *R. appendiculatus* were found on the hosts [$t_{0.05}$ (494) = 14.252; $P < 0.05$]. This male/female ratio was maintained up to the end of the experiment [$t_{0.05}$ (854) = 17.491; $P < 0.05$]. Male *R. appendiculatus* succumbed to the repellent effects of the two essential oils more quickly than their female counterparts, with the essential oil of *T. minuta* showing a stronger repellent effect than the essential oil of *T. diversifolia* (Fig. 2a, b). Specifically, residual efficacy time of the essential oil of *T. minuta* was 8 days for female *R. appendiculatus* (Fig. 2b) and 12 days for male *R. appendiculatus* (Fig. 2a). The essential oil of *T. minuta* consistently kept the levels of male *R. appendiculatus* significantly lower than those of the control during 17 days, while this essential oil only kept the female *R. appendiculatus* infestation lower than controls during 9 days. For the essential oil of *T. diversifolia*, these values were 11 and 6 days for males and females, respectively. In the treatment with the essential oil of *T. minuta*, significant re-infestation started 11 days post-treatment for female *R. appendiculatus,* whereas for male *R. appendiculatus*, it started 14 days post-treatment. With the essential oil of *T. diversifolia*, re-infestation started 8 days post-treatment for female *R. appendiculatus* and 2 days later for males.

Other livestock tick species were also affected by treatments with the essential oils of *T. minuta* and *T. diversifolia*. Essential oils of *T. minuta* and *T. diversifolia* kept the infestation levels of other livestock tick species significantly lower than the control for 11 and 5 days, respectively. Re-infestation started 11 and 8 days post-treatment with the essential oils of *T. minuta* and *T. diversifolia*, respectively (Fig. 2c).

Protection of host animals with essential oils of *T. minuta* and *T. diversifolia*

Essential oils of *T. minuta* and *T. diversifolia* protected the experimental animals from tick re-infestation on average for 12.53 days (range 4–18 days) and 7.87 days (range 4–12 days) post treatment, respectively (Table 2). Host animals treated with the essential oil of *T. minuta* were also significantly protected from re-infestation with other tick species during 12 days post-treatment ($P < 0.05$) (Fig. 2c). The duration of protection from tick re-infestation on the

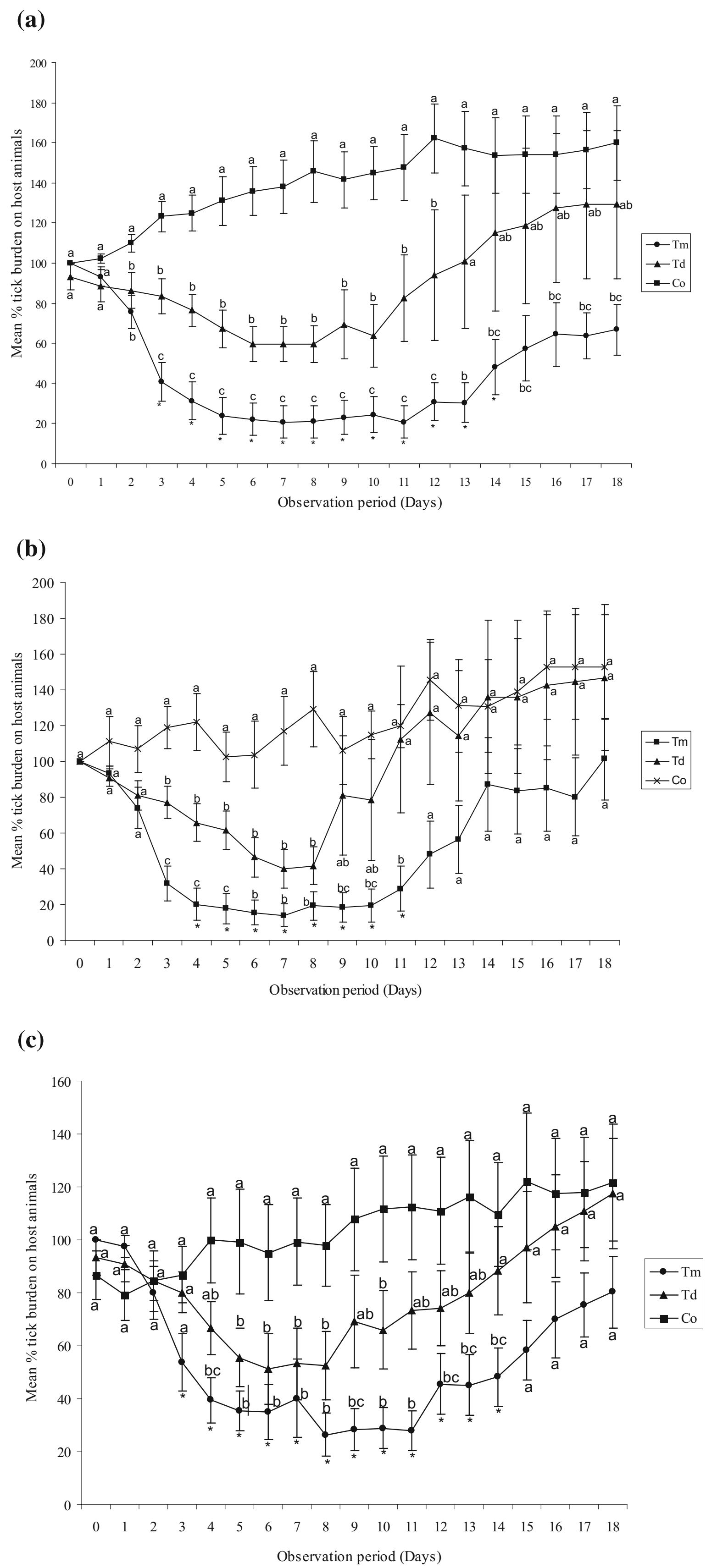

Fig. 2 Mean (±SE) percentage of tick infestation on the hosts treated with essential oil of *Tagetes minuta* (*Tm*) and *Tithonia diversifolia* (*Td*) or petroleum jelly (*Co*) (control) and monitored daily for 18 days. The effects of these treatments were evaluated on **a** male *Rhipicephalus appendiculatus*, **b** female *Rhipicephalus appendiculatus* and **c** other livestock tick species (such as *Amblyomma variegatum*, *R. evertsi* and *Boophilus* spp., with authenticity confirmed from voucher specimens of Zoology Museum of the University of Nairobi). On each day, *values capped with the same letters* are not significantly different at $\alpha = 0.05$ (Student–Newman–Keuls *H* test). *Asterisks* on the SE *bars* indicates that the mean percentage of tick infestation levels of the hosts were significantly lower than the initial tick infestation levels following treatment at $P = 0.05$ level of significance (Student–Newman–Keuls *H* test; $n = 45$)

Table 2 Observed post-treatment time (days) over which individual hosts treated with the essential oils of *Tagetes minuta* and *Tithonia diversifolia* were protected from tick re-infestation on 15 different farms ($n = 30$)

Farm	Post-treatment time (days) over which hosts were protected from tick re-infestation	
	Hosts treated with essential oil of *Tagetes minuta*	Hosts treated with essential oil of *Tithonia diversifolia*
1	16	9
2	5	5
3	11	7
4	13	5
5	10	8
6	4	6
7	10	12
8	18	10
9	15	8
10	16	4
11	13	10
12	18	9
13	14	6
14	14	11
15	11	8
Range	4–18	4–12
Mean ± SE	12.53 ± 1.07	7.87 ± 0.61

hosts by the two essential oils of *T. minuta* and *T. diversifolia* was significantly different [$t_{0.05}$ (14) = 4.336, $P = 0.001$], with essential oils from *T. minuta* giving the strongest protection.

Discussion and conclusion

Results obtained from this study confirm the previous laboratory results (Wanzala et al. 2014, 2004) and semi-field results (Wanzala 2009) in which essential oils of *T. minuta* cause a strong and consistent repellent effect on *R. appendiculatus*. The effect of essential oil from *T. diversifolia*, although significant, is less strong compared to that of *T. minuta*. The two essential oils showed a significant difference in their repellent effects on *R. appendiculatus* and on other tick species. The essential oil of *T. minuta* repels a larger proportion of *R. appendiculatus* and more quickly than that of *T. diversifolia*. In the treatment with the essential oil of *T. minuta*, an optimum effect is attained within a shorter period of time and maintained for longer than in the treatment with the essential oil of *T. diversifolia*. The results on duration of protection showed that re-infestation started early in hosts treated with the essential oil of *T. diversifolia* compared with those treated with the essential oil of *T. minuta*. These results suggest that the essential oil of *T. minuta* may contain compounds that confer a longer-lasting residual efficacy than the essential oil of *T. diversifolia*.

The mean protection time coincided with the acaricide dipping regime of 1–2 weeks for indigenous zebu animals and once per week for pure- and cross-breed animals (Norval et al. 1992). Although acaricide dipping is expensive in terms of labor input and cost and can cause side effects, it effectively controls all the ticks on the cattle, thus maintaining a tick-naïve population of cattle fully susceptible to more than one tick species (McCosker 1993). Moreover, effective tick control contributes to tick-borne disease control, and eventually to a healthy livestock population. However, 100% control of ticks on livestock may lead to a complete loss of immunity to vector-borne pathogens and this is a disadvantage, because the accidental exposure to ticks and tick-borne pathogens can potentially cause a destructive situation in susceptible animals. This was once witnessed in Zimbabwe during the civil war between 1975 and 1980 (Norval et al. 1992). By contrast, therefore, the advantage of repellents is that they do not create a tick-naïve population of cattle.

The rate of reduction of tick infestation was greater than the rate of build-up of the tick infestation in animals treated with *T. minuta*, while it was the reverse in animals treated with *T. diversifolia*. This further confirms the stronger repellent effect of the essential oil of *T. minuta* compared with that of *T. diversifolia*. This may explain why tick infestation levels following re-infestation on the animals treated with the essential oil of *T. minuta* were maintained at a lower level than they were previously, while the tick infestation levels following re-infestation on the hosts treated with the essential oil of *T. diversifolia* reached a higher level than they were before.

The results obtained in this study were comparable to those obtained elsewhere in Kenya with a 10% formulation of the essential oil of *O. suave* in liquid paraffin, as well as a 25% formulation of neem oil against attaching larvae and adults of *R. appendiculatus* (ICIPE 1998/99; Mwangi et al. 1995a). Results of recent field tests of natural repellents (5% nootkatone and carvacrol) against *Ixodes ricinus* L. by Dolan et al. (2008) were also comparable to the results obtained from the present study. By the 5th day post treatment, more than 75 and 60% of adult *R. appendiculatus* and other tick species were affected by the essential oils of *T. minuta* and *T. diversifolia*, respectively. The two essential oils not only affected the target species *R. appendiculatus* but also other livestock tick species such as *A. variegatum*, *R. evertsi* Neumann and *Boophilus* spp. This broad-spectrum repellent bioactivity against livestock ticks may have important implications for the practical use of the essential oils as tick repellent products for a variety of economically important tick species found in the host's environment. These findings warrant further research to establish to which extent each livestock tick species is affected by each type of essential oil. This approach will facilitate a more rapid incorporation of the essential oils into integrated tick control strategies. This may be a suitable approach for most African livestock farmers, who often lack the resources for appropriate and sustainable tick control. Moreover, should future studies prove these two plants (*T. minuta* and *T. diversifolia*) to have the abilities to repel ticks while in the field, like the case of *Melinis minutiflora* Beauv. (Mwangi et al. 1995a; Fernandez-Ruvalcaba et al. 2004) and *G. gynandra* (Malonza et al. 1992), then further studies are needed to evaluate how they can be incorporated into livestock pastures as tick-repellent plants.

As the essential oils protected the hosts against tick infestation for several days only, there is a need to stabilize the active ingredients so as to make them provide longer protection times in the field, comparable to substances such as pyrethroids, which give 2½ months' protection when impregnated in ear tags (Young et al. 1985). A more stable formulation, which would control the individual compounds' rate of vaporization, is needed as this could offer controlled release of repellent volatiles and more long-lasting protection. Furthermore, the performance of the oils may be affected by environmental factors such as strong sunlight, relative humidity and/or wind, as well as activities of the target host animals (Carroll 2007). Thus, improvement of the formulation may also need to address ways of dealing with these constraints, e.g., micro-encapsulation of the oils to protect the active ingredients.

The treated animals freely interacted with other animals in the experimental herd and environment (WW, personal observations in the field). Whether or not the treated animals conferred some protection to non-treated animals by virtue of their presence in any one given herd was not evaluated and is not known. However, it may be interesting to investigate this question, as was recently established with some tsetse fly repellents at ICIPE (Saini and Hassanali 2002–2003). Whether the observed increase in tick infestation in the control animals in all 15 herds was caused by an environmental factor or by the presence of repellent-carrying animals within the herds is not known. If the increase is hypothesized to be due to the presence of repellent-carrying animals, then we would have expected a decrease in tick infestation levels in the control hosts during the re-infestation period, but this trend did not appear. It would be interesting to monitor tick infestation levels on the hosts in another set of controls (negative controls) away from the potential influence of repellents.

The phenomenon of the biased male/female ratio of *R. appendiculatus* and other tick species in favor of males agrees with previous reports on sex ratios of blood-feeding ticks (Londt et al. 1979; Kaiser et al. 1982; Mwangi et al. 1985). Several possibilities to explain this phenomenon can be given. As observed by Hoogstraal (1956a, b) and Kaiser et al. (1982), it may be possible that male ticks remain on the hosts longer than the female ticks. There may also be a role for pheromones in the biased male/female ratio of *R. appendiculatus* and other tick species on the hosts. Recent studies show that, in the majority of hard ticks, feeding females produce a sex pheromone containing 2,6-dichlorophenol, which attracts males to the feeding site (Rechav et al. 1976; Sonenshine 2006). In addition to the daily activities of the hosts, weather, grass status and the amount of vegetation also affect tick population survival both on the host and in the environment (Wilkinson 1957; Thompson et al. 1978; Davey et al. 1982; Barnard 1986; Thadeu et al. 1989; Mwangi et al. 1995b; Fernandez-Ruvalcaba et al. 2004). But it is not clear whether these factors may preferentially affect ticks at sex level. As male ticks succumb to repellent effects of the essential oils significantly more than their female counterparts, we expected this sex ratio to change in favor of female ticks following essential oil treatment but surprisingly it did not. It is not clear whether or not this sex ratio phenomenon corresponded to the normal biological behavior of ticks: feeding and mating, followed by females dropping off the host body to lay eggs (Sonenshine 1991; Amoo 1992). Establishing facts about this phenomenon is important in livestock tick control and management strategies, particularly in our studies as it can lead to the development of an effective repellent dose for both female and male ticks. This information is also crucial in developing effective on-host tick control models.

The two essential oils of *T. minuta* and *T. diversifolia* are possible candidates to be considered for integration into livestock tick management programmes in the study area and in other areas with similar ecological conditions. The essential oils of *T. minuta* and *T. diversifolia* may periodically be applied alone (at intervals of 7–10 days) or integrated with acaricides and/or other alternative tick control methods suitable in that particular environment, as previously described for the essential oil of *O. suave* (Mwangi et al. 1995a). However, follow-up studies to know what happens to the surviving ticks off-host are recommended.

Author contributions

WW conceived the research idea. AH, RWM and WT contributed in the development of the research proposal, execution of the project, collection and analysis of data and interpretation of the data. WW coordinated data collection and drafted the first version of the manuscript. AH, RWM and WT participated in the review of the first version of the manuscript. All authors read and approved the manuscript for publication.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of any interests regarding the publication of this manuscript in any ways.

Declaration Authors declare that this manuscript is our own original research work and that have read and confirmed the content. Authors further confirm that the content of the manuscript has not been presented for publication in any other Journal and in any other form of this nature and that all sources of materials used for the research have been fully acknowledged accordingly.

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