

**Ethnobotanicals for management  
of the brown ear tick  
*Rhipicephalus appendiculatus*  
in western Kenya**

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**Ethnobotanicals for management  
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## Summary

This thesis describes the results of a study to assess the effect of ethnobotanical products on the behaviour of the brown ear tick *Rhipicephalus appendiculatus*, the main vector of East Coast fever in sub-Saharan Africa. Ethnoknowledge of the Bukusu people in western Kenya on tick control and management was evaluated to identify plants that affect livestock ticks, using participatory action research approaches. More than 150 plant species spread over 110 genera and 51 families were identified and documented. From these, eight plants were selected and their essential oils extracted and used for screening in the laboratory on their behavioural effects on ticks. From these, the plants *Tagetes minuta* and *Tithonia diversifolia* were chosen for further studies. The essential oils of these two plants were further extracted and used in laboratory and field bioassays.

From the laboratory assay, using a dual-choice apparatus, it was found that essential oils of both *T. minuta* and *T. diversifolia* affect tick climbing behaviour, representing a repellent response. Dose response effects were observed. On steers, differential effects to the essential oils were observed with *R. appendiculatus*, which prefer to feed mainly inside the ears of the host animal. It was found that treatment of the ear region with the essential oils of both *T. minuta* and *T. diversifolia* significantly deterred ticks from reaching the ear. The essential oils of *T. minuta* and *T. diversifolia* were evaluated in the field and significantly shown to affect *R. appendiculatus* and other ticks naturally attached to the host animals. The essential oil of *T. minuta* affects *R. appendiculatus* and other ticks more than the essential oil of *T. diversifolia*.

The results suggest the potential for essential oils to be incorporated in the on-host "push" and "push-pull" strategy for the control and management of *R. appendiculatus*, other affected livestock ticks and associated tick-borne diseases among the resource-limited livestock farming community in tropical Africa.

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# 1

## General Introduction

### Research concept

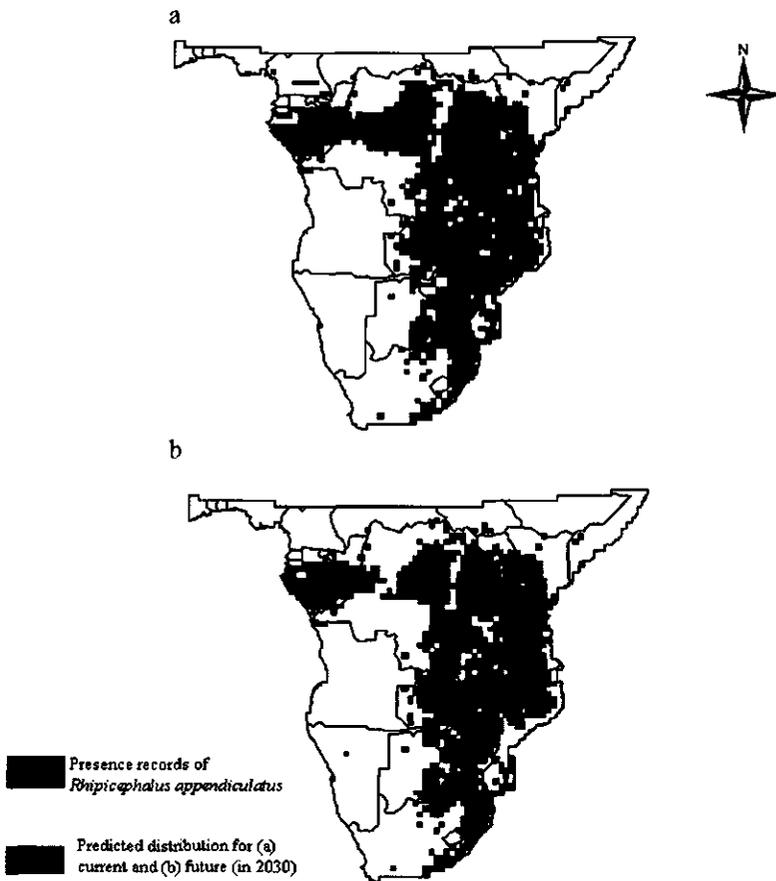
Globally, knowledge, skills, innovations, techniques and practices of indigenous and local communities have evolved so well over human generations that they are inherent in people's cultural life. These centuries' old practical ethnoknowledge and cumulative ethnoexperiences have been acquired by trial and error, which has caused many a fatality in the process (Le Strange, 1977). The evolution of ethnoknowledge involved the invention and development of ethnoremedies for almost every existing problem affecting humans and their livestock (Marina et al., 2001; Guèye, 2002). Succeeding generations and civilizations improved upon these ethnoremedies to suit their local needs and aspirations and to ensure that they passed them on to the succeeding civilization (Marina et al., 2001). Today, indigenous knowledge<sup>1</sup> is a resource to facilitate sustainable development and improve the standards of living of the poor people (IK and DM, 2004; Akall, 2003). Indigenous knowledge is considered an underutilized source of information that may be used to foster agricultural and economic development in less developed countries (Singh, 2002; Akall, 2003).

In tropical Africa, livestock farming is seriously affected by many indigenous infectious diseases. These diseases are a major constraint to sustainable rural livelihoods (Wanyangu et al., 1996). Many of these diseases are transmitted by ticks, which serve as disease vectors (Norval et al., 1992). In the course of many centuries, African livestock farmers have developed ethnoknowledge on various methods of tick-borne disease control and management, for instance by selecting plants that serve as pharmaceuticals or as anti-tick substances (Marina et al., 2001). However, few studies have been done to confirm the underlying science of these community-specific ethnobotanical products.

In the current thesis, the ethnoknowledge of the Bukusu people in western Kenya on tick control and management is evaluated. This knowledge is integrated with the conventional knowledge of on-host behaviour of the brown ear tick *Rhipicephalus appendiculatus* Neumann and considered for developing an effective on-host tick control and management strategy. The studies include laboratory and field evaluation of strategic deployment of essential oils with repellent effects designed to intercept foraging and attachment behaviour of *R. appendiculatus* by masking host-derived attractants. By comparing the repellent effects of selected essential oils, the impact of the essential oils with the strongest effect on tick behaviour is evaluated, so that effective protection can be afforded.

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<sup>1</sup>The sum total of the knowledge, techniques and skills, which people in a particular geographic area possess and enable them to get the most out of their environment. Most of these ethnoknowledge practices, techniques and skills have been passed from earlier generations uncritically but individual men and women in each new generation adapt and add to this body of ethnoknowledge, techniques and skills in a constant adjustment to changing circumstances and environmental conditions. They in turn pass on the body of the ethnoknowledge, techniques and skills intact to the next generation, in an effort to provide them with survival strategies. Indigenous knowledge evolves in response to the changing environmental conditions including exposure to more formal knowledge systems (CTA, 2008).

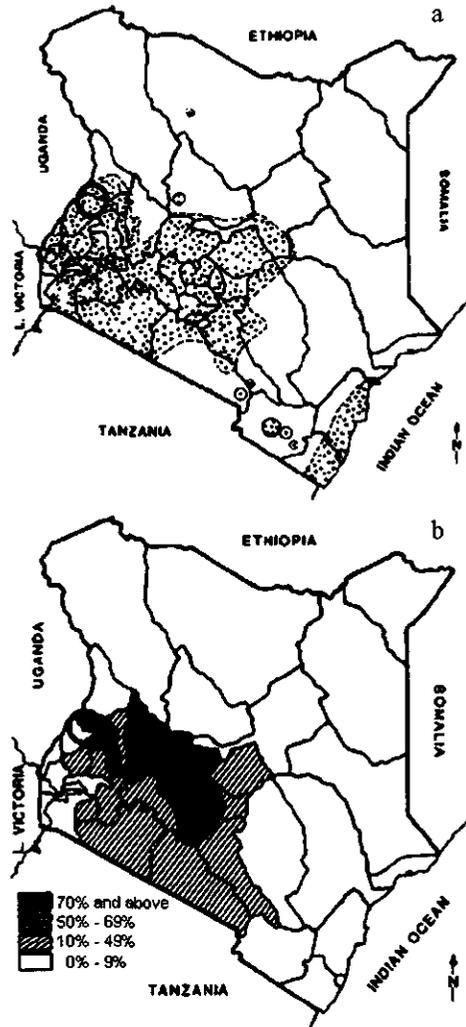


**Fig. 1.1.** The current and future predicted probability of the occurrence of the *R. appendiculatus* in sub-Saharan Africa obtained by using the predictive species model (Erasmus et al., 2002) and the DARLAM climate surfaces model: (a) current, (b) future-2030. (Adopted from Olwoch et al., 2008)

### Geographical distribution of *Rhipicephalus appendiculatus* and related rhipicephalids

Rhipicephalid species occur in Eurasia and northern Africa (15 species) and in sub-Saharan Africa (~55 species) (Walker et al., 2003). The distribution of *R. appendiculatus* and other rhipicephalids in Africa is by no means continuous, even in those countries in which they are known to occur commonly (Fig. 1.1). Their occurrence is influenced by several factors, the most important of which are climate, vegetation and host availability (Norval et al., 1992). Using a predictive species model (Erasmus et al., 2002) and DARLAM climate surfaces model (Olwoch et al., 2008) *R. appendiculatus* distribution in sub-Saharan Africa is shown in Fig. 1.1. In Kenya, the CLIMEX model was used to calculate the ecoclimatic index (EI), which shows the coincidence of distribution pattern of the dairy cattle with that of *R. appendiculatus* (Fig. 1.2). In Fig. 1.2, there is a very close correlation between the EI and the recorded *R. appendiculatus* distribution in much of the affected areas and this also applies to the affected areas of the African continent [from southern Sudan through to the south-eastern coast of South Africa (Fig. 1.1)] with some exceptions influenced by low cattle density and other herbivore hosts due to tsetse fly infestation (Perry et al., 1990; Randolph, and Rogers, 1997). Relative humidity, associated

**Fig. 1.2.** The distribution of *Rhipicephalus appendiculatus*, the vector of *Theileria parva*, in Kenya (a). The distribution of exotic beef and dairy cattle breeds (most preferred cattle, with high productivity, by rural farmers) in Kenya, showing the percentages by District (b). The two figures show the coincidence of the distribution pattern of dairy cattle with that of *Rhipicephalus appendiculatus*, thus posing great challenges on the sustainability of these breeds in ECF endemic zones. The ringed area on the two maps shows the study area (modified after Kariuki, 1989).



with rainfall and altitude, restricts *R. appendiculatus* to relatively cool and humid biotopes of less than 30 °C daily maximum and at least 400 mm annual rainfall, preferably upland savanna with a vegetation cover existing of woodlands or grasslands (Yeoman and Walker, 1967). The extent of *R. appendiculatus* distribution in the coastal regions of Mozambique is, however, unknown and no populations of this species have been reported in West Africa (Walker et al., 2003).

The occurrence and abundance of *R. appendiculatus* are affected by the amount of vegetation cover (Norval, 1977), the abundance of suitable ruminant hosts (Norval and Lightfoot, 1982) and acaricides used (Howell et al., 1981). The vegetation cover affects microclimate (Minshull and Norval, 1982), which is important for the survival of the free-living stages of the tick (Branagan, 1973). Where overgrazing and the removal of trees reduce the vegetation cover, *R. appendiculatus* tends to disappear (Norval et al., 1992).

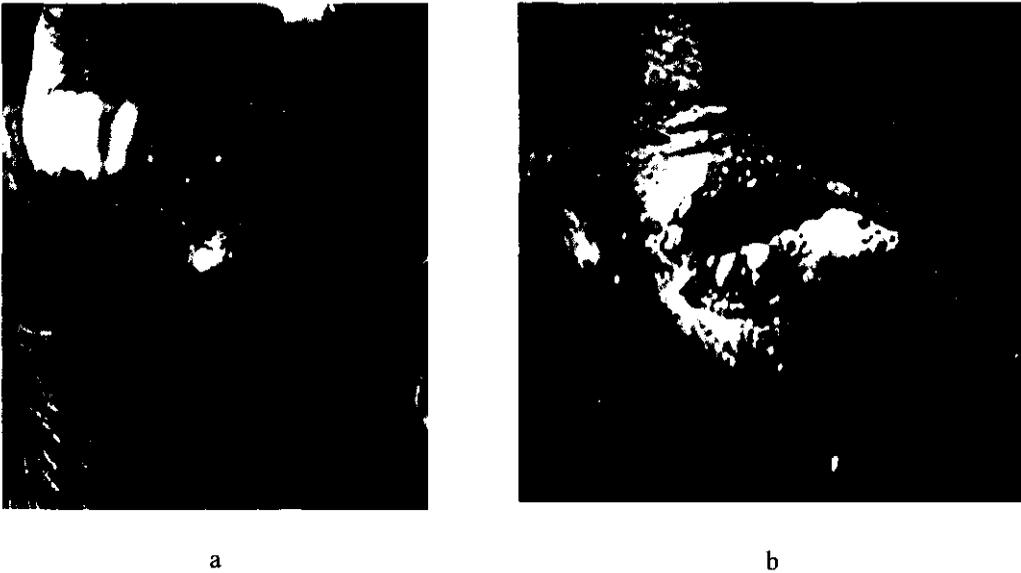
The species becomes abundant in the presence of hosts that have a low level of resistance to it (Lightfoot and Norval, 1981). Prolonged intensive acaricide treatment of livestock can cause local eradication of *R. appendiculatus*, but the tick can spread again if control measures are stopped (Norval et al., 1992).

Under certain circumstances, *R. zambeziensis* Walker, Norval & Corwin replaces *R. appendiculatus* in several of the hotter, drier areas of central and southern Africa, while *R. duttoni* occurs only in Angola and Zaire (Norval et al., 1992). However, nothing is known about the factors that limit the distribution of *R. duttoni* Neumann. *Rhipicephalus appendiculatus* shares geographical distribution and host range with a number of other rhipicephalids such as *R. evertsi* Neumann, *R. lunulatus* Neumann, *R. muhsamae* Morel & Vassiliades, *R. praetextatus* Gerstäcker, *R. pravus* Donitz, *R. pulchellus* Gerstäcker, *R. sanguineus* Latreille, *R. simus* Koch, *R. senegalensis* Koch, *R. turanicus* Pomrantsev, and *R. zambeziensis* (Walker et al., 2003).

### **Economic importance of tick-borne diseases transmitted by *Rhipicephalus appendiculatus***

Tick-borne infections of livestock are widespread in Africa and present a greater constraint to livestock development, particularly improvement of local breeds, than in any other region of the world. This is largely due to the fact that many different tick-borne infections occur on the continent, the most important of which are *Theileria parva parva* Theiler [East Coast fever (ECF)], *T. p. bovis* Neitz (January disease or Zimbabwe malignant theileriosis), *T. p. lawrencei* Neitz [Corridor disease (CD)], *T. annulata* Dschunkowsky and Luhs [Mediterranean Coast fever (MCF)], *Cowdria ruminantium* Moshkovski (heartwater), *Babesia bigemina* Smith and Kilborne, *B. bovis* Babes (babesiosis) and *Anaplasma marginale* Theiler (anaplasmosis) (Norval et al., 1992). This problem is compounded by the high susceptibility of foreign breeds of livestock being used to improve livestock productivity in many African countries (Norval et al., 1992). *Rhipicephalus appendiculatus* is undoubtedly the most economically important tick of the 40–70 African tick species (Norval et al., 1992; Walker et al., 2003). This is due to the fact that it is a highly efficient vector of *T. p. parva*, the pathogen of the most important and complex tick-borne disease, ECF (Theiler, 1904; Norval et al., 1992; Dolan, 1999). The prevalence of ECF is normally restricted to central, southern and eastern Africa where the cattle hosts, *Bos taurus* L. and *B. indicus* L., the tick and the parasite share the same geographical location (Norval et al., 1992). The disease causes high rates of mortality and morbidity in livestock populations, productivity losses in animals that recover and is the cause of exclusion of the much desired exotic breeds of cattle of high productivity from endemic areas (Kariuki, 1989). *Rhipicephalus appendiculatus* is also an efficient vector of *T. p. lawrencei* from African buffalo to cattle, causing Corridor or buffalo disease in the latter (Neitz, 1955). *Theileria parva bovis* of cattle and *T. taurotragi* of eland and cattle are also transmitted by *R. appendiculatus* (Fivaz et al., 1989) as is *Ehrlichia bovis* of cattle (Matson, 1967; Norval, 1979). The Nairobi sheep disease virus (NSDV) and Kisenye sheep disease virus (KSDV), causing hemorrhagic gastroenteritis and high mortality in sheep and goats, are transmitted primarily by *R. appendiculatus* (Bugyaki, 1955; Buisch et al., 1998). *Rhipicephalus appendiculatus* can also spread *Babesia bigemina*, Dhoi virus and Thogoto virus to both animals and humans (Jones and Nutall, 1989; Walker et al., 2003).

In wild animals such as antelopes, infestations of *R. appendiculatus* have caused toxicosis problems (Lightfoot and Norval, 1981). Heavy infestations of *R. appendiculatus* have caused the death of eland calves as a result of both acute and chronic anaemia (Lewis, 1981). The tick also transmits bacteria, *Rickettsia conori* and *R. aeschlimanii* causing tick typhus (Larisa, 2001) and virus causing louping ill (Alexander and Neitz, 1935) in humans.



**Fig. 1.3.** Heavy burdens of *Rhipicephalus appendiculatus* infestation. The ticks, having taken all available space on the animal's ears (their preferred feeding site), and becoming fully engorged with host blood (a), get attached in the neighbourhood of predilection site, the eyelid (b). Fig. 1.3a is a photograph by Prof. Dr. F. Jongejan, Coordinator, ICTTD-3 Newsletter, issue 29, 2006. Fig. 1.3b produced by ICIPE, Nairobi, Kenya.

Heavy infestations of *R. appendiculatus* on cattle (Fig. 1.3) may result in severe damage to the attachment sites, a fatal toxæmia, suppression of host immunity and re-emergence of tick-borne diseases may cause Tzaneen disease. It has been shown that for each fully engorged female, there is a loss of 4.0 g of potential growth of cattle (Irvin et al., 1996). Tick bites such as shown in Fig. 1.3 cause cutaneous effects such as focal dermal necrosis, irritation, haemorrhage, inflammatory response often involving eosinophils and development of wounds that become infected with bacteria such as *Staphylococcus* causing local cutaneous abscesses or pyæmia (Wall and Shearer, 1997). Heavy tick infestation may also cause significant blood loss, reduced productivity in terms of poor production of milk, meat, hides and skins, reduced weight-gain and restlessness (Norval et al., 1988; Pegram et al., 1989a). For instance, in an ECF-infested area of Kenya, de Castro et al. (1987) recorded a decrease of live weight of cattle of 12.8 kg for a 200 kg animal. Milk production was reduced by 9 g per each engorging female *R. appendiculatus* in indigenous sanga cattle (Norval et al., 1997). Secondary infections in wounds caused by tick bites such as infections of *Dermatophilus congolensis*, may result in high mortality of livestock (FAO, 1998). Tick-bite lesions also predispose animals to screwworm myiasis (Wall and Shearer, 1997).

In sub-Saharan Africa, 76 million cattle live in the ECF-affected region covering an area of about 156 million hectares (Mukhebi et al., 1991). East Coast fever puts at risk the lives of about 25 million cattle in Burundi, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe (Norval et al., 1992). The disease has been reported to cause half a million deaths of cattle per year in East Africa (VIE, 2002). In Kenya alone, it has been estimated that 50–80% of the national cattle population of about 10 million animals, are exposed to tick infestation, and of these animals 1% die of ECF each year (Mbogo et al., 1995; VIE, 2002). A micro-economic analysis completed in 1988 on eight large and medium-sized farms, with a total of 37,779 head of cattle, in Na-

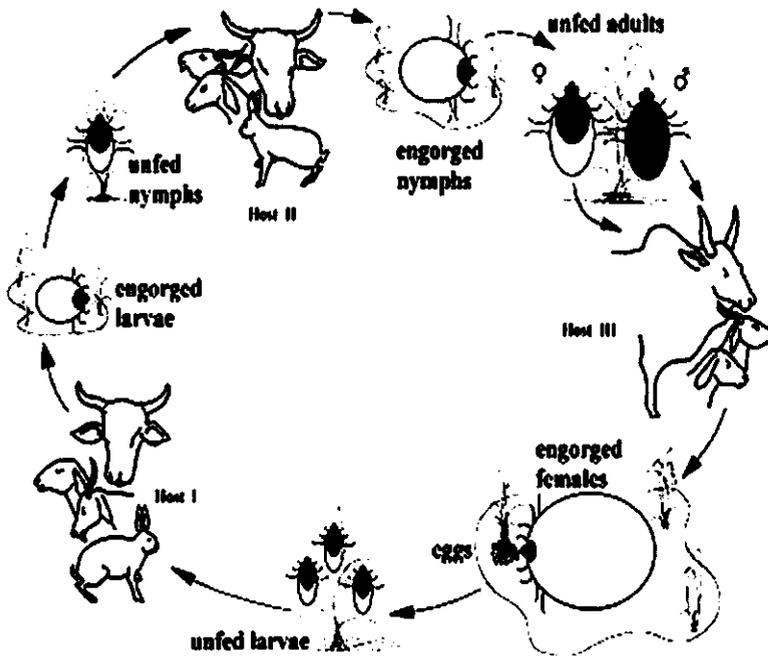


Fig. 1.4. The life cycle of *Rhipicephalus appendiculatus* displaying the teleotropic type of behaviour (the three-host cycle)

kuru District, Central Rift Valley, Kenya, showed that the cost of acaricides, production losses and losses due to clinical theileriosis and other tick-borne diseases amounted to approximately US\$ 515, 305 or \$13.64 per animal per year (Kariuki, 1989).

Exotic cattle, *B. taurus* are highly susceptible to ECF with a mortality of 90–100% while indigenous cattle, *B. indicus*, suffer a much lower fatality of about 10–40% in calves due to acquired immunity (Sutherst et al., 1978). Upon recovery, cattle become reservoirs of ECF (Young et al., 1986) as observed too in wildlife (Young et al., 1981). This, however, maintains the disease in cattle populations longer, particularly where cattle and wildlife share grazing fields with the availability of suitable vectors (Norval et al., 1992).

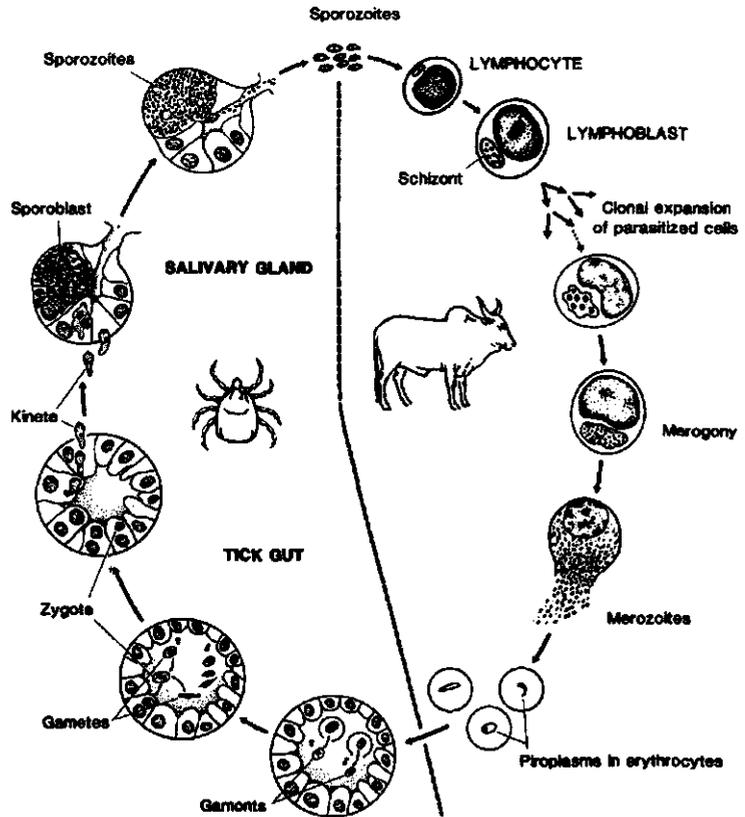
Besides cattle morbidity and mortality as well as severe economic losses as a result of direct and indirect tick parasitism, stock management also requires large financial inputs. For example, costs incurred for control operations have been estimated at US\$ 7.02/head/year or US \$1.08 per hectare (Mukhebi et al., 1993). Countries exposed to the threat of ECF also face a large financial burden due to importation of acaricides, training of local personnel, treatment of infected animals, maintenance of infrastructure and investments into research on appropriate solutions to ticks and TBDs. Such economic constraints have been estimated to cost millions of US dollars annually in many African countries (Mukhebi et al., 1993).

### The biology and behaviour of *Rhipicephalus appendiculatus*

#### *Life cycle of Rhipicephalus appendiculatus*

Ticks are obligate, blood-feeding ectoparasites of vertebrates: mammals, birds, reptiles and amphibians, more particularly mammals and birds. Adults of most tick species parasitize wild and domestic artiodactyls, perissodactyls, or carnivores (Cumming, 1998). Bovine cattle are the main host of *R. appendiculatus*, but goats, buffaloes, elands, waterbucks,

**Fig. 1.5.** A generalized life cycle of *Theileria parva* in the animal host and in the vector, the *Rhipicephalus appendiculatus* as understood today. (figure adopted from the International Laboratory for Research on Animal Diseases, Annual Report, 1980).



nyalas, greater kudu and sable antelopes serve as non-domestic hosts, while dogs and sheep are also infested. *Rhipicephalus appendiculatus* become well adapted to domestic cattle and can be maintained by all stages feeding on cattle, but immature ticks may feed on smaller antelopes and scrub hares, thus showing a telotrophic type of behaviour (three-host cycle) with a tendency to the monotrophic type (one-host cycle). On cattle, the immature stages of *R. appendiculatus* attach mainly on the neck and dewlap, the cheeks, eyelids, muzzle and ears. The adult *R. appendiculatus* prefer to feed on the ear pinna of bovids but not in the ear canal. In heavy infestations, adults are also found around the eyelids (Fig. 1.3b) and horns, on the upper neck, in the tail-brush and around the anus. The telotrophic type of behaviour of *R. appendiculatus* is summarized in Fig. 1.4. Only about 2–5% of tick's lifespan is spent on a host and the majority of their life cycle is spent on the ground or vegetation (Branagan, 1973).

#### **The interaction of the parasite *Theileria parva parva* with the vector *Rhipicephalus appendiculatus* and the vertebrate host**

The interactions of the tick, host and parasite exhibit a complex relationship whose understanding remains key to the successful control and management of tick and TBDs (Norval et al., 1992). The parasite undergoes sexual development in the vector (definitive host) and asexual development in the mammalian (intermediate host) host (Fig. 1.5). The sporozoites of *T. parva*, produced in large numbers in the acinar cells of the salivary glands of the in-

fectured tick, are inoculated along with saliva during blood feeding of *R. appendiculatus* to initiate its asexual cycle of development in the mammalian host. Using specific receptors, the sporozoites rapidly enter target lymphocytes, which become transformed after the *Theileria schizont* is formed. The entry of *T. p. parva* sporozoites into bovine peripheral blood lymphocytes is temperature-dependent and requires the participation of live and intact sporozoites and host cells (Shaw et al., 1991). This process involves a sequence of events (recognition and attachment of sporozoite to lymphocyte by binding, formation of a very close continual junction between the sporozoite and lymphocyte membranes, zippering of sporozoite to lymphocyte membrane, separation of enclosing host cell membrane from the sporozoite, entry of sporozoite into lymphocyte cytoplasm and finally the formation of an orderly array of host cell-derived microtubules around the sporozoite) (Fig. 1.5). The parasite does not use rhoptries and microspheres for entry into the host cell, but uses them to destroy the surrounding host cell membrane after entry into the host cell (Norval et al., 1992). This prevents the host cell from being able to use lysosomal activity against the parasite. The infected lymphocyte is transformed into a lymphoblast and divides in conjunction with the schizont, giving rise to two schizont-infected daughter cells at frequent intervals. This process has been termed "parasite-induced reversible transformation" because, if the cells are treated with antitheileria drugs, the transformed cells revert to quiescent lymphocytes (Ole-Moiyoi, 1989).

Within the infected lymphocytes, schizonts are associated with microtubules involved in spindle formation during host cell division (Norval et al., 1992). Clonal expansion of infected lymphoid cells occurs with an approximate tenfold increase of schizonts every 3 days. Schizonts, traditionally called macroschizonts or Koch's blue bodies, vary in size and in the number of nuclei. Early detectable forms are small with nuclei that, when Giemsa-stained, appear as chromatic granules. The clonal expansion of the *Theileria*-infected lymphoid cells with the concomitant destruction of the infected tissues appears to give rise to the main pathogenic effects of the disease (Dolan et al., 1984). Cattle that recover from ECF acquire protective immunity and become resistant to re-infection with a stock of *T. p. parva* homologous (parasite-specific Major Histocompatibility Molecules-class I-restricted cytotoxic T-lymphocyte responses) to that which induced initial infection, but they may die if challenged with a heterologous stock (McKeever, 2001).

*Rhipicephalus appendiculatus* become infected with *T. parva* when feeding on an infected host having piroplasms in erythrocytes (Konnai et al., 2007a). Piroplasm-infected erythrocytes are ingested by ticks of the larval or nymphal stages and undergo a sexual development cycle in the gut of the replete tick to produce zygotes, which in turn develop into motile kinete stages that infect the salivary gland acini of the next instar, the nymph or adult (Fawcett et al., 1985). In the salivary glands of the tick, the kinete develops into infective sporozoites (sporogonic phase) and this repeats the cycle when the infected tick with infective sporozoites in its salivary glands takes a blood meal from a susceptible cattle host (Fig. 1.5). *Theileria parva* only mature and enter the saliva after the tick attaches to a host for a considerable period of 3–4 days (Martin et al., 1964). This period has been recently shown to be between 24 and 72 hours (Ochanda et al., 1988; Konnai et al., 2007a). However, if environmental temperatures are high, infective sporozoites can develop in ticks on the ground and may enter the host within hours of attachment (Ochanda et al., 1988). The time from entry in the tick to sporozoite development in the salivary glands is on average 19–20 days for feeding nymphs and 20–21 days for adult females (Ochanda et al., 1996; Watt and Walker, 2000).

From day 14 after *T. p. parva* infection of cattle by a tick bite, individual schizonts undergo merogony to produce merozoites (traditionally called microschantons). Merozoites

invade the erythrocytes to become piroplasms, which may subsequently undergo limited division also by merogony (Conrad et al., 1986).

### **Behaviour of *Rhipicephalus appendiculatus***

#### ***Host-seeking behaviour of Rhipicephalus appendiculatus***

Blood-feeding arthropods such as ticks, have, over time, developed a complex relationship with their mammalian hosts. Broad variations occur in host-specificity of ticks, duration and multiplicity of contacts, and in host-location behaviour (Gibson and Torr, 1999). The results obtained from analysis of a quantitative data set of 43,615 individual collection records of ticks in Africa suggest the existence of a spectrum in host-specificity but with the edges of this spectrum readily demarcated (Cumming, 1998). From this broad range of a spectrum in host-specificity, generalists and specialists in host/prey location can be discerned (Steidle and van Loon, 2003). Nevertheless, what induces host-seeking behaviour of the vector and its subsequent finding and selection of suitable hosts by different tick species has not been fully understood. This behaviour has been considered either as the result of evolutionary adaptation processes to the host-derived stimuli (Cupp, 1991; Steidle and van Loon, 2003), pathogen-induced behaviour in the vector, normal feeding habits, visual cues, host food and or its products such as faeces, urine or exuviae (Steidle and van Loon, 2003) or combinations of these factors. In other arthropods such as mosquitoes, the role of olfaction in host-seeking behaviour has been explained (Takken, 1991), including cues as human breath, body odours etc. (Mukabana, 2002). It is assumed that host-seeking behaviour of ticks is affected by similar cues. The combined knowledge demonstrates that the host-vector-parasite relationship is complex in nature, and whose pattern of responses and sequence of behavioural events, particularly of the vector, have to be clearly understood and strategically integrated in epidemiological tools in order to achieve sustainable control and management of vector-borne diseases.

Previous studies indicated that various attractive host-derived stimuli (e.g., host texture, host skin humidity, host body temperature and chemical factors (kairomones/allomones/synomones<sup>2</sup>) such as skin emanations, breath, urine and faeces, influence host-seeking behaviour in ticks (Sika, 1996). Kairomones are the main sensory cues used by haematophagous organisms to find their hosts (Mordue and Mordue, 2003). Environmental factors complement these kairomones in influencing host-seeking behaviour in ticks (Speybroeck et al., 2003). Adult *R. appendiculatus* search their hosts for a blood meal when they are active early in the day. They become active under specific sets of temperature, rainfall, humidity, length of the rainy season, number of rainy and cloudy days, and day length (Pegram et al., 1989b). Numbers of adult *R. appendiculatus* on the host increase after the onset of the rains (Berkvens et al., 1998). The main factor responsible for this phenology is thought to be day length, where a long photoperiod terminates the state of diapause and induces host-seeking behaviour in the wet months (Madder et al., 2002). Diapause in ticks is considered to be a pre-adaptive behaviour to allow the ticks to survive unfavourable conditions of a given season. Near the equator, ticks are non-diapausing and may usually feed throughout the year and their numbers vary less (Speybroeck et al., 2004).

<sup>2</sup>An allomone is a chemical substance produced by an organism, which induces in a member of another species a behavioral or physiological reaction favorable to the emitter; may be mutualistic or antagonistic. If the benefit is to the recipient the substance is referred to as a kairomone but if both organisms benefit then it is a synomone (Sbarbati and Osculati, 2006)

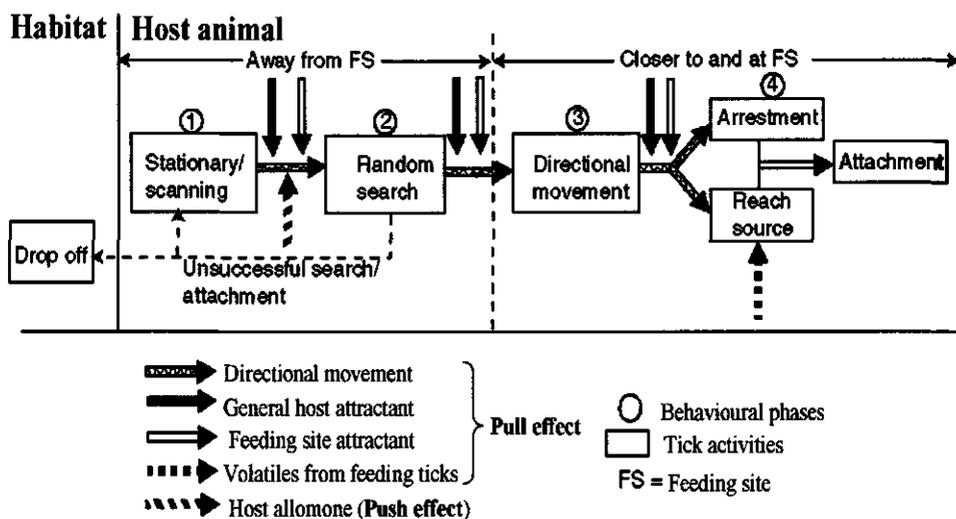


Fig. 1.6. A sequence of orientation behavioural activities of *Rhipicephalus appendiculatus* on the host. The process is characterized by different behavioural patterns (after Sika, 1996).

Once the weather conditions become favourable for the ticks to become active, the chemical cues elicit long-range responses while the physical cues elicit short-range responses in the host-location processes (Mordue and Mordue, 2003). The integration of physical factors and kairomones in the light of the pivotal role played by environmental factors in influencing host-seeking behaviours of ticks has not been explored in building up tick control device(s) that suit different climatic conditions.

#### **Differential selection of predilection feeding sites by *Rhipicephalus appendiculatus***

Once on the host animal, many tick species will not probe until they have arrived at the preferred feeding site and are not at risk of being removed from the host. This preference for feeding site may serve to avoid competition amongst the tick species feeding on the same host animal and perhaps inter-species mate confusion between closely related species, thereby enhancing their survival and reproductivity (Chilton et al., 1992). This may be as a result of evolutionary adaptations of certain tick species to the attraction of specific stimuli originating from the predilection feeding site on the host animal. Once on the host animal, even very closely related species demonstrate predilection for feeding at different body sites as demonstrated for *R. appendiculatus* and *R. evertsi* (Sika, 1996; Wanzala et al., 2004). The mechanisms underlying these species-specific interactions and selection of the feeding site on the host have not yet been understood. For instance, adult *R. appendiculatus* showed marked preference for feeding in the inner part of the bovine ear pinna while the immature ones showed less selectivity by feeding on many other parts of the host in addition to the ear pinna (Walker, 1974).

<sup>3</sup>A pheromone (from Greek φέρω phero "to bear" + 'ορμόνη "hormone") is a chemical 'message' secreted externally by an insect or other animal through which it communicates with another individual of the same species through a sense of smell to influence or trigger specific natural behavioral or physiological responses (especially of sexual behaviours) (Vet and Dicke, 1992). Pheromones are used to attract members of the opposite sex for reproduction, to mark food trails or location and territory, and are used as warnings or alarm systems. Each insect (or animal) has its own set of complex chemical pheromone, some of which have been identified and used in traps to monitor and sometimes control pest populations. In other cases, pheromones are used to confuse or lure insect pests away from target crops.

Just as during host location, a wide range of factors ranging from physical to chemical are involved in the feeding site location on the host by ticks. The role of semiochemicals (pheromones<sup>3</sup>, host kairomones and some allomones and host-microbial odours) does not help the tick to identify a suitable host and reach it (Sonenshine et al., 1986), but continues while on the host to help it identify a suitable feeding site (Norval et al., 1989a; Wanzala et al., 2004). The selection of the feeding site is not a random activity or chance effect but is systematically brought about by a well-coordinated stereotyped sequence of behavioural events (Fig. 1.6) elicited by host-derived semiochemicals (Sika, 1996). Often, ticks have to travel relatively long distances on the host to reach the targeted feeding site (McDowell and Walade, 1985). Routine observations showed that this phase is characterised by different behavioural patterns, which include runs, stops, bouts, turns, scanning etc. (Wanzala et al., 2004). The settlement at 'preferred feeding sites' is also related to physical characteristics of the host (texture, body temperature, skin humidity, etc.) as well as chemical factors (skin emanations, host-derived microbial odours, breath, faeces and urine), which are likely to provide optimum conditions for the attachment of ticks (Doube and Kemp, 1979). Observations have shown that ticks tend to avoid desiccation by choosing to occupy less exposed areas or parts on the host (Roberts, 1971). Micro-environmental conditions specific to certain body areas of the host e.g., the hygrometric index in the ear cavity, skin temperature and humidity have also been suggested to play a role in the feeding site preference by certain tick species (Waladde et al., 1991). The complex odorous environment met by individual ticks on their way to the preferred feeding site is important in maintaining the oriented course of the ticks. This offers an odour-permeated background (resulting from multiple secretions and volatile emissions on the skin surface of bovine host). Chemical compounds in sweat and other skin secretions, detectable by olfactory and/or tactile receptors, are believed to facilitate the selection of suitable feeding sites (Waladde and Rice, 1982). Earlier, Balashov (1972) reported electrophysiological evidence on the detection of glucose and sucrose by the type A sensilla in the olfactory organs of the tick.

More recent studies suggest that different tick species themselves also play a role in guiding other ticks of the same species to the suitable feeding site. This is the case in certain *Amblyomma* species where an aggregation of the ticks at specific feeding sites is supported by the male-emitted attraction/aggregation attachment pheromone (ortho-nitrophenol) secreted by attached and feeding ticks, attract conspecific males, females and nymphs (Maranga et al., 2003). In the majority of the hard ticks studied so far, feeding females produce an attractant sex pheromone containing 2,6-dichlorophenol, which attracts males to the feeding site (Rechav et al., 1976). In the case of *R. appendiculatus*, olfactometric experiments using swabs impregnated with ear emanations evoked attraction of adults but repelled nymphs and larvae of the same species (Akinyi, 1991). In the same study, ear swabs mixed with male tick extracts inhibited responses of these immature stages. No explanation was given to account for these results. Likewise, for *R. evertsi*, although its feeding site propensity is known (around the anal region) (Elbl and Anastos, 1966), adequate data are lacking to provide insights into its feeding site selection and the nature of the semiochemical signal(s) involved. Stimuli present in the host's anal region, which attract *R. evertsi*, are of interest, as in addition to body surface volatiles, and may include effluvium from the gut and volatiles from dung. In addition, the pheromones emitted by male *R. evertsi* while in the preparasitic as well as in the parasitic phases, may play a significant role in feeding site location by host-seeking conspecifics (Goethe and Neitz, 1985).

The migratory bouts therefore, which *R. appendiculatus* and a closely related species, *R. evertsi* perform during search and location of the predilection feeding sites, are guided by attracting and repelling host-derived semiochemicals in “push” and “push–pull” modes (Wanzala et al., 2004). With this pheromonal and allelochemical (kairomonal/allomonal) knowledge coupled with that of environmental factors, it is possible to explore on–host tick control interventions involving: (i) tactical use of repellent botanicals near/around the feeding sites to confuse the ticks (the ‘push’ tactic), (ii) divert ticks (the ‘pull’ tactic) to simple traps strategically placed on the host body and contaminated with a killing agent such as pathogenic fungi or acaricide botanicals, and (iii) concurrent protection of feeding sites with repellents and diversion of ticks to the traps (the “push” and “push–pull” tactics). As much as differential selection of predilection feeding sites studies are shown to be important in the control and management of ticks on the hosts, only casual attention has been given to the feeding site location behaviour of relevant arthropods.

***Responses of Rhipicephalus appendiculatus and other ticks to host-derived kairomones***  
Semiochemical (kairomonal as well as allomonal) communication is quite well known and demonstrated in ticks (Yoder et al., 1993). Host-derived odours influence on–host orientation of the tick and their selection of feeding sites. Host-borne kairomones fall into four main classes based on their nature and the corresponding behaviour evoked: (i) CO<sub>2</sub> and other bovine breath volatiles, (ii) skin emanations (iii) specific feeding site attractants (host-derived cues that elicit responses, which help ticks to locate a specific feeding site on the host) as previously explained and (iv) volatiles from excretory products (urine and faeces). Carbon dioxide (5–10% of expired air of mammalian breath) is among host-originated odorants influencing the behaviour of several tick species, either as an attractant (Garcia, 1962) or a stimulant (Norval et al., 1989b). Sauer et al. (1974) reported that CO<sub>2</sub> doses of 1–8% enhanced host-seeking behaviour in the American lone star tick, *Amblyomma americanum*. Other compounds of interest found in trace amounts in ox breath are acetone, 1–octen–3–ol (octenol) and butanone. A blend of octenol and CO<sub>2</sub> was found to enhance catches of tsetse flies (Vale, 1980), but these components didn’t evoke any attraction in *Amblyomma hebraeum* (Norval et al., 1987). Other detailed studies showed that CO<sub>2</sub>’s role in host location by ticks is more intricate, as elevated CO<sub>2</sub> in combination with other stimuli (e.g., heat, odour and temperature) elicit greater responses from ticks than when these stimuli were presented singly (Howell, 1975). Carbon dioxide has also been shown to be a phagostimulant for *R. appendiculatus* and *Amblyomma variegatum*. Using a 5% CO<sub>2</sub> atmosphere, the larvae and nymphs of *A. variegatum* can be induced to fully engorge in an in vitro system in the total absence of a host animal, achieving engorged weights comparable to those achieved on mammalian hosts and with a high moulting rate. *Rhipicephalus appendiculatus* larvae, nymphs and females as well as female *A. variegatum* can be fed in an artificial system when the CO<sub>2</sub> concentration is 7% (Anon., 2008).

Ticks locate their host by responding to host-derived odours. For example, adult *Ixodes scapularis* Say have been shown to travel  $\geq 10$  m in response to host-derived kairomonal cues (Carroll and Schmidtman, 1996). Stimuli from hosts serve as a multi-purpose indicator for host proximity, to guide to a potential host and to elicit feeding responses once the contact with the appropriate site has been made (Howell, 1975). Waladde and Rice (1982) distinguished two types of inherent appetite behaviour used by a tick vis-à-vis its host. In the questing type, passive ticks would await for a passing host to cling on. The hunting type, in contrast, would actively move to a potential host and is likely to make use of host odours for orientation, alone or in conjunction with other cues. There is, therefore, a great variation in host location behaviour between tick species. In general, the pres-

ence of various stimuli has made possible a hierarchy with regard to whether these act at close, mid- or long-range distances during the host-finding process as observed for some insect groups (Visser, 1986). Attraction and attachment of a tick to host skin are believed to be influenced chiefly by olfactory stimuli augmented by thermal stimuli (Howell, 1975). Rare cases of visual and/or acoustic stimuli have been proposed (Waladde and Rice, 1982). Host skin emanations are assumed to function as kairomones. Olfactometric experiments have indicated that many host parts do not play a kairomonal role as swabs of calves from legs, back, perineum and belly were unattractive to adults, nymphs and larvae of *R. appendiculatus* (Akinyi, 1991). Swabs of calves collected from inside the ear, on the inner and upper sides of the ear pinna and around the ear base, however, were attractive. In related laboratory and field studies, adult *I. scapularis*, *Dermacentor variabilis* Say, 1821 and *Amblyomma americanum* Linnaeus, 1758 have been shown to exhibit similar behaviour in response to substances rubbed from their respective hosts and non-host animals (Carroll et al., 1995).

While on the host, different tick species exhibit various locomotory and exploratory behavioural patterns, which relate to either mate finding or selection of feeding sites (Sonenshine et al., 1986). For species such as *R. appendiculatus*, whose mating takes place on the host after attachment, females merely move to feeding sites and remain there until engorgement is completed. The pre-mating behaviour of the male is comparatively more intricate. Observations on *D. variabilis* and *A. americanum* showed that males first do not move very much from their attachment point (Gladney and Drummond, 1970). It is only after they become sexually active following a blood meal that they disperse, sometimes extensively, over the host to seek the females (Hamilton et al., 1994). This mate seeking behaviour may be mediated by pheromones, which may also attract unfed conspecifics of both sexes to the feeding site. It has been assumed that stimuli from preferred feeding sites may play a similar role as attractants.

### **Some developments in livestock tick control and management: successes and failures**

Tick control is practiced in a wide variety of circumstances involving different tick and host species. The main reasons for tick control are to protect hosts (livestock) from irritation and production losses, formation of lesions that can become secondarily infested, damage to hides and udders, toxicosis, paralysis, and of greatest importance, infection with a wide variety of disease agents (Norval et al., 1992). Control also prevents the spread of tick species and the diseases they transmit to unaffected areas, regions, or continents (Aiello and Mays, 2003). The resultant product being the increased livestock production manifested in improved livelihood and economic development and growth of livestock holders (Mukhebi et al., 1991). The control of ticks has been under taken by chemical means, cultural measures including use of botanicals, mechanical, biocontrol and immunological techniques. These will be considered below.

#### ***Chemical control***

The first application of ixodicides to control ticks on cattle was made by treating the infested cattle with various oils—including paraffin but without much success (Harrison et al., 1973). An effective chemical control of livestock ticks began with the introduction of arsenical solutions as cattle dips in South Africa in 1893 and in Australia in 1895. The use of chemical ixodicides against livestock ticks has continued until today. In Africa, seven basic acaricide groups have been indiscriminately used to control livestock ticks and these are: (i) arsenicals, (ii) organochlorines, (iii) organophosphates, (iv) carbamates, (v) amid-

nes, (vi) pyrethrins and (vii) synthetic pyrethroids (Mitchell, 1996). Mode of application of these acaricides have included the use of dip tanks, knapsack hand sprayers, hand dressing, squirting acaricide on predilection feeding sites of ticks, dusting, etc. (Awumbila, 1996). In recent years, several other methods of acaricide application have been tested, including the slow release of systemic acaricides from implants and boluses; the slow release of conventional acaricides from impregnated ear-tags; 'pour-ons', which are applied on the backs of livestock and spread rapidly over the entire body surface; and 'spot-ons', which are similar to 'pour-ons' but have less capacity to spread (Norval, 1989).

Acaricide application may either be directed against the free-living stages in the environment or the parasitic stages on the hosts. In either case, the problems associated with the use of acaricides in livestock industry are very challenging without imminent solutions. Acaricides are costly and out of reach of poor rural livestock farmers who also do not have sufficient technical know-how of managing and handling them (Norval et al., 1992). The high costs of acaricides have become a major problem for smallholder farmers who constitute the majority in the livestock industry on the continent of Africa (Kariuki, 1996). The control of ixodid ticks by acaricidal treatment of vegetation has been done in specific sites to reduce the risk of tick infestation to susceptible hosts. This method is however not sustainable and has not been recommended for wider use because of associated environmental pollution problems and the high costs involved in the treatment of large areas (Aiello and Mays, 2003). In addition, inappropriate drainage of dip liquid causes water pollution and indiscriminate damage to the fauna and flora in the environment. Few quantitative data are available on the impact of these acaricides on flora and fauna but it can be assumed to be substantial at the local level (de Haan et al., 1996). Acaricides are a health hazard as well. They cause food poisoning (through meat, blood and milk) and residual toxicity. For instance, organochlorine products have been demonstrated to leave residues in meat and milk (Mitchell, 1996).

Intensive application of acaricides to livestock creates an enzootically unstable disease situation<sup>4</sup> in the population (Norval et al., 1992). When tick control breaks down, large losses can occur. An example of this was in Zimbabwe, where a compulsory dipping policy had been in force since 1914 and when dipping infrastructure broke down during the war of independence between 1974 and 1979, an estimated one million cattle died, mainly of tick-borne diseases (Lawrence et al, 1980).

The ticks have consistently shown themselves to possess a genetic pool containing the potential to resist a wide range of chemical poisons. This has been compounded by illegal cattle movement, civil unrest in some areas, poor management and inadequate maintenance of cattle dips and poor use of manufacturers' instructions (Matthewson, 1984; Nolan, 1990). It has been shown that the indiscriminate use of acaricides may affect future tick control as this has the consequence of facilitating rapid development of tick resistance to the active compounds used in the acaricide formulations (Fraga et al., 2003). There is therefore a very real danger that unless new acaricides of different chemical structures are forthcoming, tick resistance to existing compounds will spread. But the problem is that the development of new acaricides is a long and very expensive process (Graf et al., 2004).

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<sup>4</sup>A pre-immune status to tick-borne infections, so-called endemic stability, often establishes in indigenous cattle through a continuous contact with the infectious agents from early in life. This status is normally removed during excessive application of acaricides. The presence of endemic stability is claimed to be the main factor in limiting losses, mainly expressed in terms of low or absence of mortality due to tick-borne infections in indigenous cattle populations. Cattle that are exposed to *Anaplasma*, *Babesia* or *Cowdria* organisms early in life do not usually develop the clinical disease and are subsequently immune. This form of age-related immunity does not occur with *T. parva* (Latif, 1992).

As chemical control is still the main method of tick control worldwide but has become unsustainable, it may jeopardise the envisaged target of revolutionizing livestock production to meet the needs of food security by 2020.

### ***Mechanical control: handpicking***

In certain East African pastoral communities, livestock is communally organized, brought together and held in a crutch facility where ticks are picked off the animals one by one and either buried or thrown into fire (Marina et al., 2001). This practice was also conducted during milking and cleaning of livestock sheds by women (Marina et al., 2001). Some ticks, after being picked from the respective host animals, were given to chickens/birds at home as a food supplement. However, this method is tedious, time consuming and involves much labour in order to serve a big herd of cattle and hence is not sustainable.

### ***Host grooming***

Today, there exists evidence to strongly support the concept that grooming (cleaning the fur of an animal), which occurs amongst animal communities, is beneficial (Hart, 2000; Park, 2008). In Africa it has been observed that many species of wild bovids live in tick infested environments but usually with low levels of tick loads. This is the result of a behavioural defence against infestations with ectoparasites such as ticks as was experimentally demonstrated and observed in free-ranging adult female impala (Mooring, 1995; Mooring et al., 1995;).

It is reasonable to assume that much of the vulnerability of cattle to ticks reflects a relatively reduced predisposition to groom, a behaviour that in turn stems from their derivation from European (and more recently from North American) stock, where the environment has been relatively free from ticks (Hart et al., 1996). Thousands of years of low exposure to ticks may have led to a selection against frequent grooming in favour of reducing the cost associated with frequent grooming. The grooming in many wild bovids observed in a number of studies provide evidence that systemic chemical cues (chemical signals affecting the whole body) may be modulating the grooming rate (Hart, 1997). Thus, there might be some way to enhance the grooming of European-derived cattle through appropriate administration of biological substances that are found to increase grooming in wild bovids. Given the potential value of grooming in the removal of attached ticks, another possibility is that cattle might be selectively bred (or genetically engineered) to show high rates of grooming and, hence, be less susceptible to tick-borne diseases. However, before embarking upon a costly and time consuming breeding programme, effective and protective values of grooming for tick control first needs to be ascertained.

In Trinidad, studies by Smith in 1974 showed that grooming was effective in reducing the infestation of cattle with the tick, *Amblyomma cajennense* Fabricius (Smith, 1975). In Kenya, adult female *R. appendiculatus* have been observed to die as a result of increased grooming by cattle because of increased dermal irritation by the ticks (Essuman et al., 1991). There have been studies to show that of the interspecific and intraspecific determinants that modulate grooming in animals, some of the intraspecific determinants appear

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<sup>2</sup>Parasitoids differ from parasites in their relationship with the host. In a truly parasitic relationship, the parasite and host live side by side with little or no damage to the host organism while the parasite takes enough nutrients to live on and reproduce without draining the host's reserves. In a parasitoid relationship, the host is usually killed after the full development of the other organism (parasitoid). This type of relationship seems to occur only in organisms that have fast reproduction rates (such as insects or mites) (Godfray, 1994).

to be mediated by systemic physiological influences, suggesting there may be ways to improve grooming activity in weak groomers, such as cattle (Hart, 2000).

### **Biological control**

Biological control of ticks is the use of natural enemies (parasitoids, pathogens, parasites and predators) that can reduce the density of the target population or even eliminate it. The development of biological control of ticks is more neglected than that of most other animal pests (Rutz and Patterson, 1990) and lags behind that of plant pests by several decades (Samish, 2000). In the literature, however, more than 257 tick biocontrol agents are mentioned, comprising 100 species of pathogens, seven parasitoids and 150 predators (Samish and Alekseev, 2001). The subject has been extensively reviewed by Hu et al. (1998) and Samish (2000), who shed some light on the way forward and reflect on the previous failures and successes. Only a few studies have as yet been conducted on pathogens, parasitoids<sup>5</sup>, and predators of ticks. A first remarkable field trial was made with the introduction of parasitic wasps originating from France in the USA in the late 1920s and early 1930s and in the early 1940s in Russia (Hu et al., 1998). However, in both the USA (Smith and Cole, 1943) and Russia (Alfeev, 1946) they were unsuccessful in controlling target tick populations, similar to a previous trial conducted in the USA between the years 1927 and 1932 (Cooley and Kohls, 1934). During the past decades, interest in developing biological methods for tick control using birds (Couto, 1994), parasitoids (Hu et al., 1998), entomopathogenic nematodes (Samish, 2000), entomopathogenic fungi, arthropods (Samish and Alekseev, 2001) and bacteria (Hassanain et al., 1997) has gained momentum worldwide, because of the limited impact of these organisms on the environment.

### **Host resistance**

Cattle host resistance— a measure of the host's ability to limit the establishment, growth rate, fecundity and/or persistence of a parasite population (Coop and Kyriazakis, 1999), has been reported from numerous studies that zebu (*B. indicus*) and sanga (a *B. taurus* and *B. indicus* cross-breed) cattle, the indigenous breeds of Asia and Africa, usually carry significantly fewer ixodid ticks than exotic European (*B. taurus*) breeds of cattle (Utech and Wharton, 1982; Aiello and Mays, 2003). It has been shown that tick infestation increases as the proportion of European genes in an animal increases (Lemos et al., 1985). Studies have also shown that the magnitude of losses due to tick infestation varies with the genotype of cattle (Lemos et al., 1985). Within a genotype, losses per tick unit increase with the number of attached ticks (Pegram et al., 1989a; b). The tick resistance of zebu breeds and their crosses is increasingly being considered for exploitation as a means of control of tick ectoparasitic stages on livestock. The introduction of zebu cattle to Australia has positively revolutionized the control of *B. microplus* on that continent as zebu breeds were successfully exploited in cattle breeding programmes to develop tick resistant cattle breeds that limited the impact of *B. microplus* infestation (Seifert, 1984). Use of resistant cattle as a means of tick control is also becoming important in Africa, Asia and the Americas (Fraga et al., 2003; Silva et al., 2007).

Host resistance, reviewed by Latif and Pegram (1992), manifests itself as the rejection of ticks that attach to the host because of host-specific physiological and immunological reactions. Highly resistant cattle keep overall tick populations very low in contrast to cattle with low resistance in the same herd that harbour more ticks in certain seasons (Solomon and Kaaya, 1996). Cattle can be ranked for resistance on the basis of natural tick counts and about 10% of cattle ranked as of low resistance carrying 50% of the total tick population infesting the herd (Latif et al., 1991). Comparison of the respective tick infesta-

tion on calves and cows showed that calves had a lower tick infestation than cows (Jongejan et al., 1987). In most parts of Africa studied, zebu and sanga cattle are found to be considerably more resistant to TBDs (Tatchell and Easton, 1986; Bakheit and Latif, 2002) and the application of intensive dipping with the purpose to increase weight gain is therefore not justifiable (Norval et al., 1992). The use of naturally tick-resistant cattle biotypes should be incorporated in tick control schemes as a means to contribute to the control of tick infestations on livestock (Silva et al., 2007). Although assessing the levels of host-resistance in different breeds of cattle by selection, breeding or gene alteration (de Castro, 1991) may take some time, the feasibility of this method has been demonstrated at the International Centre of Insect Physiology and Ecology, Kenya (Latif, 1992).

### **Vaccination**

A novel approach of tick control is to make vaccines against the vectors rather than against all the individual disease agents they carry (Labuda et al., 2006). Previous studies have shown this approach to be feasible (Burke et al., 2005; Labuda et al., 2006). Research has focused on identifying proteins from the whole tick macerates, the ticks' salivary glands and digestive tract as candidate broad-spectrum tick vaccine targets (Trimnell et al., 2005). Efforts have so far yielded potential vaccines against *Boophilus spp.* (Willadsen et al., 2006). Prospects of developing similar vaccines against other ixodid ticks of major veterinary importance have not been forthcoming. *Boophilus spp.* are one-host ticks and show a marked preference for bovine hosts, which act as the principal reservoir of perhaps the most important group of disease agents (*Babesia spp.*) that *Boophilus* ticks transmit. By contrast, most other ticks of medical importance are three-host ticks, which infest not only cattle but also wild ungulate species. For these reasons, vaccines against non-boophilid ticks may not be feasible and a development of the near future. Anti-tick vaccines, however, remain one of the most promising prophylactic measures against tick bites and transmission of tick-borne pathogens (de la Fuente and Kocan, 2006).

### **Integration of semiochemicals in tick management**

Unlike in insects where pheromones have been used extensively to control certain crop and orchard pests (Judd and Gardiner, 2005), a semiochemical-based strategy has made little headway in the control of ticks and, where attempts have been made, it is only on trial basis. For example, CO<sub>2</sub>, which is found in vertebrate breath, acts as a tick kairomone, and has been tested for sampling and control under different synthetic preparations such as dry ice, compressed gas (Gray, 1985). Similarly, sex pheromones have been evaluated in a number of ways. Norval et al. (1989b) evaluated a combination of CO<sub>2</sub> with o-nitrophenol (ONP) in the field. According to these workers, increased catch of *A. hebraeum* was obtained by the activating effect of CO<sub>2</sub> in synergy with the inherent attractant action of the pheromone. Other attempts have explored the use of pheromone/acaricide-treated areas of the host. This method proved to be very effective in killing ticks that were lured by the baits (Rechav and Whitehead, 1978). A significant progress in the latter approach involves the use of impregnated objects or decoys with a slow release delivery system (Sonenshine et al., 1992). Another technique, which has achieved some degree of effectiveness, is the confusant-killing method tested by Ziv et al. (1981). These authors incorporated the 2,6-DCP-sex attractant pheromone in gelatine microcapsules (applied to host's fur) and mixed with a pesticide (Propoxur) against the dog tick, *Dermacentor variabilis*. As a result, a majority of lured males were killed, while surviving females were left unmated by the induced pheromone-permeated background. This successful disruption of mating was achieved with 5.6 µg/ml of microencapsulated 2,6-DCP. Mixtures of an acari-

cide with an aggregation pheromone or a sex pheromone impregnated on plastic decoys were among other options showing promise. Hamilton and Sonenshine (1989) have patented a decoy coated with natural Mounting Sex Pheromone (MSP) and a pesticide. The decoys dispersed on the hair coat of the host were found highly lethal to males within 30 minutes and mating attempts by males with decoy resulted in 89% of death (Sonenshine et al., 1992). A modified method of the above was tested by Norval et al. (1992) who used plastic bands impregnated with components of the attractant-aggregation-attachment pheromone (AAP) mixed with the pesticide flumethrin. The poisonous bands were attached to the tails of the cattle and as a result, there was a good killing of ticks as those attracted aggregated around the animal's rear and came into close contact with toxic chemicals.

The combination of an attractant and acaricide may not sound as ecologically suitable for a pesticide-free environment. However, since this method utilizes a behavioural trait that occurs regularly and predictably, its advantage lies on a reduced amount of acaricides being used and their tactical application on the host (Sonenshine, 2006).

## **Cultural control**

### ***Habitat interference and host removal***

Habitat interference and host removal can be directed against both the free-living and parasitic stages of ticks (Aiello and Mays, 2003). The free-living stages of most tick species, both ixodid and argasid, have specific requirements in terms of microclimate and are restricted to particular microhabitats within the ecosystems inhabited by their hosts. Destruction of these microhabitats reduces the abundance of ticks. Alteration of the environment by removal of certain types of vegetation has been used in the control of *A. americanum* in recreational areas in south-eastern USA and in the control of *Ixodes rubicundus* Neumann, 1904 in South Africa. Control of argasid ticks such as *Argas persicus* Oken, 1881 and *A. walkerae* Kaiser and Hoogstraal, 1969 in poultry can be achieved by eliminating cracks in walls and perches, which provide shelter to the free-living stages.

Removal of alternate hosts or hosts of a particular stage of the life cycle can also reduce the abundance of tick species as this may starve ticks to death depending on starvation period (Aiello and Mays, 2003). This approach has occasionally been advocated for the control of three-host ixodid ticks such as *R. appendiculatus*, *Amblyomma hebraeum* Koch, 1844 and *I. rubicundus* in Africa, and *Hyalomma spp* in south-eastern Europe and Asia. In Kenya, the Somali community uses this method as one of their tick control strategies (Pers. Commun. with Somali pastoralists).

Rotation of pastures or pasture spelling has been used in the control of the one-host ixodid tick *Boophilus microplus* Canestrini, 1888 in Australia (Sutherst et al., 1979). The method could also be applied to other one-host ticks in which the duration of the spelling period is determined by the relatively short life span of the free-living larvae. However, it has minimal application to multi-host ixodid ticks or argasid ticks because of the long survival periods of the unfed nymphs and adults. Burning grazing pastures directly kills ticks, while ploughing grazing fields buries them and eventually they die.

### ***Ethnoknowledge in tick management***

Traditional preventive management measures and practices employed in livestock tick control and management have long been used by various ethnic groups engaged in traditional animal husbandry. These measures were aimed at reducing the animals' risk of infestation and have included the following. (1) Practicing appropriate herd distribution, in which,

certain areas were used only by cattle and small ruminants, or only by camels depending on the varying spread of tick infestation specific to particular types of animals (Marina et al., 2001). (2) Avoidance of specific pastures at particular times of the day or year, or throughout the year. This has involved areas with a large population of ticks, mosquitoes and livestock-biting flies during the rainy season, which are only used for grazing during the dry season or not at all. Areas infested with ticks in various stages of development (e.g., abandoned paddocks) are avoided for a number of months (Mathias-Mundy and McCorkle, 1989). (3) Keeping livestock away from shady areas that may provide micro-habitats for ticks in pastures (Mathias-Mundy and McCorkle, 1989). (4) During migratory herding, areas infested with ticks and other parasites are avoided at the times of year when the population is at its largest (Sutherst 1987, Sykes 1987). (5) Burning of pastureland suspected to be infested with ticks in various stages of development (West, 1965; ITDG and IIRR, 1996). (6) Feeding animals on a certain type of soil nutrients to prevent tick infestation (Marina et al., 2001). (7) Hanging a bouquet of flowers/leaves at the doors, windows and in the roofs of cattle shade (boma) and growing certain plant species with repellent properties around the boma (ITDG and IIRR, 1996). (8) Selective breeding of livestock with particularly high resistance to tick vectors thus, making it possible to introduce a particular livestock species into areas where it could not be kept in the past (Sutherst 1987). (9) Avoiding common grazing ground with wild animals serving as a "reservoir" for pathogens and causing particular tick-borne epizootic diseases and ticks per se (Mathias-Mundy and McCorkle, 1989). And (10) Bush clearance to keep the tick population low by destroying their micro habitats, particularly those close to bomas or frequently visited grazing grounds (Marina et al., 2001).

In livestock herds infested with ticks, a number of methods are used involving the release of predator chickens and birds, kitchen ash mixed with fresh grass, smoke directed in the herd or boma and application of various ethnobotanical preparations as concoctions, powder/dust, bolus, paste, oil etc. In addition, handpicking of ticks was conducted as a communal cultural practice to reduce tick burden on heavily infested animals (Mathias-Mundy and McCorkle, 1989). Of all these methods, applications of ethnobotanical products play a major role in tick control and management. For example, in Kenya, the Somali people take heavily infested herds with ticks to graze in vegetations with *Commiphora spp.* which have repellent effects and cause ticks to get detached from their animals (Beentje, 1994). This traditional claim has been confirmed by Maradufu (1981).

Pastoralists in the rugged, harsh terrain of northern Kenya are increasingly using indigenous medicine rather than modern veterinary services to care for the health of their livestock. Instead of relying on regular dipping and spraying with acaricides, which are expensive and out of their reach or unavailable in such isolated areas, the herders use locally available herbs to control ectoparasites, particularly ticks. Surveys and field trials carried out in the Samburu and Turkana communities of Kenya by the Intermediate Technology Development Group-Eastern Africa (ITDG-EA), revealed that these two pastoral communities use a combination of three traditional herbal remedies for tick control, namely, tobacco (*Nicotiana tabacum* L.) mixed with sodom apple (*Solanum incanum* L.), labai (*Psidium punctulata* (DC) Vatke.) mixed with Aloe (*Aloe secundiflora* Engl.) and Africa olive tree [*Olea europea* (L.) ssp. *africana* (Vent.) P. Green] mixed with Ilkerereai (*Cordia purpurea* (G. Piccioli) Aiton) The results from these field trials were promising, as no tick (*R. appendiculatus*, *R. evertsi*, *B. decoloratus* and *A. variegatum*) could be found on the cattle with a mixture of either *A. secundiflora* and *P. punctulata* or *A. secundiflora* and *O. europea* 4 days post-treatment (Akall, 2003). However, in all the field trials, cattle treated with these traditional anti-tick remedies, had tick reinfestation starting on the tenth day

post-treatment. Previous work at ICIPE involving the interaction of local communities and conventional researchers indicated that components of many plants could act as allomones for some local livestock tick species (Chiera et al., 1977; Malonza et al., 1992; Mwangi et al., 1995a, b; Mwangi, 1996; Ndung'u et al., 1995; Lwande et al., 1999). It was observed that *R. appendiculatus* preferred to ascend drier vegetation than a fresh green one, possibly because of an unknown allomonal factor(s) (Mwangi et al., 1995b). Some tropical legumes, especially those of the genus *Stylosanthes* spp. have been reported to exude sticky substances from their trichomes that trap and kill ascending larvae of *B. microplus* ticks (Sutherst et al., 1982; Zimmerman et al., 1984; Sutherst and Wilson, 1986; Wilson and Sutherst, 1986; Sutherst et al., 1988; Wilson et al., 1989). In addition to these trichomes, glandular emanations of *Stylosanthes* spp. were also found to kill ticks (Sutherst et al., 1982; Norval et al., 1983). *Melinis minutiflora* has also been identified to have strong anti-tick properties (Menendenz, 1924; Chiera et al., 1977; Thompson et al., 1978; Mwangi et al., 1995a, b). Although it has been conclusively shown that crude extracts from a number of plants have acaricidal, repellent and growth regulating properties against a variety of livestock tick species, the active compounds and blends are yet to be characterized and evaluated in both laboratory and field under varying conditions in order to allow their integration into the strategies of tick control and management.

### Current strategies and views on tick management

Initially the main uses of acaricides were tick eradication, prevention of spread of ticks and tick-borne diseases and eradication and control of tick-borne diseases. The eradication programmes were successful in some ecologically marginal subtropical areas, such as southern USA and central Argentina where *Boophilus* spp. and babesiosis were eradicated and southern Africa where East Coast fever (caused by *Theileria parva parva*) was eradicated. The programmes were less successful in the tropical areas of north-eastern Australia, Central America, the Caribbean Islands, and eastern Africa.

In the areas where ticks and tick-borne diseases eradication programmes were not achieved, the costs of maintaining intensive tick control programmes have become prohibitive. For this reason, integrated control strategies are being encouraged. From previous experiences, the future of livestock tick control and management lies in the integration of the available classical and modern technologies (indigenous knowledge, vector and host semiochemicals, host-vector associations, tick biocontrol agents, host grooming behaviours, host genetics, chemicals, vaccines, ecological, immunological and the management of acaricide resistance), as it seems unlikely that any of the technologies (as reviewed above) is robust enough to stand alone (Willadsen, 1997; Grossard, 1998). The effectiveness of these cost-containment strategies requires better knowledge of the dynamic associations among the disease agents, their vertebrate hosts, the tick vectors and the environment. Control of tick-borne diseases will require the utilization of the principles of endemic stability and development of improved recombinant vaccines.

Strict quarantine measures need to be enforced in order to prevent re-introduction of ticks and tick-borne diseases in countries where they have been eradicated. Climate-matching models, geographic information systems, and expert systems (models based on expert knowledge and artificial intelligence) are being used to identify currently unaffected areas in which ticks may establish if introduced (Aiello and Mays, 2003). Integration of community-based ethnobotanicals in tick control and management is increasingly being recognised as an effective means of livestock tick management at local level (ICIPE's livestock tick control and management strategy, 2002-2003).

## Statement of problem

Although the application of traditional knowledge in tick control and management is widespread, every community has its own specific and unique knowledge. For this knowledge to be useful, each community therefore, needs to develop its own knowledge-derived practice within its cultural framework. However, as much as this realization exists, a considerable amount of this indigenous knowledge is fast disappearing, while still in undocumented and unevaluated forms because of: (1) untimely deaths of persons with ethnoknowledge, (2) extinction of plant and animal species, the sources of the much needed ethnopharmacologically active substances, (3) encroachment of development and modernization, (4) adoption of new lifestyles and education systems that do not embrace indigenous knowledge, (5) intermarriages, (6) changes in farming systems, (7) changes in human settlement patterns, (8) religious beliefs and western faith that label African ethnoknowledge as inferior, (9) exposure to foreign cultures, which believe that western type of products are always superior to domestic ones, and (10) failure of governments/states to support and develop a legal framework and clear working policy for sustainable utilization of indigenous knowledge (Munyua et al., 1998).

Much as the aforementioned information reveals the importance of indigenous knowledge as well as the dangers and threats facing it, a lot of it is only known to few individuals (healers, traditional medicine experts, village elders, spiritualists, herbalists, ritualists) in their respective communities (Martin, 2004). The results of some documented and evaluated indigenous knowledge lies on the shelves of libraries and laboratories in many institutions and therefore not many outside people know about it or use it. Effective use of indigenous knowledge and its products have been affected by poor formulation, packaging, storage, standardization and determination of optimal concentration and application regimes (Martin, 2004). These problems are compounded by the facts that ethnopractitioners do not receive formal training and their informal education is never systematic, documented and standardized and ethically accepted by all stakeholders in the community. This has raised fears of overdosing and poisoning leading to death and other undesirable side effects amongst the users. From a practical point of view, therefore, identification, documentation and scientific evaluation of ethnobotanicals with anti-tick properties available to and used by different communities and the way they affect target tick species, may provide an understanding of the underlying science so that better ways of using effective ones are developed and improved to benefit the local communities (Fig. 1.7). Moreover, the strategic deployment of anti-tick ethnobotanicals that takes into account on-host behaviour of target tick species, and particularly their feeding site location behaviour, may open up effective, efficient, simple, accessible and sustainable methods of controlling target tick species suitable for different rural communities with minimal external input.

### *Why study the Bukusu community in western Kenya?*

Research in ethnoveterinary knowledge is mainly focused on pastoralists. Workshops held in Kenya in 1996 (ITDG and IIRR, 1996) and in India in 1997 (McCorkle et al., 1997) challenged this focus and revealed that many arable farming communities such as the Bukusu once led a pastoral life and therefore possess a large amount of ethnoveterinary knowledge just like the pastoralists. Indeed the Bukusu community has a vast amount of knowledge about farming practices, rich pastoral vocabulary and the broad variety of legends connected with pastoral life (Makila, 1978).

Secondly, during my childhood I watched my late maternal and paternal grandfathers apply several ethnopractices on tick-infested cattle, including the use of plants as

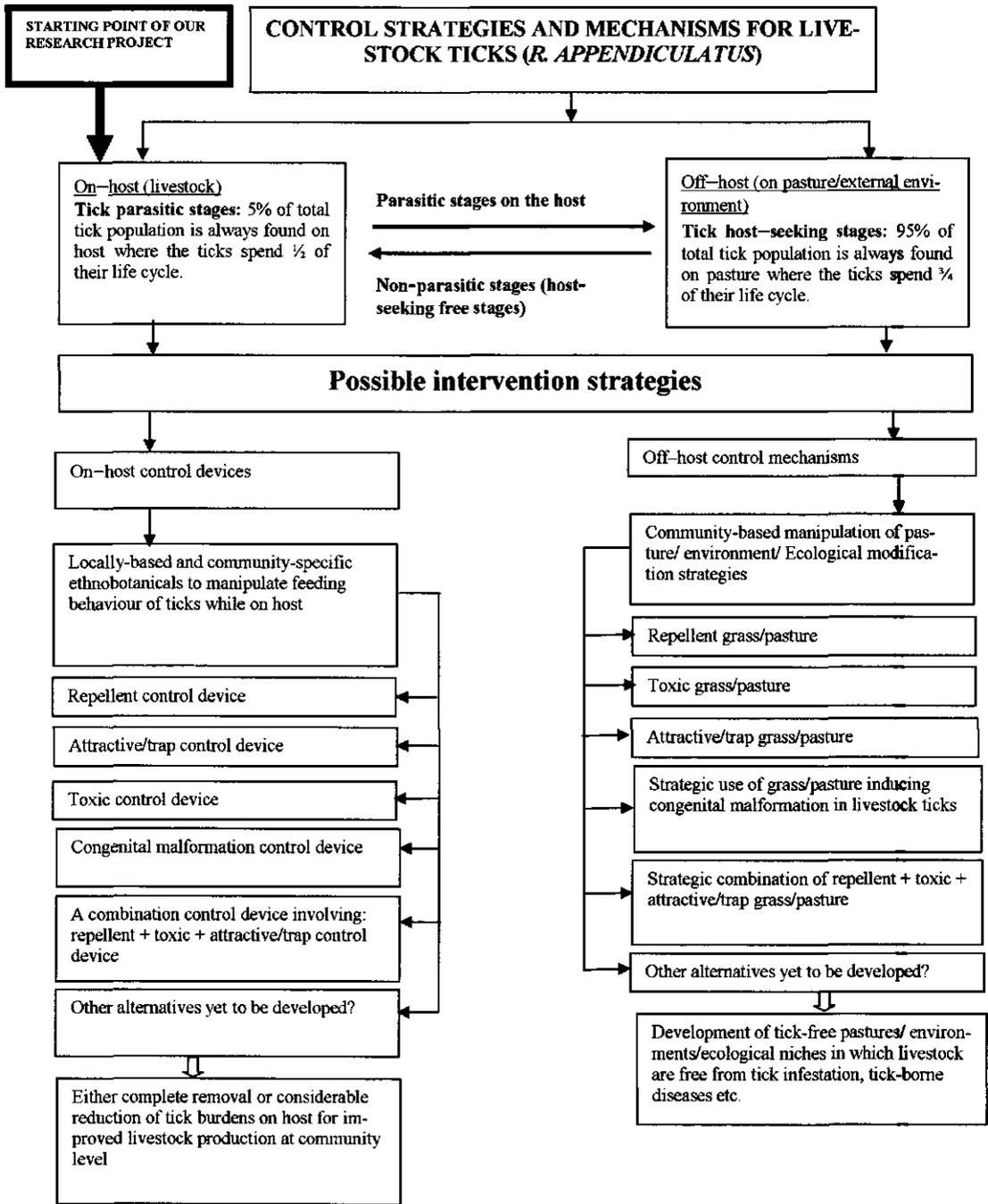


Fig. 1.7. Possible interplay of factors suitable for the development of on-host and off-host community-based integrated tick control and management strategies, which are effective and sustainable under local

biological acaricides, in the Bukusu community. These could be applied as dusting powders, pastes, juice extracts, and decoction substances. Other plant treatments included bolus or infusions or smoke. I would often accompany my grandfathers on their consultation missions in the neighbourhood. They were fondly known as *Abalesi ba efiayo*, literally meaning 'the maids of the livestock', which translates to mean persons who provide primary healthcare to the animals in the village and are consulted in major cases involving livestock ill-health. As a result, ethnopractices caught my eye. I was fascinated by the rich repertoire of traditional knowledge and techniques applied in almost all spheres of people's lives, be it in agriculture, healthcare, delivery of children, or in livestock or fisheries. And therefore, the prerequisite familiarity with the research community when one is beginning to develop a career in ethnobotany was fulfilled and took up the challenge (Martin, 2004).

#### ***Why start first with on-host and not off-host approach?***

This question stems from the information illustrated in the conceptual framework of the project in Fig. 1.7 as to which of the two alternative approaches is appropriate to start with. One need just to imagine what may happen if we started this research project with off-host tick control and management approach and we neither kill 95% of ticks always present in the host environment nor have in place appropriate on-host control and management strategies and mechanisms! Ticks and tick-borne disease epidemics and related problems may develop and wipe out large numbers of herds of cattle within a short period of time. Being guided by our research question regarding the type of tick control technology that should be developed and put in place for rural livestock farmers to use, we wanted to know what the local communities know about tick control strategies and mechanisms (ethnopractices for tick control and management) and how what they know can best fit in an on-host tick control tactic.

#### ***Why local community-driven tick control intervention?***

In the past, many effective tick control interventions have been implemented and failed. One reason for this scenario is that the end users (the rural local community livestock farmers) together with their centuries' old useful ethnoknowledge are not involved in developing, planning and execution of such interventions. The current project considers this as a priority. It will be easier and cheaper to implement any new technology for tick control and management within the local communities because of prior familiarity and acceptability of local materials and ethno-knowledge used if the approach is community-driven. This will make sense only if the people themselves together with their ethnoknowledge are directly involved in the project. Secondly, they will learn and train to use their own ethnoknowledge in a better way and will not require external personnel to train them on how to use the technology after its development. Thirdly, planning and evaluation of animal health programmes require a clear understanding of both the epidemiology of the diseases in question and the livestock production systems involved (Putt et al., 1988), which are clearly understood in rural communities who are the majority livestock keepers (Kariuki, 1996). Such an understanding can only be achieved through the availability of reliable and up-to-date information at all stages of the planning and evaluation processes, which involves close interaction of rural communities and researchers.

## Objectives of the studies

### *Main objective*

The work under taken in this thesis had as its main aim to evaluate ethnopractices affecting livestock ticks from the Bukusu community in western Kenya and investigate their potential, strategic use and, in the longer term, integration with the knowledge of on–host behaviour of the brown ear tick *R. appendiculatus* for developing an effective on–host tick control and management strategy.

### *Specific objectives*

1. To survey, document and evaluate potential anti-tick ethnobotanical(s) and other practices as used by the Bukusu community in western Kenya (Chapter 2).
2. To extract essential oils from selected plants, evaluate their effects on adult *R. appendiculatus* and determine their chemical compositions (Chapters 3 and 5)
3. To study the effects of the essential oils at the feeding sites on the navigation and attachment behaviour of *R. appendiculatus* (Chapters 4 and 6).
4. To evaluate an on–host “push” tactic with the repellent ethnobotanicals for the control of *R. appendiculatus* (Chapters 7 and 8).

## Hypotheses

1. The selection of certain plants for tick control in traditional rural communities is based on trial and error process, which has led to the discovery of effective ethnobotanicals. Thus, there is an empirical rationale for their current use.
2. The location of preferred feeding sites of ticks on the host is mediated by host-derived semiochemicals. It is possible to manipulate these behavioural patterns through the appropriate deployment of selected repellent ethnobotanicals leading to the prevention or reduction of tick bites on cattle.
3. The deployment of traditional tick control practices in the current strategies of integrated tick control and management may significantly be improved if well integrated with scientific knowledge of on–host tick behaviour.

## Outline of the thesis

**Chapter 1.** The general knowledge about the vector, the host and the pathogen is reviewed, while focusing on the control and management strategies of the vector, *R. appendiculatus*. The research questions together with the justification of the research, objectives and hypotheses are stated.

**Chapter 2.** In the recent past, research into ethnoveterinary knowledge is focused on pastoral and nomadic communities and neglected arable and mixed farming communities. In this chapter, a survey of indigenous anti–tick knowledge of the Bukusu, who combine both arable and pastoral life forms for economic survival, is presented. About 154 candidate anti–tick plant species spread over 110 genera and 51 families were identified and documented, together with 10 non–botanical anti–tick ethnoagents. Following an extensive literature search on these plants, eight plants are selected for laboratory and field studies.

**Chapter 3.** This Chapter comprises laboratory assays of essential oils extracted from the eight selected plants (*Tagetes minuta*, *Tithonia diversifolia*, *Juniperus procera*, *Solanecio manii*, *Senna didymobotrya*, *Lantana camara*, *Securidaca longepedunculata* and

*Hoshundia opposita*). Repellent effects of these essential oils on adult *R. appendiculatus* are evaluated using a dual choice climbing assay. The essential oils of two plants, *T. minuta* and *T. diversifolia* with a relatively high repellent effect on adult *R. appendiculatus* are selected for an indepth scientific study, comparison and chemical characterization using GC and GS-MS technology.

**Chapter 4.** In this Chapter, I describe the behavioural biology of the vector on two Friesian steers. On-host behaviour of adults of two sympatric tick species, the brown ear tick (*R. appendiculatus* Neumann, 1901) and the red-legged tick (*R. evertsi* Neumann, 1897), which prefer to feed mainly inside the ears and the anal regions of bovids, respectively, is studied. Up to 95% of *R. appendiculatus* and 85% of *R. evertsi* oriented toward and located their respective feeding sites from different parts of the host body. An odour-based 'push-pull' pair of stimuli is suggested to account for efficient orientation and navigation behaviour of the two tick species to their respective predilection feeding sites. It is hypothesized that such concurrent deployment of repulsive and attractive cues may be quite widespread among arthropods and related organisms that specialise on specific hosts or microenvironments in the performance of their biological functions.

**Chapter 5.** A comparative study is made between a dual-and no-choice assay arrangement on the climbing response of adult *R. appendiculatus* exposed to the essential oil of *T. minuta*. Both assays show a significant repellent effect of essential oil on climbing behaviour of *R. appendiculatus*. However, in contrast to our predictions, using a no-choice tick climbing apparatus during the assay did not increase the sensitivity. The dual-choice assay proves a more sensitive assay than the no-choice assay, although the no-choice assay provides greater residual effects.

**Chapter 6.** In this Chapter I discuss the on-host behaviour of *R. appendiculatus*, which was studied in the semi-field experiments to evaluate the repellent effects of the essential oils of *T. minuta* and *T. diversifolia* at its predilection feeding site. This is amongst the few studies in which tick repellent essential oils are evaluated in the presence of host-derived semiochemicals. The disruption effects of the two essential oils on the dispersal, orientation and attachment behaviour of the ticks are compared. The previously-suggested mediation of specific host-derived attractive and repellent orientation and navigation signals are masked by the essential oils as initially hypothesized. Both essential oils therefore may offer potential for inclusion in the integrated tick control and management particularly following the laboratory and field studies of individual constituent compounds and selected blends.

**Chapter 7.** In this Chapter, the site on the host animal and a delivery format suitable for application of essential oils were evaluated. Further, this in vivo study also compared the repellent ability of the two essential oils of *T. minuta* and *T. diversifolia* in intercepting the movement of *R. appendiculatus* toward its predilection feeding site. For both the essential oils, legs + tail sites of essential oil application, followed by ear smear and then ear tube, had significant effects on orientation to the host and attachment site preference of adult *R. appendiculatus* on the host animal, in that order. As treatment of legs + tail is tedious, time consuming, and requires more essential oil than the other two sites, we recommended the ear smear site for treating host animals with essential oils for field experiments in Chapter 8.

**Chapter 8.** In this Chapter, I provide the results of a field study that evaluates the effects of the essential oil of *T. minuta* and *T. diversifolia* on *R. appendiculatus* infesting livestock in Bungoma District, western Kenya. The ear smear site for treating host animals with essential oils as recommended in Chapter 7 was used to study a "push" tactic for on-host control of *R. appendiculatus* in the field. Within 1-4 days post-treatment, the number

of ticks on animals treated with the essential oils was reduced by more than half that of the original population. By the 5th day post-treatment, more than 75 and 60% of adult *R. appendiculatus* and other tick species, respectively, were affected by the essential oils so that they 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*. Both *T. minuta* and *T. diversifolia* essential oils affected several other less dominant but economically important tick species such as *Amblyomma variegatum*, *R. evertsi* and *Boophilus spp.*, although these tick species have different feeding sites. The results suggests potential for appropriate essential oil formulations in reducing tick burden and associated tick-borne diseases among the resource-limited livestock farming community in tropical Africa.

**Chapter 9.** In the final Chapter, I discuss the circumstances surrounding the tick control mechanisms. The discussion focuses on the question: why the livestock tick is still a serious pest in livestock industry after more than 150 years of tick research? Can the integration of plant-derived repellents in tick control and management strategies provide a solution to the chronic tick-borne problems in livestock industry? The discussion reflects on the results obtained from this research, pinpointing the general contribution made and the specific gaps in knowledge and need for follow up research and development. Relevant conclusions based on the research results are outlined.



## **Part I**

# **Use of ethnobotanicals for tick control**



## 2

**Traditional knowledge on tick control within the Bukusu community in Bungoma District, western Kenya<sup>1</sup>**

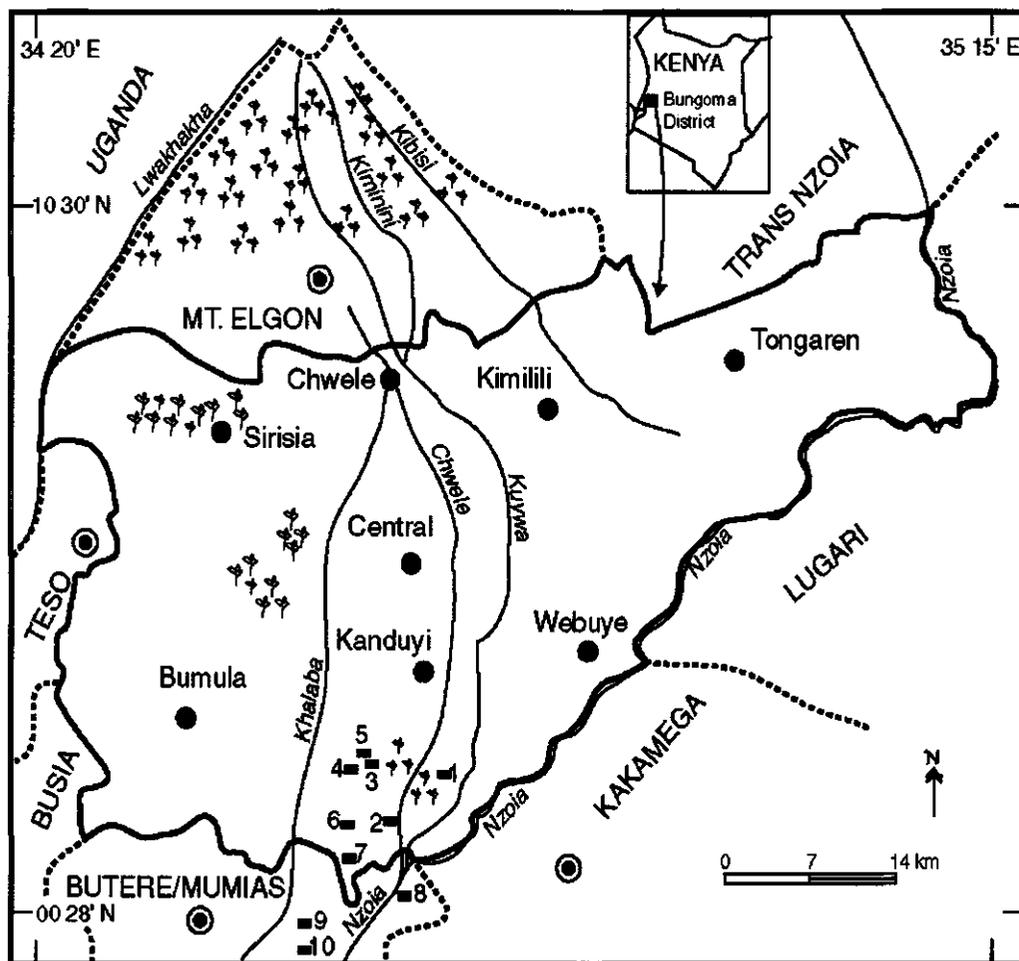
**Abstract:** In the recent past and currently, research into ethnoveterinary knowledge is focused on pastoral nomadic communities and neglected settled arable and mixed farming communities such as the Bukusu. We conducted a survey of indigenous tick-management knowledge of the Bukusu, who combine both arable and pastoral life forms for their economic survival, used participatory action research approaches involving 272 women and men aged between 18 and 118 years. The information was collected from a school-based questionnaire, group discussions, and individual interviews as well as discussions with some key respondents, veterinarians and field staff from the Ministry of Agriculture, Rural and Livestock Development. Traditionally, ticks have been controlled and managed using a variety of methods, such as applying ethnobotanicals, hand picking, burning pastures, livestock quarantine, grazing practices, cleaning cattle sheds, burning or burying residues of cattle shed, bird predation, feeding animals on natural salty soils locally called '*silongo*', and applying kerosene, soap, fish residues, cattle dung and urine, grease, ash, magadi soda, and sisal juices on cattle. Ethnobotanicals have been applied by a variety of methods, e.g., fumigation, pouring a decoction on the animal, steaming or dusting the animal's skin, hanging plant bouquets in cattle sheds, rubbing a bolus or paste on the animal, and grazing animals in pasture of identified anti-tick plants. About 154 candidate anti-tick plant species spread over 110 genera and 51 families were identified and documented, together with 10 non-botanical anti-tick ethnoagents. From an extensive literature search, we found that 11 of the 154 plants documented had been scientifically evaluated elsewhere and found to have either acaricidal, insecticidal or bioactive ingredients or were closely related to plants with acaricidal or tick-repellent properties. This information was crucial in providing leads to identifying suitable acaricidal or tick-repellent botanicals available in different local communities and probable scientific rationale underlying their use.

**Key words:** Bukusu, traditional tick control and management, ethnoknowledge, ethnobotanicals, livestock industry, ticks

**Introduction**

Bungoma District is located in Western province of Kenya between latitude 00°28' and 10°30'N and longitude 34°20' and 35°15'E (Fig. 2.1). The District occupies an area of 3 074 km<sup>2</sup>, with a population of about 2 million people on the southern slopes of Mt. Elgon. It lies in an agro-ecological zone stretching from tropical alpine zones to lower mid-land

<sup>1</sup>This chapter will be published as: Wanzala, W., Takken, W., Pala, A.O., Mukabana, R.W., Hassanali, A., Traditional knowledge on tick control within the Bukusu community in Bungoma District, western Kenya, when submitted.



- Key:
- Collection sites of plant samples in Bungoma District (●) and neighbourhood (⊙)
  - Sampled primary schools (1 – 10) used to identify key respondents
  - Forested mountain/bushy hills (✦✦)
  - Rivers/streams (~~~~)

Fig. 2.1. The location of Bungoma District in Kenya. Note the positioning of the District within the Lake Victoria Basin and towards the southern slopes of Mt. Elgon.

zone with tea, wheat/maize–pyrethrum, coffee, sunflower–maize, sugarcane and cotton plantations (Martina, 1998). The predominant *off-farm* vegetation patterns are riverine forests, rocky forested hillsides, hedgerows, wooded grassland relicts, woodlands or col-line forest relicts and tree groves whereas the noticeably tree-rich *on-farm* management units are home gardens, homesteads, live fences, coffee- and banana-groves and annual cropping fields (Martina, 2001). The location of the District on the slopes of Mt. Elgon

influences rainfall and mitigates temperatures. Average annual rainfall ranges from 1 600–2 000 mm, while mean annual temperatures in the southern parts, away from the mountain, are about 21–22 °C and in northern areas closer to the mountain are in the range of 5–10 °C because of altitude. The District is within the Lake Victoria Basin, rising from 1 200 m above the sea level in the West and Southwest to over 4 000 m a.s.l. to the North. The slopes of Mt. Elgon are generally gentle, although in some areas they rise abruptly in the form of cliffs up to 70 m in height and are cut by deep river gorges with frequent waterfalls. The rest of the area consists of a gently sloping surface falling from 2 100 m elevation in the northeast to 1 200 m elevation in the southwest. The surface consists of wide, nearly flat land separated by shallow river valleys. Several inselbergs and ranges stand above the general level, forming the Kavujai Hills, Lucho Hills, Sang'alo Hills, Mwibale and some small hills, especially around Sirisia Division. The Nzoia River and its tributaries, Chwele, Khalaba, Kibisi, Kuywa, Kimilili, and Lwakhakha mainly drain the District. All the streams, except the Nzoia River originate from Mt. Elgon (Fig. 2.1).

Of the 19 ethnic groups of the Luhya people in western Kenya, the Bukusu community holds livestock keeping/farming and livestock per se in high esteem, as livestock is used as a measure of one's wealth and the animals' blood and meat are required and highly valued in all their traditional and cultural practices. Examples include funeral ceremonies (where cattle skin is used as a coffin), circumcision, agricultural festivals, bride wealth exchanges, and during cleansing ceremonies and sacrifices to appease the spirits.

In the recent past and currently, research into ethnoveterinary knowledge is focused on pastoral and or nomadic communities whereas settled arable and mixed farming communities such as the Bukusu have been neglected. Indeed, almost all of these neglected communities, the Bukusu included, largely led nomadic lifestyles before settling down to either arable, or mixed arable/pastoral farming systems (Makila, 1978). They therefore have ethnoveterinary knowledge that is just as rich as nomadic communities preferred in the ethnoveterinary research (ITDG and IIRR, 1996), making the Bukusu community ideal for current ethnoveterinary research. Useful ethnoveterinary information has not been documented to date in many communities such as the Bukusu and is at risk of being lost (Kofi-Tsekpo and Kioy, 1998).

In this study, we discuss the results of a survey conducted to document and analyse anti-tick ethnoveterinary practices of the Bukusu community in western Kenya. The study further helps to demonstrate the link between plant biodiversity and livestock healthcare ethnoknowledge systems in the Bukusu community.

### **The Bukusu community**

Bungoma is a district including a wide diversity of populations in western Kenya. This is partly due to its establishment as the last stop on the Kenya–Uganda railway in the 1920s, rapidly growing into a complex commercial and colonial and post-colonial administrative center along the railway line (Port, 2000). A majority of the residents of Bungoma belong to the indigenous Bukusu, who live near the border with Uganda, along the southern slopes of Mt. Elgon ever since their migration from the Sudan/Egypt border during the second half of the last century (Were, 1967; Makila, 1978). The Bukusu community is the largest single ethnic group of the 19 ethnic groups (sub-tribes) of the Luhya people of interlacustrine Bantu group of East Africa, with 16.35% of the Luhya population (Central Bureau of Statistics, 1996). The Community speaks the Bukusu language, whose current diversity of pronunciations and intonations, and even the lexical varieties, has been heavily influenced geographically and with time by immigrants of both Bantu and Nilotic origin (Makila, 1978; Wandibba, 1998; Roach, 2003). It has been observed that the Bukusu continue to

maintain a strong attachment to their traditions, cultures, and superstitions (Corbit and Wanyama, 2004). However, in spite of these observations, there is some evidence that there is a growing erosion of the value of the Bukusu ethnoscience knowledge (Wandibba, 1998; Roach, 2003; Corbit and Wanyama, 2004). This is believed to be the result of a combination of factors: (1) deaths of persons with ethnoknowledge, (2) extinction of plant and animal species and ritual practices, (3) encroachment of development and modernization, (4) adoption of lifestyles and education systems that do not embrace ethnoknowledge, (5) bias in religious beliefs, (6) perception of certain socio-cultural practices as unhygienic, and (7) expenses and risks involved in socio-cultural practices. Moreover, to date, little written information is available, little or no information is being passed on to the current generation and there is a lack of generalized use of this ethnoknowledge across the Bukusu community. This situation underlies the importance of the present study.

In the Bukusu community, plants were historically and are still highly valued for nutritional and medicinal applications, education, agro-ecological requirements and functioning of ecosystems, economic value and in socio-cultural life (Martina, 1998; Mann, 2001; UNEP World Conservation Monitoring Centre, 2003). For centuries, plants have been part of the Bukusu's land-use system. This is demonstrated by the practice of selective clearing of trees, forests, and bushes, leaving useful trees and woodlots to grow in the arable fields for fodder, timber, food, medicines, windbreaks, fences/boundaries, supporting crops, shrines, etc. (Martina, 1998). A significant proportion of Bukusu poems, riddles, and proverbs are expressed in association and with symbolic meaning of indigenous plants (Martina, 1998; Mweseli, 2004), summarising daily life experiences as well as portraying philosophical observations, which elders considered to be essential skills with which to equip growing children. Some plants were never used at all and were specifically reserved for ancestors, others for certain functions only, while yet others were used by a certain class of people and during certain occasions only (Martina, 1998). These socio-cultural practices and human-environment interactions are essential today for the recognition of ethnobotanical knowledge, and its meaningful utilization as well as the development of community-specific conservation strategies (Juma, 1989). A great deal of information regarding the use, management, and socio-cultural values of plants is remembered particularly by the older generation (45–108 years of age).

The livelihood of the Bukusu community depends solely on the combined pastoral and arable economies (Martina, 1998). Being sedentary pastoralists, they had time for arable farming; women and young girls were known to grow millet, *Eleusine coracana*. Early in the last century, they lived on millet, cassava and small livestock, but now raise mostly maize, beans, potatoes, cassava, bananas and cabbage for food and grow sugarcane, cotton, tobacco and sunflower for cash. They also keep cattle, goats, sheep, pigs and chickens. In fact, arable farming activities (sugarcane, maize and millet plantations) have dominated the land use and currently threaten the survival of grasslands, woodlands, bushes and forests, which form the source of ethnopharmacologically active agents upon which ethnoknowledge is based (Biosafety News, 2002). Men and young boys look after cattle along the river valleys on patches of grasses along arable lands, and in grasslands and bushes surrounding forests. Women and young girls are responsible for domestic work. Women till the land and grow crops for consumption such as millet, squashes, cassava and potatoes. They also look after calves, sheep, birds and goats. However, the Bukusu customary rights only allow the women to use land but not to own it, despite their significant contribution to agriculture and animal production (Kenya National Archives, 1954–1956; Nasimiyu, 1985; Nangendo, 1994). It is this division of labour that has determined the distribution patterns of a given aspect of ethnoknowledge within the Bukusu

community.

Many families live in houses made of mud with straw/grass roofs. The straw/grass roofs provide for shade from the hot sun in the summer months and are totally waterproof for protection in the rainy season. A few people live in cement houses with iron sheet roofs. Both types of housing suffice and are reasonably comfortable. Most rural homesteads do not have electricity or running water. Lighting is with candles or kerosene-powered lanterns and water is drawn from a well on the farm and is either filtered for drinking or heated for bathing. Beds usually consist of a wooden frame with a thick mattress.

### **The significance of archiving and valuing ethnoveterinary knowledge**

The pastoral lifestyle was initially the mainstay of the Bukusu economy before arable farming overtook it, as evidenced today by the rich archaeology, nomadic and pastoral vocabulary, traditions and sociocultural anthropology of the Bukusu people (Makila, 1978). Moreover, the community has well-established ethnoveterinary practices in curing, controlling and managing livestock diseases and related livestock vector-borne problems. A rich history of anti-tick ethnoveterinary practices has evolved to avert tick-related economic losses and as a way of survival in a tick-prone environment (Lewis, 2001). Such traditional applications, which have withstood the test of time, have a number of advantages over modern equivalents. They are holistic in action, cost-effective, easily applied, locally suitable, easily accessed, environmentally friendly and community-specific (Mathias-Mundy and McCorkle, 1989; Hamburger et al., 1991; Martin et al., 2001). Moreover, these ethnopractices are an integral part of the people's daily lives and are based on inherent indigenous knowledge developed from experience gained over centuries and adapted to local culture and environment. The knowledge has been acquired through such practical life experiences as observations, dreams and visions, friendly exchanges, trade and sales, ceremonies, inheritance, and annual exchange programmes of ethnopractitioners. Because this is mainly an oral tradition, ethnoknowledge is largely transmitted by word of mouth from generation to generation (Kokwaro, 1993), and only sketchily recorded in books (Abegaz and Dемissew, 1998). It is also stored (based on the collective memory of the community members) and passed on through songs, poems, drawings, paintings and stories (Mweseli, 2004). In certain communities, the collective knowledge is believed to be owned by the ancestors and kept under the custody of presently-living elderly men and women. There is a danger however, that this method of vesting knowledge in the humans (elderly) can be undermined by their mortality, thereby causing a loss of important information for future generations.

This study therefore was carried out to survey, document and generate a database of anti-tick ethnopractices and knowledge. This resource may be used to develop a new framework for effective tick management in sub-Saharan Africa and the possible deployment of some of the ethnopractices on a wider scale (Alghali, 1992). The ethnoknowledge generated may be integrated with modern science to sustainably control and manage livestock ticks of poor livestock farmers. In so doing, value-added knowledge will be provided back to the community in the form of useful products (e.g., the development of an on-host tick control device is envisaged) and services, and help ethnopractitioners regain confidence in their ethnoknowledge and establish links with modern science for sustainable use and management of local environment and its resources.

## Materials and methods

### The source of anti-tick ethnoknowledge

Anti-tick ethnoknowledge was surveyed from 13 different sources in the study area. The survey involved a sample of 272 key respondents of mixed sex and age (18–118 years old) and was drawn from a number of sources. These sources included primary school pupils who provided leads to key respondents and local school teachers/education officers who helped select candidate schools, gave permission to distribute the questionnaire to pupils, and helped clarify what information was being asked of pupils. Local veterinarians, para-veterinarians, and agricultural extension officers responsible for providing extension services to farmers were also able to identify some key respondents. We also attended public meetings organized by local administrators to identify more possible respondents. Local livestock traders and dealers, as well as individual livestock farmers, contributed their knowledge on tick control based on their professional and economic activities, whereas community/village/clan leaders/elders had information on communal tick control and management programmes funded by the Government and NGOs. Local ethnopractitioners, including traditional healers/herbalists/spiritualists/ritualists, formed a particular subset of knowledgeable people from whom key respondents were drawn. Other sources of data included NGOs such as the Council for Human Ecology–Kenya, the German Development Service and the Bungoma Indigenous Trees Conservation Club, which had prior experience and close interaction with local livestock farmers and had earlier documented part of their ethnoveterinary knowledge. Centres known for preparation of a local brew (*Busaa*) were the most important meeting points of old men and women and formed a venue for the discussion sessions. Secondary data was obtained from the Bungoma District Veterinary Office (DVO) records on tick control. All these groups were consulted because each was associated with a specific aspect of ethnoknowledge relevant to the study.

### Key steps to accessing anti-tick ethnoknowledge

#### *Constitution of sampling group*

The first step was the generation of a purposive sample of key respondents from the 13 sources mentioned above. Key respondents are local experts or people in the study area with a profound knowledge of a particular issue or technology of interest (in this case, anti-tick ethnoknowledge) (Etkin, 1993; Waters–Bayer and Bayer, 1994, McCorkle et al., 1997). They have a more extensive understanding of local social and veterinary–cultural systems than others in the community. A purposive sample referred to a particular subset of knowledgeable people in the area of traditional control and management of livestock ticks. Intensive and extensive collaboration and interaction with these key respondents was considered to be an effective research strategy (Oakley, 1981; Warry, 1992). A random sample would not have been appropriate for this sort of socio–cultural set-up, in which we depend on folk knowledge (Etkin, 1993), as not everyone sampled randomly may have the required knowledge (Martin, 1996).

#### *Local primary school pupils: the questionnaire method*

This was the main method used to identify key respondents and obtain local and remote anti-tick ethnoknowledge from largely illiterate people. The use of children to collect such information has had precedents in the field (Lans and Brown, 1998; Lans, 2001), but there is very little published literature about this method (Campell, 1994).

From a list of local primary schools at the District Education Office (DEO) in Bungoma, 10 schools were selected for participation in the survey studies. The selection of schools was based

on variables such as rurality vs. urbanization, ethnicity, gender, on-going teaching activities and geographical spread of the schools within the study area. In selected schools, pupils were visited and the basics of participatory research (Baldwin and Cervinkas, 1993) were explained to them, and the importance of their contribution was stressed. Pupils were asked to interview parents, friends, neighbours, etc. about traditional practices of tick control and fill the answers of the respondents in a well-structured questionnaire. The questionnaire consisted of 15 questions: (1) the location where questionnaire is administered, (2) identification of the person being interviewed, (3) type and number of livestock kept by the person interviewed, (4) what the tick means to the livestock farmer, (5) the kind of tick-related problems experienced by the livestock farmers, (6) responses to tick-related problems, (7) association between ethnoremedies and identified tick-related problem, (8) tick ethnocontrol remedies (ethnobotanicals) (9) how these ethnobotanicals are: harvested, processed, and applied, (10) when do livestock farmers apply their ethnoremedies, (11) mode of application of these ethnoremedies, (12) monitoring of cases after application and (13) any observations made regarding side-effects of these ethnoremedies, (14) any collaboration sought during tick control, and (15) personal observation of tick infestation made by the interviewer in the candidate livestock herd of the interviewee.

From the questionnaires, key respondents were identified basing on whether the essay and or the questionnaire indicated that a respondent had potentially useful information on anti-tick ethnoknowledge. This method was considered very useful and robust because it reduced the following bias: (1) modelling bias, which was the projection of the interviewer's views onto those studied, (2) strategic bias, which was the expectation of benefits by the subject, (3) familiar relationships between interviewer and interviewee (children and parents/neighbours) reduced resistance to the questioning and responding with rote answers, as well as outsider bias (Sutton and Orr, 1991) and (4) reduces bias for the selection of key respondents (Etkin, 1993; Waters-Bayer and Bayer, 1994).

#### *Personal interviews with key respondents*

The third step was conducting the interviews/discussions with key respondents, followed by plant specimen identification and collection from the field and transportation to the University of Nairobi Herbarium for further identification and taxonomic studies. The interviews were guided discussions, semi-structured by a mental checklist of relevant points to confirm the validity of the questionnaire information.

#### *Focus-group discussions*

The fourth step involved holding joint focus-group discussions with all stakeholders. A focus-group discussion is an exploratory discussion designed to obtain perceptions on a specific theme from a target group in a non-threatening environment (Krueger, 1988; Etkin, 1993). This kind of group interaction produced data and insights that would have otherwise been less accessible (Morgan, 1988). The interaction between all stakeholders formed the collaborative and participatory appraisal (PA) approaches utilized to build a consensus and verify that the information from other interviewees was accurately recorded (IIRR, 1994). The group interaction also minimized the objectification of the respondents as the only source of data (Oakley, 1981). One purpose of this form of collaborative research was to return decision making, based on theoretical knowledge, to the community, rather than conceding this role to the conventionally-trained expert (Warry, 1992). Empowerment of research subjects can take place when theory is allowed to inform practical action and by returning knowledge for use to its point of origin (Warry, 1992), through consultative community-based training workshops and manual booklets translated into local languages.

### ***Collection of secondary data***

The fifth step involved the collection of secondary data on anti-tick plants and ethnopractices from the District Veterinary Office (DVO). This was followed by an extensive literature search on plant taxonomy and ethnobotanical applications from the internet, livestock research institutions, NGOs, East Africa and University of Nairobi Herbaria libraries and laboratories.

### ***Enumerations of documented plants with effects on livestock ticks***

An extensive list of ethnobotanicals, including their scientific names as well as vernacular names, growth habits, family names and other information about their usage can be found at the end of this dissertation (Appendix 1). The term ethnobotany is used in the context of how people of a particular culture and region make use of their indigenous plants: in this case, communities, tribes, clans and sub-clans living in Bungoma District. Because of the ethnic diversity amongst communities living in Bungoma District, more than one vernacular name may be used to refer to a particular plant and/or any other related plant within a given genus or family. On the other hand, two or more different plant species could have one vernacular name depending on their geographical locations, uses, and associated ethnic group(s). Classification of growth life forms and or habits was defined according to Yumoto et al. (1994).

## **Results and discussion**

### ***Local primary school pupil—The questionnaire method***

The questionnaire method was instrumental in allowing the participation of a cross-section of local residents in the research survey. This helped us to obtain much useful information and ensured that plants with more than one vernacular name were correctly identified and their uses accurately recorded throughout the studied community. In addition, it allowed a considerable number of illiterate people, and those ethnopractitioners and other knowledgeable key respondents living in remote areas to be reached and to meet the targets of the survey (Sutton and Orr, 1991). Many of these people gave useful leading viewpoints as shown in Tables 2.1–2.4.

### ***Interviews with key respondents and focus-group discussions***

Focus-group discussions were instrumental in enabling us to transcribe the local Bukusu language accurately, especially with regard to local plant names and their meanings. This method of group discussions furthermore yielded key information for the selection of the anti-tick plants that merit further scientific investigation. The discussion sessions helped to resolve the controversies among interviewees of multiple names referring to one plant and vice versa. Following numerous meetings and interactions, a consensus was built amongst the discussion groups on the correct naming of plants and their local uses and preparation. It also became evident that there was need for conventionally trained scientists to: (1) respect the ownership, source and origin of ethnoknowledge and the needs and sensitivities of its holders (2) take the time to establish a strong, trusting relationship based on honesty, openness and cooperation, (3) work on projects of common interest and benefit, (4) continuously foster communication between partners, and (5) provide value-added knowledge back to the community in the form of useful products (such as reports) and services, and share equitably with the holders any benefits arising from the use of ethnoknowledge. Ethnopractitioners should be made aware of legislation protecting their ethnoknowledge base through relevant state ministries, so as to defend the communities' indigenous knowledge from biopiracy that may threaten it.

**Table 2.1a.** Tick description by colour, size and life cycle type in relationship to on-host feeding site and habitat preference as a percentage of the responses (n = 987)\*

Tick description	Site preference of the described ticks										NMAS	% total	
	On host animals					Habitat							
	Ear	Head	Dewlap/ neck/ brisket	Fore- legs	Belly	Back/ body sides	Hind legs	Tail/ anal region	Udder/ scrotum	Bush			Grass
<i>Colour</i>													
Red	5.37	1.32	2.43	1.52	0.51	1.11	1.62	2.74	3.34	0.00	0.51	0.30	20.77
Brown	5.88	1.93	3.34	2.13	0.61	1.01	2.23	4.05	4.56	0.00	0.30	0.30	26.34
Blue	6.08	2.13	1.95	2.03	0.61	1.11	2.13	3.55	4.76	0.00	0.41	0.10	24.86
Black	0.61	0.10	0.20	0.10	0.00	0.00	0.10	0.41	0.41	0.00	0.00	0.10	2.03
Multicolour	1.11	0.00	0.20	0.30	0.00	0.20	0.41	0.10	0.10	0.00	0.10	0.10	2.62
Grey	0.10	0.00	0.00	0.10	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.10	0.40
Yellowish	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
<i>Size</i>													
Small	1.32	0.10	0.51	0.41	0.20	0.10	0.51	0.30	1.01	0.00	0.00	0.41	4.87
Big	1.52	0.10	0.30	0.41	0.30	0.10	0.41	0.30	1.22	0.00	0.00	0.30	4.96
<i>Life cycle type</i>													
1-host tick	0.71	0.41	0.30	0.00	0.00	0.00	0.20	0.61	0.51	0.20	0.20	0.10	3.24
2-host tick	1.52	0.51	0.51	0.20	0.20	0.00	0.30	1.11	0.71	0.20	0.20	0.10	5.56
3-host tick	1.11	0.51	0.41	0.10	0.20	0.00	0.20	0.61	0.71	0.10	0.10	0.20	4.25
<b>% total</b>	<b>25.43</b>	<b>7.11</b>	<b>10.15</b>	<b>7.30</b>	<b>2.63</b>	<b>3.63</b>	<b>8.21</b>	<b>13.78</b>	<b>17.33</b>	<b>0.50</b>	<b>1.82</b>	<b>2.11</b>	<b>100.00</b>

\*More than one response was received from each of the 272 respondents NMAS, no mention of association with any site of either host or habitat

**Table 2.1b.** Observation records by interviewees on individual animals of their herds (n = 233)

Categories of observations	No. of herds observed	Mean No. of ani-mals per herd	% of interviewees who made an observation
Many ticks (> ~20 per animal)	19	11 ± 1	8
Few ticks (~5 – 20 per animal)	21	10 ± 2	9
Very few ticks (< ~5 per animal)	16	8 ± 1	7
No tick was observed on any animals in the candidate herd	37	7 ± 1	16
No observation was made on individual animals in the candidate herd	140	10 ± 1	60

### Responses to questionnaires and results from the discussion groups

Despite the initial constraints in accessing anti-tick ethnoknowledge, there was sufficient goodwill among most of the main collaborating and participating stakeholders to ensure substantial success in the research process. From the survey studies, it became apparent that although for a long time the role of ethnoveterinary medicine and its potential contribution to livestock health has been neglected (Kofi-Tsekpo and Kioy, 1998), a large proportion of people still depend on it. This was evidenced by the complex composition of the purposive sample (n = 272) of key respondents, and their voluminous and high-quality responses (Tables 2.1a and b). Even more important was the commitment and determination of all the stakeholders to have their local anti-tick ethnoknowledge recognized, documented and evaluated. They too were interested in using their ethnoknowledge in an improved and sustainable manner.

From discussions during one of the focus-group sessions held in Bungoma District under the auspices of Bungoma Indigenous Trees Conservation Club (BITCC) and Bungoma Herbalists Association (BHA), it was evident there was concern that the community could lose its rich socio-cultural heritage. This was further confirmed by a lack of generalized use of this ethnobotanical knowledge across the Bukusu community (Wandibba, 1998; Corbit and Wanyama, 2004; Buteyo, 2004). There was a consensus, therefore, that efforts to conserve ethnobotanical knowledge of the Bukusu be intensified. Throughout the colonial era, indigenous knowledge was equated to witchcraft and wholly dismissed and actively suppressed. The practice continued, however, in a clandestine manner to avoid punishment by the colonial administration and only after independence did trends towards strengthening national and socio-cultural identities began re-emerging (Mubukusu, pers. comm.). Succeeding governments encouraged the integration of indigenous knowledge into existing health services. However, there was no clear legal framework for its operation. The conventionally-trained doctors and various religious faith groups took the advantage of lack of legal recognition of indigenous knowledge to denounce it. This attitude has encouraged exploitation, without due recognition of indigenous knowledge. Indeed, during our study, Bungoma ethnopractitioners reiterated that they are being exploited by malpractices of bioprospecting and biopiracy by some conventionally trained scientists, some of whom attempt to lay claims on the ethnoknowledge without giving the indigenous community proper recognition. This behaviour has caused fear among ethnopractitioners and has contributed to their reluctance to share indigenous knowledge with foreigners. It also accounts for a discrepancy between knowledge availability and its use, as well as mistrust and dishonesty among stakeholders (Simon, 1998; Hempel, 2002). It is hoped that the present study, based on participatory research, will help

**Table 2.2.** Tick-borne problems experienced by livestock farmers as percentage of respondents (n = 272)

Tick-related problems	% respondents identifying problems
Livestock diseases <i>per se</i> without specifications	16.50
Anaemia	13.97
Poor performance of the animals	12.87
Inflicted wounds and damage to skin	12.13
Irritation to animals	9.56
East Coast Fever (ECF)	7.72
Deaths	6.62
Weakening animals	5.88
Anaplasmosis	2.94
Heartwater	2.57
General economic losses	2.57
Fever	1.84
Gall sickness (disease)	1.25
Non-respondents	3.58

to reinforce ownership and recognition of indigenous ethnobotanical knowledge amongst the Bukusu. Moreover, local community approval and active involvement in projects conducted by conventionally-trained scientists helps to restore confidence in the ethnopractitioners and provide insights into conservation and management of biodiversity and local ecosystems in rural settings.

#### *Associated tick and tick-related problems*

The Bukusu community is informed and aware of livestock ticks and associated tick-borne problems. The community participants involved in the survey described to the locals the nature of ticks and the objectives of the study in layman's language that was well understood. From their responses, it was clear that the interviewees consistently recognized the parasitic (on-host) tick stages, which were more commonly seen than the non-parasitic stages (off-host) of ticks (Tables 2.1a and b). They observed ticks on their livestock and categorised the intensity of infestation (Table 2.1b).

Results from Table 2.1b correspond to tick-related problems identified in Table 2.2. This confirms that every domestic animal hosts a variety of tick species. Ticks were traditionally recognised by their colour, size, type of life cycle and on-host feeding sites and habitat preference (Table 2.1a). A considerable proportion of local people were familiar with the various ticks that are found attached on ears, udder, scrotum, tail/anal region, dewlap/neck, between legs and head. They also recognized tick and tick-related problems (T&TRPs) as serious constraints to livestock production in the study area (Table 2.2). The description of ticks on the host animals (Table 2.1a) together with the problems caused by ticks (Table 2.2) reflected the different tick species available in the study area.

Ticks are known to cause a number of serious livestock problems in the study area (Table 2.2). Descriptions of the ticks, including their predilection feeding sites on their host animals (Table 2.1a) were sufficient to confirm their taxonomic status to genus level using voucher specimens from the Kenya National Museums (KNM) as *Rhipicephalus* spp., *Amblyomma* spp. and *Boophilus* spp. A majority of respondents classified ticks by

**Table 2.3.** Frequency of ethnomethods used by respondents in tick control and management (n = 272)

Ethnomethods	% respondents using ethnomethod
Handpicking	34
Ethnobotanicals	20
Pasture spelling/ grazing practices	14
Burning pastures	14
Fumigation with smoke	6
Application of kerosene, magadi soda, urine, soap, fish resi-dues, cow dung and grease (alone or mixed)	3
Livestock quarantine	3
Predation by birds	3
Cleaning cattle shed and burning residues believed to contain ticks	2
Dusting with ash	1

**Table 2.4.** Methods of ethnobotanical application (n = 272)

Method of application	% of respondents using method
Pouring on of the suspension	74
Fumigation	11
Rubbing	5
Dusting	5
Anti-tick pastures	2
Steaming	1
Hanging bouquet	1
Others	1

their colours (77.1% of the responses), followed by type of life cycle (with 13.1% of the responses) and last by size (with 9.8% of the responses). Classification by type of life cycle is influenced by modern scientific knowledge, however. The highest number of responses identified the ear (25.4% of responses) as the most preferred site of tick attachment on the host animals (Table 2.1a). A considerable proportion of respondents knew that ticks cause diseases to livestock (16.5%), poor productivity in affected animals (12.9%), deaths to livestock (6.6%) and some people (2.6%) were also aware of economic losses resulting from tick infestation in livestock populations (Table 2.2). The generation of ethnoknowledge through this interaction of community ethnopractitioners and western trained scientists yielded valuable information on historic and present patterns in tick infestation and distribution, in land use economies, biodiversity and other aspects of local ecosystems. On this basis, therefore, sustainable utilization and management of local resources can be developed as a value-added return to the local community (Hempel, 2002).

#### *Ethnocontrol of livestock ticks in the community*

While community-based traditional knowledge of tick control and management was not widely shared, it was still an important piece of the puzzle. Understanding local communities' ethnoknowledge regarding pest management can provide information on broad trends such as pest and host species distributions, abundance and seasonal behaviour patterns of ticks, and as a result, provide useful information for scientists' field work (Hempel, 2002). From our survey, it became apparent that ticks have been traditionally controlled and managed by a variety of ethnomethods (Table 2.3). All of these ethnocontrol strategies comprised a traditionally integrated system, equivalent to modern integrated pest

management (IPM), aimed at livestock tick control and management in the community.

A number of plants and plant products with the potential to protect livestock against ticks were documented (Appendix 1), including anti-tick plants that have been studied in other laboratories. The ethnobotanicals used were applied using a variety of methods (Table 2.4). A detailed literature search on suggested and documented anti-tick plants and their properties are presented in Appendix 1, as one of the bases for selecting the best anti-tick ethnobotanicals that merit more detailed scientific studies. Integration of some of these anti-tick ethnopractices in tick control and management has the potential to improve the local livestock industry and give the impoverished rural economy a fresh impetus.

#### ***Secondary data and documented anti-tick ethnobotanicals***

In total, 154 plants were documented and the literature regarding each one was evaluated in detail (Appendix 1). In addition, four non-botanical anti-tick agents were documented. Based on key respondents' information, acaricidal, insecticidal, pesticidal and bioactivity information from literature, eight plant species were selected for an in-depth scientific studies in the laboratory. However, there were many plants mentioned several times during the field survey for which the literature search revealed very little or no leading pesticidal, insecticidal, acaricidal or bioactive information (Appendix 1). On the other hand, a plant species that had been proven in the laboratory to have very good acaricidal properties may only have been mentioned once or twice (as in the case of *Cleome gynandra*). This led to difficulties in selecting the leading anti-tick candidate plant species. Of the eight plants that had already been selected for laboratory studies, two were re-selected for detailed scientific studies based on the bioassay laboratory screening results and their bioactive, pesticidal, insecticidal, acaricidal and related taxonomic information from the literature search (Appendix 1). Many of these documented plant species had various medicinal and cultural applications recorded in the literature in other communities.

From our study, the database generated on anti-tick ethnoknowledge clearly showed that there is great potential for integration of anti-tick ethnopractices and knowledge in sustainable tick control and management. Furthermore, the identification, documentation, and evaluation of anti-tick ethnobotanicals and other anti-tick ethnopractices used by various communities may foster increased effectiveness and wider acceptance of these ethnoremedies by all stakeholders and thereby reduce suspicion against them.

A large number of other plants reputedly work as acaricides and insecticides, but reference to their use is difficult to uncover in the scientific literature. Popular literature, anecdotal evidence, and information gleaned from modern survivalists and seekers of historical plant knowledge contain vast amounts of information. Some tick ethnocontrol applications that were found in the lay literature and mentioned by some respondents but that did not emerge in a scientific literature search include the use of cow dung, cattle urine, fish residues, soap, and more than 101 plants in families such as the Lamiaceae, Flacourtiaceae, Acanthaceae and Ebenaceae.

#### **Conclusions**

The survey of indigenous tick-management knowledge of the Bukusu community revealed a wealth of information on plants, plant products, and anti-tick ethnopractices having the potential to control and manage ticks by rural livestock farmers. Evidence from respondents' information revealed that some of the plant uses were brought from their

original area/location to Bungoma by immigrants. Some plants and anti-tick ethnopractices had very few ethnoknowledge references in the literature; perhaps, they were truly indigenous to the Bukusu community or perhaps relevant references could not be accessed. Those plants thought to be indigenous to the Bukusu community and with traditionally claimed effects on livestock ticks numbered more than 101, in families such as Lamiaceae, Flacourtiaceae, Acanthaceae and Ebenaceae etc. Ritual anti-tick practices comprised burning pastures, livestock quarantine, types of grazing practices, cleaning cattle sheds, burning or burying residues of cattle sheds, bird predation, feeding animals on natural salty soils locally called '*silongo*', and applying kerosene, soap, fish residues, cattle dung and urine, grease, ash, magadi soda, and sisal juices on cattle. Nevertheless, some of the local claims of acaricidal properties of the ethnobotanicals have been supported by scientific studies. Elucidation of scientific rationale of these plants and anti-tick ethnopractices, however, will provide more information and understanding towards the deployment of some of them in the most needed integrated tick control and management programmes.

### **Acknowledgements**

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## **Part II**

# **Laboratory evaluation of essential oils**



## 3

## Repellent activities of essential oils of indigenous plants against the brown ear tick *Rhipicephalus appendiculatus*<sup>1</sup>

**Abstract:** Essential oils of eight plants (*Tagetes minuta*, *Tithonia diversifolia*, *Juniperus procera*, *Solanecio manii*, *Senna didymobotrya*, *Lantana camara*, *Securidaca longepedunculata* and *Hoslundia opposita*) were screened for their repellent activity against the brown ear tick *Rhipicephalus appendiculatus*. The essential oils of two plants, *T. minuta* and *T. diversifolia*, which had relatively high repellent effects compared to the other six, were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) to establish their chemical compositions. In laboratory assays, the essential oils of these two plants exhibited repellent effects which, at doses of 1 mg and neat oil (50 mg), were comparable to that of the commonly-used commercial repellent, N,N-diethyl-3-methylbenzamide (DEET). When the two essential oils were compared, the essential oil of *T. minuta* ( $RD_{50} = 0.0021$  mg) manifested stronger repellent effects against *R. appendiculatus* than the essential oil of *T. diversifolia* ( $RD_{50} = 0.263$  mg). The major constituent of *T. minuta* was *cis*-ocimene (43.78%), followed by dihydrotagetone (16.71%), piperitenone (10.15%), *trans*-tagetone (8.67%), 3,9-epoxy-p-metha-1,8(10)diene (6.47%),  $\beta$ -ocimene (3.25%), *cis*-tagetone (1.95%),  $\beta$ -caryophyllene (0.84%), bicyclogermacrene (0.62%) and AR-turmerone (0.50%). In the essential oil of *T. diversifolia*,  $\alpha$ -pinene occurred in largest amount (63.64%) followed by  $\beta$ -pinene (15.00%), isocaryophyllene (7.62%), nerolidol (3.70%), tetradecanol (1.75%), limonene (1.52%), sabinene (1.00%),  $\alpha$ -copaene (0.95%),  $\alpha$ -gurjunene (0.56%) and cyclodecene (0.54%). The implication of these results for the control of *R. appendiculatus* is discussed.

**Key words:** ethnobotanicals, Kenya, *Tagetes minuta*, *Tithonia diversifolia*, Asteraceae, essential oils, chemical compounds, GC-MS, repellents, *Rhipicephalus appendiculatus*, dual-choice essay, behaviour

### Introduction

In sub-Saharan Africa, East Coast Fever (ECF), caused by *Theileria parva parva* Theiler, 1904 and transmitted by the brown ear tick, *Rhipicephalus appendiculatus* Neumann, 1901, is one of the major constraints to the development of the livestock industry (Norval et al., 1992; Olwoch et al., 2008). Of the estimated 12.7 m head of cattle (both indigenous and exotic), 76% is at risk to ECF (East Africa, 2003). The disease is associated with up to 10% mortality in zebu calves in ECF endemic areas and can cause up to 100% mortality in susceptible exotic and indigenous breeds (Mbogo et al., 1995; East Africa, 2003).

Control and management of both vector and pathogen have continued to rely heavily on the application of synthetic chemical acaricides on the host. This has proved to

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be unsustainable in many ways (Norval et al., 1992). The acaricides can eliminate ticks from the host, but do not prevent continued re-infestation from the source environment, where ticks spend 90% of their life. For effective management of ticks, we hypothesize that there must be a mechanism to control ticks on individual hosts as well as in the host environment in order to prevent host re-infestation during grazing. One possible strategy towards achieving this is the use of tick repellents on the host and tick-repellent plants in the pasture (environment), combined with plants that are attractive to ticks surrounding the pastureland so as to develop a push-pull tick control system (Hassanali et al., 2007; Cook et al., 2007). Although the proposed strategy appears complex, it may be possible to achieve in zero grazing/semi-zero grazing livestock farming systems, small scale free-range livestock farming systems and tethering livestock farming systems. For others, such as pastoralism and large-scale livestock farming systems, the deployment of well-formulated, controlled-release dispensers may be more practical.

A commonly used commercial arthropod repellent, N,N-diethyl-3-methylbenzamide (DEET), is still considered the best available product, repelling a wide variety of insects, ticks and mites (Fei and Xin, 2007). In humans, however, the repellent may cause insomnia, mood disturbances, impaired cognitive functions, seizures, toxic encephalopathy and allergic reactions (Robbins and Cherniack, 1986; Qui et al., 1998; Lewis et al., 2000). Though DEET is not expected to bioaccumulate, it has been found to cause considerable environmental pollution (Seo et al., 2005). Furthermore, the ability of DEET to dissolve some plastics, rayon, spandex, other synthetic fabrics, leather and painted or varnished surfaces has led to a search for alternative repellents.

Studies have shown that plant-based repellents can be comparable to DEET or even better (Panda, 2004; Trongtokit et al., 2004; Chauhan et al., 2005; Bond, 2007). The potential of some local plants and plant products for use as tick repellents on hosts, and tick-repellent plants in grazing areas to protect livestock from tick bites has been demonstrated (Menendez, 1924; Thompson et al., 1978; Carroll et al., 1989; Wilson and Sutherst, 1990; Webb and David, 2002). One such repellent is the Flea and Tick Granular Repellent, which is made from essential oils of cedar, cinnamon, mint and lemon grass and has a pleasant odour and can be safely used outdoors for flea and tick control (Nixalite of America, 2005). *Melinis minutiflora* (molasses grass), a tropical grass already in use as livestock fodder (Lersten, 1983; Juarez Lagunes et al., 1999), cover crop and mulch (Duke, 1983) and for thatching houses (Chiera et al., 1977), has been shown to be toxic to ticks (Thompson et al., 1978) and to repel ticks (Menendez, 1924; Hernandez, et al., 1990; Mwangi et al., 1995b) as well as insects and snakes (Duke, 1983). At the International Centre of Insect Physiology and Ecology (ICIPE), a study to develop anti-tick pastures using molasses grass was undertaken at Mbita and Kuja in Nyanza Province, Kenya, to control *Amblyomma variegatum* and *R. appendiculatus*, vectors of the livestock diseases heartwater (cowdriosis) and ECF, respectively (Mwangi, et al., 1995b). The local shrubs *Cleome hirta* and *Gynandropsis gynandra* have been shown to be possible tick-repellent pasture plants (Dipeolu et al., 1992; Malonza et al., 1992; Ndung'u et al., 1999). Studies have also shown the tick-repellent properties of essential oils from *Commiphora erythraea* and *C. myrrh* (Maradufu, 1981), *Cleome monophylla* (Ndung'u et al., 1995), *Ocimum suave* (Mwangi et al., 1995a), *Cleome hirta* (Ndung'u et al., 1999) and *G. gynandra* (Lwande et al., 1999) against *R. appendiculatus*.

The practical application of tick-repellent plants and essential oils and their integration with other tick control measures either on the host or in pasture lands could be a practical and economical way of controlling not only livestock ticks but other arthropod vectors as well (Gupta and Rutledge, 1994; Copeland et al., 1995; WHO, 1995). This alter-

native is very suitable because the source plants and essential oils are locally accessible and familiar to resource-poor livestock farmers, meaning that they therefore may not require the input of external personnel during their application, as is often the case with synthetic chemical acaricides. Studies on the formulation, mode of action and possible toxicity of these plant materials have not yet been done, however. Furthermore, other problems may manifest themselves if the plants and their products are used on a wider scale.

Our objective in the present study was to identify indigenous plants from Bungoma District in western Kenya as sources of tick repellents with the potential to reduce contact between ticks and their livestock hosts. Essential oils were extracted from these plants and subsequently evaluated on-host for their ability to intercept feeding-site location behaviour of the vector by masking the natural host odours.

## Materials and methods

### Selection of plants

An ethnobotanical survey conducted in the Bukusu community in Bungoma District, western Kenya, identified 154 plant species having possible effects on livestock ticks. Each documented plant was evaluated in detail (Chapter 2 of this thesis), and based on this evaluation, eight plant species were selected for further studies of their repellent effects on adult *Rhipicephalus appendiculatus* (Table 1). The selection was based on the four levels of validity established by Heinrich et al. (1992) as follows:

0. If no information in the literature supports the Bukusu traditional claim for use, this indicates that the plant might have no effect and hence has no validity. Or may be the plant could be the true indigenous species of the community and has not been evaluated anywhere.
1. A plant (or another species of the same genus) which is used in geographically distinct areas for the control of the same livestock tick, attains the lowest level of validity if no further phytochemical or pharmacological information validates its popular use. The use in more than one area increases the likelihood that the plant is active against the tick.
2. If in addition to the ethnobotanical data, phytochemical or pharmacological information also confirms its use in the Bukusu community, the plant is assigned a higher level of validity. Plants in this category may exert a physiological action on the target tick species and are more likely to be effective remedies in livestock tick control and management than those at the lowest level of validity.
3. If ethnobotanical, phytochemical and pharmacological data support the traditional use of the plant, it is grouped in the highest level of validity and is most likely to be an effective tick control and management remedy (Heinrich, et al., 1992) and therefore selected (Table 1).

Plants were collected from Bungoma District, western Kenya along the southern slopes and foothills of Mount Elgon at altitudes ranging from about 1300 m in the south to about 3 500 m in the north. The district is located between latitude 0° 25'S and 0° 53'N and longitude 34° 21'W and 35° 04' E. All aerial parts of the plants were collected. The plants were identified in the herbarium at the School of Biological Sciences, University of Nairobi, Kenya. Voucher specimens of the selected plant species were deposited at the University of Nairobi Herbarium: *Tagetes minuta* L. (029-BGM-Mwi/2002), *Tithonia diversifolia* (Hemsl.) A. Gray (015-BGM-Muf/2002), *Juniperus procera* Endl. (134-BGM-Elg/2002), *Solanecio manii* (Hook. f.) C. Jeffrey. (106-BGM-Mwi/2002), *Senna didymobotrya*

**Table 3.1.** The eight plants with anti-tick activity selected for screening from the Bukusu community in western Kenya

Botanical name	Local Bukusu name	Family name	No. times mentioned for local use	Plant parts used	Reported plant characteristics	Reference
<i>Togetes minima</i> L.	Nanjaka	Asteraceae	9	Leaf/flower/bud (used to make suspension and hanging bouquet)	T, P, A, B, I	Getahun, 1976; Berger, 1994; Anon., 2001 Njoroge and Bussmann, 2006; Adekunle et al., 2007
<i>Tithonia diversifolia</i> (Hemsl.) Gray	Kamang'ulic/ Kamaua/ Kiming'ulite	Asteraceae	7	Whole plant used to make mainly paste for application	I, P, T, B, F, Af	Adoyo et al., 1997 Anon., 1997; Adeidire and Akinneye, 2004; Njoroge and Bussmann, 2006
<i>Solanecio manii</i> (Hook. f.) C. Jeffrey.	Nandebe	Asteraceae	5	Whole plant used in many different forms	A, C	University of Nairobi Herbarium records
<i>Juniperus procera procera</i> Hochst. ex Endlicher.	Kumutanakwa	Cupressaceae	5	Leaf/root/back	I, B	Getahun, 1976; Anon., 2001
<i>Securidaca longepedunculata</i> Fres.	Kumulondamwombe / Kumunyakasia/ kunny-anjabakeni	Polygalaceae	2	Whole plant	I, C, B, F, Ps	Jayasekara et al., 2002; Rukanjira, 2001
<i>Lantana camara</i> L.	Lantana/ Mukheke	Verbenaceae	2	Whole plant (used to make concoction for pour on or for steaming)	B, F, C, I,	Adebayo et al., 1999
<i>Hosaindia opposita</i> Vahl.	Bifwofo	Labiatae	1	Whole plant	T, A	Jembere et al., 1995
<i>Senna didymobotrya</i> (Fresn.) Irwin & Barneby	Kumubunbu/ Kumuphuphu	Caesalpiniaceae	1	Leaf/back/root (used to make suspension)	T, B, I	Fernandes et al., 1985; Mansingh and Williams, 1998 UWMAG, 2003

**Key**

- A Plants that were found in the literature to exhibit acaricidal properties
- B Plants that were found in the literature to have bioactive compounds
- P Plants that were found in the literature to exhibit pesticidal properties
- I Plants found in the literature to be resistant to attack by insects or fungi
- T Taxonomic affinity to plant species (at genus and family levels) known to possess bioactive, insecticidal or acaricidal properties
- F Human food plants, unless otherwise stated
- Af Plants with antifeedant properties
- C Plants with cultural applications
- Ps Poisonous plants to either animals or both humans and animals

(Fresen.) H.S. Irwin & Barneby (132-BGM-Web/2002), *Lantana camara* L. (043-BGM-Mwi/2002), *Securidaca longepedunculata* Fres. (018-BGM-Mec/2002) and *Hoslundia opposita* Vahl. (133-BGM-Bul/2002).

#### **Extraction of essential oils**

The plant materials were left in a well-ventilated room for 1-2 weeks before hydrodistillation. The materials were cut into small pieces and about 1 kg of each plant was hydrodistilled using a Clavenger-type apparatus for 8 h (Clavenger, 1928). Pure oil was collected from every plant into 2 ml vials and stored at  $-20^{\circ}\text{C}$  in a freezer until required for analysis and bioassays.

#### **Determination of the composition of the essential oils**

Both qualitative and quantitative characteristics of the various essential oils were studied using gas-chromatography (GC) and gas-chromatography/Mass Spectrometry (GC-MS) techniques (Tholl et al., 2006). The constituents of the essential oils were identified by analysis of their mass spectra, direct comparison of their mass spectra to the Wiley NBS and NIST databases or library of mass spectra, and co-injection with authentic standards on the GC.

GC analyses were performed with a Hewlett Packard HP 5890A Gas Chromatography equipped with a flame ionization detector (at  $230^{\circ}\text{C}$ ). A fused silica capillary column (Hewlett Packard, 50 m x 0.22 mm x 0.33 mm CD) coated with methyl silicon (0.3  $\mu\text{m}$  film thickness) was used with nitrogen as the carrier gas. All GC analyses were performed in the splitless mode with the injector temperature at  $270^{\circ}\text{C}$ . The oven temperature was programmed from  $60^{\circ}\text{C}$  isothermal for 7 min, to  $120^{\circ}\text{C}$  at  $5^{\circ}\text{C}$  per min, then to  $180^{\circ}\text{C}$  at  $10^{\circ}\text{C}$  per min, and finally to  $220^{\circ}\text{C}$  at  $20^{\circ}\text{C}$  per min, where it was maintained for 10 min. Peak areas were calculated using a Hewlett Packard 3393 B series integrator and together with their GC retention times, compared to those of authentic samples.

GC-MS analyses were performed with a VG Masslab 12-250 quadrupole gas chromatography-mass spectrometer. Chromatographic separations were achieved using a fused silica capillary column (Hewlett Packard, 50 m x 0.32 mm ID) coated with Carbowax 20M (0.3  $\mu\text{m}$  film thickness) with helium as the carrier gas. All the GC-MS analyses were made in the splitless mode with helium as the carrier gas. The GC column was temperature programmed as in the case of GC analysis. Compounds were identified by their electron impact (EI) mass spectral data, order of elution and relative GC retention times, and by comparison of their mass spectra and GC retention times to those of authentic samples.

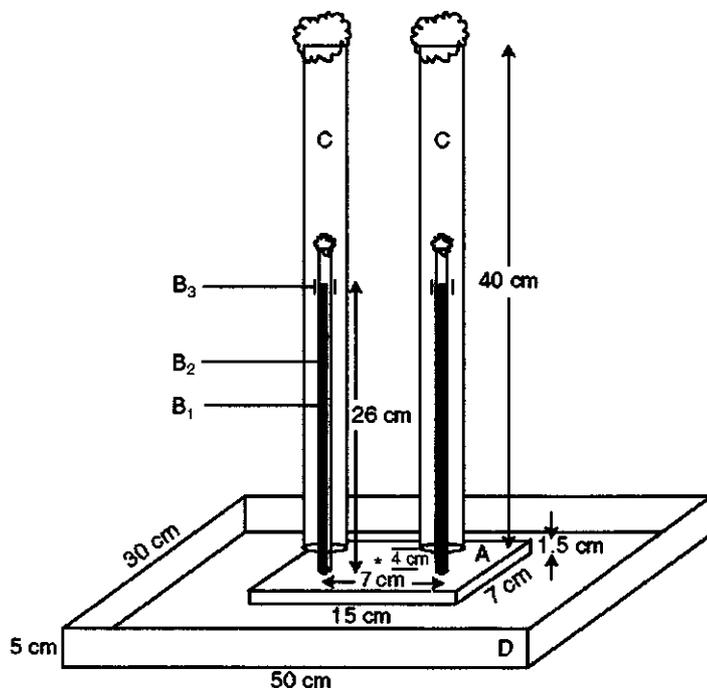
The computer on the GC-MS system records a mass spectrum for each scan and has a library of spectra that can be used to identify an unknown chemical in the sample. The library compares the mass spectrum from a sample component with mass spectra in the library. It then reports a list of likely identifications along with the statistical probability of the match.

#### **Synthetic chemicals**

Synthetic standard chemicals (authentic samples) used in GC co-injections were obtained from Sigma Chemical Company, Poole, UK and Aldrich Chemical Company, Gillingham, UK. All the authentic samples used were over 95% pure.

#### **Experimental ticks**

The ticks used (*R. appendiculatus*) were obtained from colonies at the International Livestock Research Institute (ILRI) and bred at ICIPE, Nairobi, Kenya. Rearing conditions and



**Fig. 3.1.** Tick climbing bioassay apparatus (placed in a tray measuring 50 x 30 x 5 cm) with water up to 1.5 cm deep; A, aluminium base; B<sub>1</sub>, aluminium rod (26 cm long x 1.7 cm in diameter); B<sub>2</sub>, 0.8-cm glass tube plugged with wet cotton wool; B<sub>3</sub>, filter paper collar; C, 4.5-cm glass tube plugged with dry cotton wool. The two aluminium rods, B<sub>1</sub> on the aluminium base, A (15 x 7 cm), were 7 cm apart. The outer tubes, C are held in position, 4 cm above the aluminium base, A by a retort stand clamp. The five ticks were introduced on the aluminium base, A, at a position marked with a star, 3.5 cm from the base of the aluminium rods, B<sub>1</sub> (modified from Browning, 1976).

management were as described previously (Bailey, 1960; Irvin and Brocklesby, 1970). All experiments were conducted with newly-emerged adults.

### Dual choice assay

A dual choice tick climbing assay apparatus was used for screening the essential oils of eight plants for their tick repellent activities, and further analyses were conducted on two of these selected plants, *T. minuta* and *T. diversifolia*. The bioassay apparatus (Fig. 3.1) [described previously by Wanzala et al. (2004)] made use of a characteristic behaviour of *R. appendiculatus*: the ticks climb up grass stems and settle for a period near the tip of the stem to await any passing potential hosts (Browning, 1976; Chiera, 1985). This experiment was set up at ICIPE, Nairobi, Kenya. An aluminium base of area 105 cm<sup>2</sup> with two stands of 26 cm in height and 7.0 cm apart was put in a basin of water, 1.5 cm deep (the water restricts the movement of the ticks to the aluminium base). Two sets of glass tubes were used, one of 4.5 cm (outer one) and the other one 0.8 cm (smaller inner tube) in diameter. A strip of filter paper (Whatmann No 7, 2 cm wide) was stapled to form a collar around the upper parts of each smaller inner glass tubes at a distance of 20 cm from the aluminium base to provide the source of either test odours or pure solvent. One collar on the pair of the tubes was treated with test odour solution and the other one with the same amount of pure solvent (dichloromethane-DCM) to serve as control. After the solvent was allowed to evaporate (about 10 min.), these tubes were shielded with the wider outer glass tubes from 4 cm above the aluminium base to facilitate a relatively uniform vertical gradient of the test odours along the 3.7 cm gap between the two glass tubes. The larger glass tubes also served to shield the smaller ones, in order to limit the diffusion of the test material and the solvent, and their upper ends were plugged with dry cotton wool. Wet cotton wool on top of the smaller glass tubes ensured a high relative humidity (>75%) within the columns.

The test materials and the solvent were dispensed using a calibrated Eppendorf pipette and equilibrated for 30 min before five adult ticks of mixed ages and sexes were released at the centre of the aluminium base. Prior to each bioassay, ticks were kept at high relative humidity (>85% RH) for 24 h in containers with moist cotton wool, so that they were not dehydrated and as a result would have less tendency to drown in the water surrounding the aluminium base. All bioassays were conducted in a room kept at  $28 \pm 1$  °C and  $75 \pm 5\%$  RH, which had an exhaust fan running continuously. The assays were run for 1 h, and the number of ticks above the filter paper strip on the control glass tube (Nc) and on the treated glass tube (Nt) were recorded at 15, 30, and 60 minutes. After each test, the apparatus was thoroughly cleaned and dried at 100 °C. Initial comparison of the responses of ticks in the setup with and without residual dichloromethane on both sides showed no bias for either side and no effects of the residual solvent on the adult ticks. Each dose of the test material was tested 20 times; each time with a fresh, naïve adult tick. The repellent effect of the essential oils was evaluated according to the formula adopted by Ndung'u et al. (1995) and Lwande et al. (1999) namely: percentage of repellency (PR) =  $[(Nc-Nt)/(Nc+Nt)] \times 100$ , where Nt and Nc represent the number of ticks that climbed on or passed the treated and control collar of filter papers on the glass tubes, respectively.

#### Selection of two plants: *Tagetes minuta* and *Tithonia diversifolia*

Screening of essential oils from eight plants and selection of two for further studies were based on a comparative analysis of assay results and ethnic knowledge on tick control. Preliminary studies were conducted on eight essential oils of plants listed in Table 3.1. *Tagetes minuta*, the plant that showed the highest tick repellency, was selected for further assays in order to select the most appropriate dose to be used for screening the remaining seven plants. From the results obtained for tick repellency bioassays of *T. minuta* essential oil, a dose of 0.1 mg was chosen. This dose and the neat oil (50 mg) were used to screen all eight plants, leading to the selection of *T. diversifolia* in addition to *T. minuta*.

#### Standard commercial synthetic arthropod repellent

The repellency bioassay results were compared with those previously obtained with DEET. This repellent is a colourless, oily liquid with a slight odour. It is still the best available product, repelling a wide variety of insects, ticks and mites and generally lasting longer than other repellents (Manson-Bahr and Apted, 1982; Chunge, 1986). Previous work at ICIPE, tested various DEET doses and determined their percent repellency against *R. appendiculatus* (Table 3.2), with which we compared the repellencies caused by essential oils of *T. minuta* and *T. diversifolia*.

**Table 3.2.** Mean percentage repellency ( $\pm$  SE) of N,N-diethyl-3-methylbenzamide evaluated in a dual-choice assay against adult *Rhipicephalus appendiculatus* at the International Centre of Insect Physiology and Ecology, Nairobi, Kenya under the same laboratory conditions as the current studies.

Factor	Categories			
	I	II	III	IV
Treatment dose (mg)	0.0998	0.00998	0.000998	0.0000998
Repellency (%)	84.0 $\pm$ 3.9	82.8 $\pm$ 3.6	75.6 $\pm$ 4.5	70.5 $\pm$ 3.6

(Sources: Ndung'u et al., 1995; Lwande et al., 1999; Ndung'u et al., 1999)

### Data management and analysis

Data were entered into an SAS database for analysis. The data (doses) were transformed ( $\log_{10}$ ) and subjected to analysis of variance (ANOVA) (SAS<sup>®</sup> Institute, 2002-2003). During analysis, percentage repellency (PR) values were converted to repellency probabilities ranging from 0 to 1 in order to fit into a probit model. The Tukey HSD test was used to compare the mean values of repellency obtained for various doses of the repellents (SAS<sup>®</sup> Institute, 2002-2003). Dose-response relationships were determined using probit analyses and repellent doses (RD) at  $RD_{50}$  and  $RD_{75}$  values obtained from the regression model:

$$\text{Probit [P(dose1)]} = \beta_0 + x \beta_1 + \hat{I}$$

Where,  $\beta_0$  = coefficient of the model representing y-intercept,

$\beta_1$  = coefficient of the model representing dose1,

dose1 =  $\log_{10}$  (dose),

$\hat{I}$  = error term in the data set of the predictor (regressor) variable (x) and

P = repellency probability.

The curves for the regression models were drawn using R software for Microsoft windows.

### Results

#### Initial screening of essential oils from selected plants

The essential oils from eight plants (*T. minuta*, *T. diversifolia*, *J. procera*, *S. manii*, *S. didymobotrya*, *L. camara*, *S. longepedunculata* and *H. opposita*) caused repellency against newly emerged *R. appendiculatus* adults of mixed sexes. The results from two different treatment levels [at 0.1 mg dose and neat oil (50 mg)] are shown in Fig. 3.2. The percent repellencies of neat oils were comparable to that of the commercial arthropod repellent, DEET, with the exception of the essential oils of *S. manii*, *L. camara* and *S. longepedunculata*. However, at the 0.1 mg dose, the percent repellencies of the essential oils were below that of DEET, except that of *T. minuta* (80.1%).

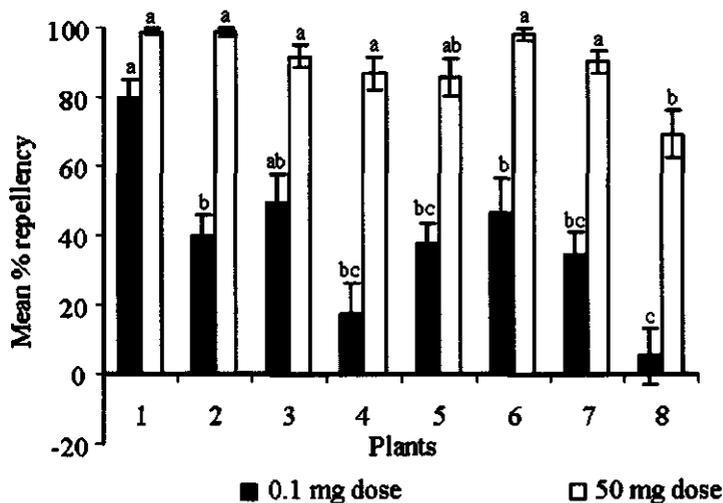


Fig. 3.2. The repellent effect of essential oils of eight plants in doses of 0.1 mg and 50 mg (neat oil) against *Rhipicephalus appendiculatus*. Plant species 1 = *Tagetes minuta*, 2 = *Tithonia diversifolia*, 3 = *Hoslundia opposita*, 4 = *Solanecio manii*, 5 = *Lantana camara*, 6 = *Juniperus procera*, 7 = *Senna didymobotrya* and 8 = *Securidaca longepedunculata*. For a given repellent dose, means capped by the same letters are not significantly different at  $P < 0.0001$  (Student-Newman-Keuls test).

**Selection of two plants for dose-response studies**

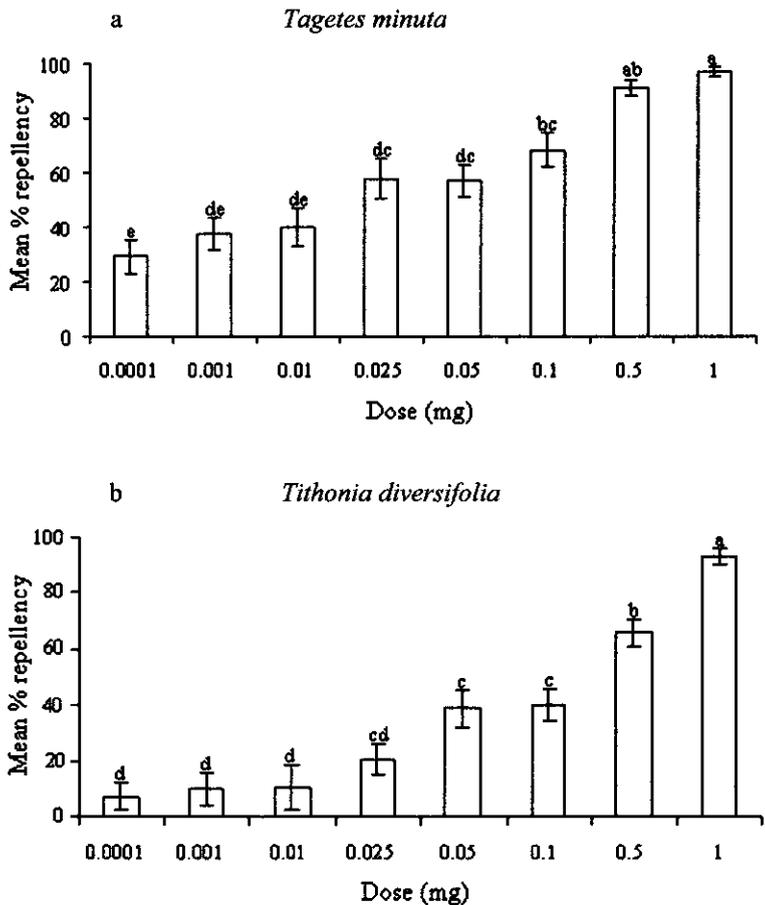
Two plants (*T. minuta* and *T. diversifolia*) were selected, based on the above results, showing relatively high repellent activities against *R. appendiculatus*. Moreover, these plants were ranked highly by traditional livestock holders (Chapter 2 and Table.3.1).

**Yield and dose response studies of essential oil of *Tagetes minuta***

The yield of the essential oil of the fresh aerial parts of *T. minuta* was 0.00029% w/w. The oil is soluble in dichloromethane (DCM), ether and ethanol and insoluble in water (El Deeb et al., 2004). The responses of *R. appendiculatus* adult ticks exposed to different doses of *T. minuta* essential oil are shown in Fig. 3.3a. The repellent effect of the essential oil at RD<sub>50</sub> and RD<sub>75</sub> levels is shown in Table 3.3, whereas the dose-response link function of the regression model was:

$$\text{Probit [P(dose1)]} = 1.1036 + 0.4132\text{dose1}$$

where dose1 = Log<sub>10</sub> (dose), and P = repellency probability function as shown in Fig. 3.4a.



**Fig. 3.3.** Mean repellency percentage of different doses of *Tagetes minuta* (a) and *Tithonia diversifolia* (b) essential oils against newly emerged adult *Rhipicephalus appendiculatus* in a dual choice assay. Means with the same letters are not significantly different at P<0.0001 (Student-Newman-Keuls test).

**Table 3.3.** Probit analysis of dose-response relationship of *Tagetes minuta* and *Tithonia diversifolia* essential oils at RD<sub>50</sub> and RD<sub>75</sub>.

Plant species	Repellence probability	Repellent dose (mg)	Upper confidence limit at 95%	Lower confidence limit at 95%
<i>Tagetes minuta</i>	0.50	0.00213	0.00237	0.00191
	0.75	0.09151	0.10124	0.08299
<i>Tithonia diversifolia</i>	0.50	0.26292	0.27120	0.25478
	0.75	0.59721	0.61157	0.58350

**Yield and dose response studies of essential oil of *Tithonia diversifolia***

The yield of the essential oil of the fresh aerial parts of *T. diversifolia* was 0.00015% w/w. The essential oil was observed to be soluble in dichloromethane (DCM), ether and ethanol and insoluble in water. The response of newly emerged *R. appendiculatus* adult ticks exposed to different doses of *T. diversifolia* essential oil are shown in Fig. 3.3b. The repellent effect of the essential oil at RD<sub>50</sub> and RD<sub>75</sub> levels is shown in Table 3.3, while the dose-response link function of the regression model was:

$$\text{Probit [P(dose1)]} = 0.6401 + 0.4962\text{dose1}$$

The repellency probability function (P) is shown in Fig. 3.4b.

**Gas chromatography analyses of the essential oils of *Tagetes minuta* and *Tithonia diversifolia***

Gas chromatography separated the chemical components in the mixtures of the essential oils of *T. minuta* and *T. diversifolia* and the representative spectral outputs are shown in Figs. 3.5a and b, respectively. The x-axis shows the retention time (RT), and the y-axis shows the intensity (abundance) of the signal. The RTs provide the qualitative aspect of the chromatogram while the chromatographic peak heights or peak areas provide the quantitative aspect of the analyte. In both the chromatograms, most chemical components that show relatively high peak heights or large peak areas are eluted in the first 30 minutes. There appear to be more different compounds in the essential oil of *T. diversifolia* than in that of *T. minuta* (Figs. 3.5a and b).

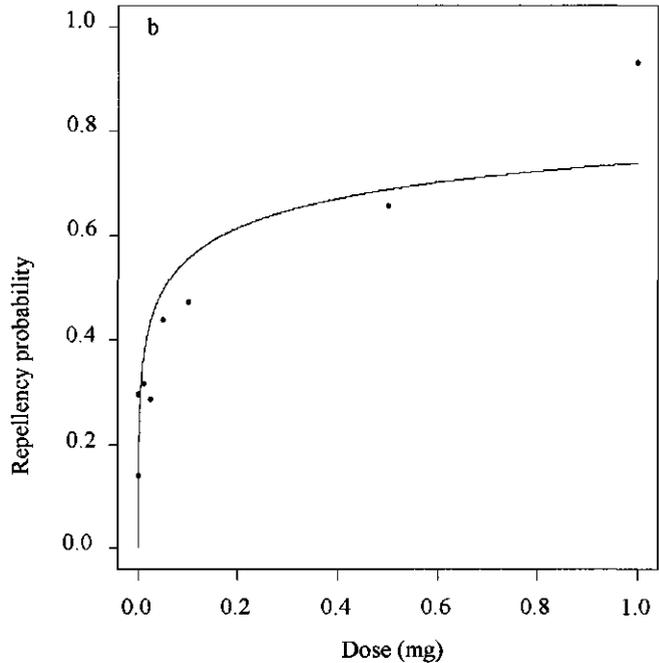
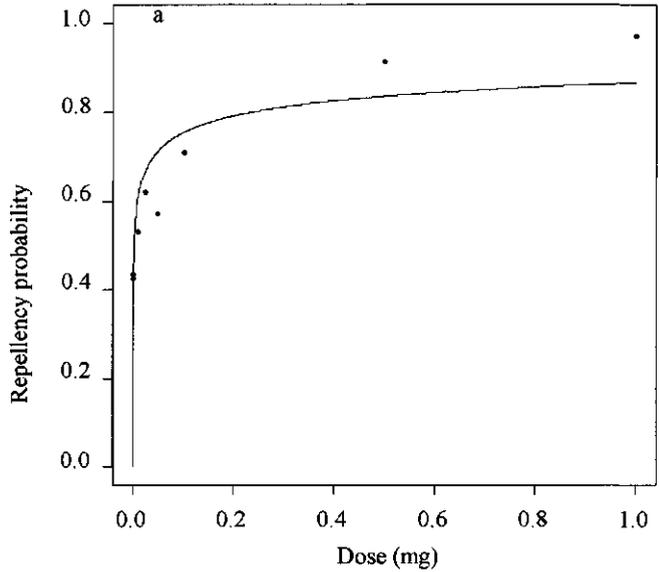
**Mass spectroscopy of the essential oil of *Tagetes minuta***

Individual components in the sample mixture of the essential oil of *T. minuta* were characterized. One hundred compounds were identified in the essential oils. Of these 100 compounds, 51% (51 compounds) were monoterpenes, 48% (48 compounds) were sesquiterpenes and 1% (1 compound) was diterpene. Thirteen of 51 monoterpenes were found in the literature to have repellence properties, while six of 48 sesquiterpenes were also found in literature to have some repellency. Of these 100 compounds, cis-ocimene occurred in the largest amount (43.78%), followed by dihydrotagetonone (16.71%), piperitenone (10.15%), trans-tagetonone (8.67%), 3,9 epoxy-p-metha-1,8 (10) diene (6.47%), β-ocimene (3.25%), cis-tagetonone (1.95%), β-caryophyllene (0.84%), bicyclogermacrene (0.62%) and AR-turmerone (0.501%) in that order (Table 3.4a).

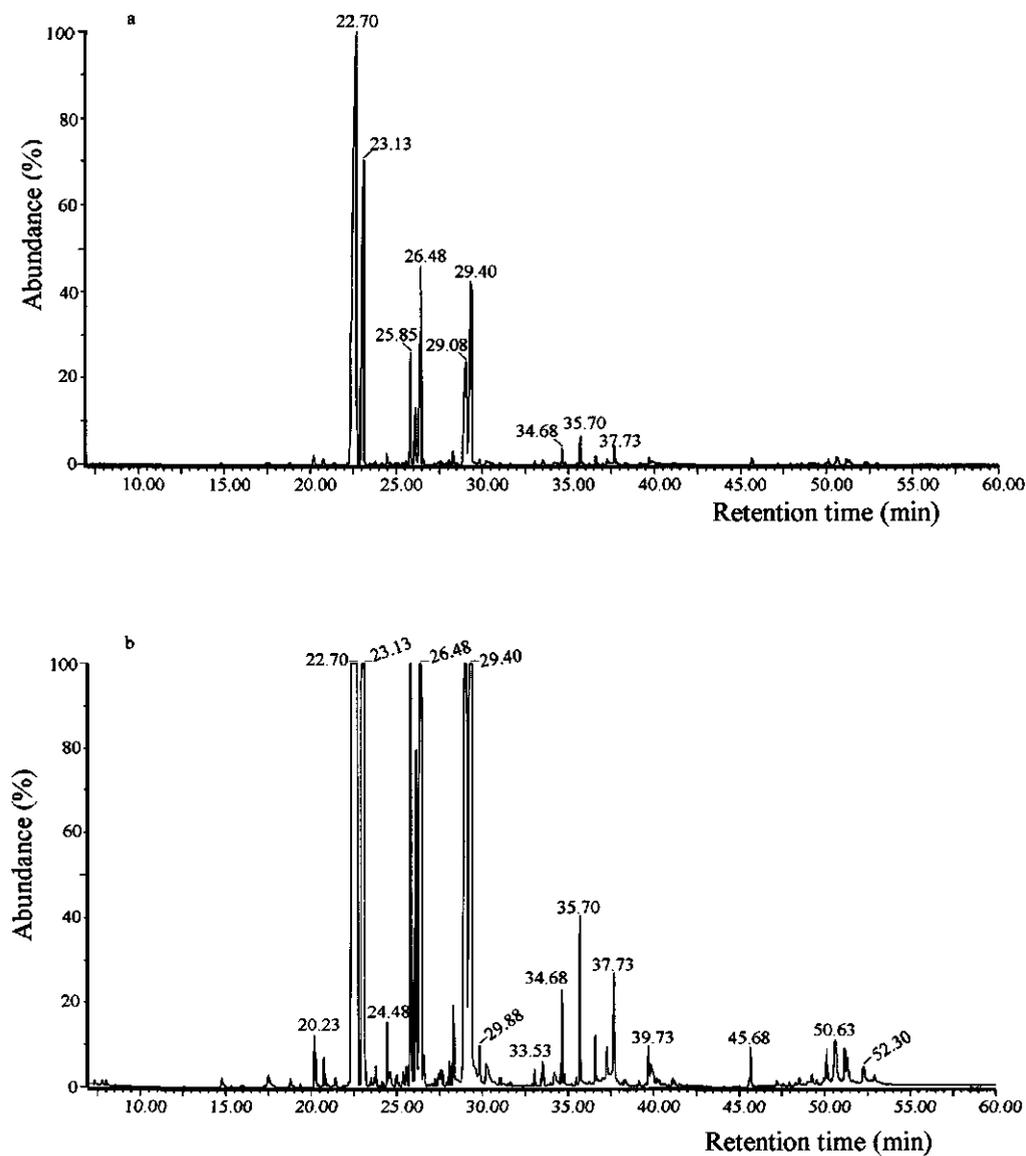
**Mass spectroscopy of the essential oil of *Tithonia diversifolia***

Individual components in the sample mixture of the essential oil of *T. diversifolia* were characterized. Fifty compounds were identified in this essential oil. Of these 50 compounds, 40% (20 compounds) were monoterpenes, while the rest 60% (30 compounds)

**Fig. 3.4.** The link function of probability repellency dose-responses with *Tagetes minuta* and *Rhipicephalus appendiculatus* as explained by the curve generated by a regression model, Probit [P (dose1)] = 1.1036 + 0.4132dose1 (a), while (b) is the link function of probability repellency dose-responses with *Tithonia diversifolia* and *Rhipicephalus appendiculatus* as explained by the curve generated by a regression model, Probit [P (dose1)] = 0.6401 + 0.4962dose1. In both models, dose1 =  $\text{Log}_{10}$  (dose), and P = repellency probability.



were sesquiterpenes. Twenty-four percent (12 compounds) of the 50 compounds and all the monoterpenes were found in the literature to have some repellence properties. Of these 50 compounds,  $\alpha$ -pinene occurred in the largest amount (63.64%), followed by  $\beta$ -pinene (15.0%), isocaryophyllene (7.62%), nerolidol (3.70%), 1-tridecanol (1.75%), limonene (1.52%), sabinene (1.00%),  $\alpha$ -copaene (0.95%),  $\alpha$ -gurjunene (0.56%) and cyclodecene (0.54%) in that order (Table 3.4b).



**Fig. 3.5.** Chromatograms generated by gas chromatography for the essential oils of (a), *Tagetes minuta* and (b), *Tithonia diversifolia*. Each of the peaks in the chromatogram represents the signal created when a compound elutes from the chromatographic column into the detector.

**Table 3.4.** GC and GC-MS identified 10 major constituent chemical compounds in the essential oil of (a) *Tagetes minuta* and (b) *Tithonia diversifolia*

(a)

Peak no.	Compound	Molecular formula	RT	M <sup>+</sup> (g/mol)	Base peak	Major peaks	Relative abundance (%)	Identification of compounds
14	<i>cis</i> -ocemene	C <sub>10</sub> H <sub>16</sub>	22.701	136.20	93	41, 79, 105, 121	43.78	GC-MS Co
15	dihydrotagetone	C <sub>10</sub> H <sub>18</sub> O	23.126	154.00	85	41, 57, 69, 97	16.71	GC-MS
42	piperitenone	C <sub>10</sub> H <sub>16</sub> O	29.401	152.00	150	41,91,107,135	10.15	GC-MS
31	<i>trans</i> -tagetone	C <sub>10</sub> H <sub>16</sub> O	26.476	152.00	95	41, 67, 109, 137, 152	8.67	GC-MS Co
41	3,9 epoxy-p-metha-1,8 (10) diene	C <sub>10</sub> H <sub>14</sub> O	29.076	150.00	135	41, 79, 122, 150	6.47	GC-MS
29	<i>β</i> -ocimene	C <sub>10</sub> H <sub>16</sub>	25.851	136.20	121	41, 79,105, 136	3.25	GC-MS Co
30	<i>cis</i> -tagetone	C <sub>10</sub> H <sub>16</sub> O	26.126	152.00	95	41, 67, 109, 152	1.95	GC-MS Co
57	<i>β</i> -caryophyllene ( <i>Trans</i> )	C <sub>15</sub> H <sub>24</sub>	35.701	204.00	41	69,93,133	0.84	GC-MS Co
61	bicyclogermacrene	C <sub>15</sub> H <sub>24</sub>	37.726	204.40	121	41, 93, 136, 161	0.62	GC-MS
93	AR-turmerone	C <sub>15</sub> H <sub>20</sub> O	50.626	216	83	41,79, 124	0.501	GC-MS

(b)

Peak no.	Compound	Molecular formula	RT (min)	M <sup>+</sup> (g/mol)	Base peak	Major Peaks	Relative abundance (%)	Identification of compounds
2	<i>α</i> -pinene	C <sub>10</sub> H <sub>16</sub>	18.975	136.24	93	39, 77, 79, 121, 136	63.64	GC-MS Co
5	<i>β</i> -pinene	C <sub>10</sub> H <sub>16</sub>	20.425	136.24	93	41, 121, 136	15.0	GC-MS Co
34	Isocaryophyllene	C <sub>15</sub> H <sub>24</sub>	35.476	204.36	93	41, 69, 105, 133, 161	7.62	GC-MS
45	Nerolidol	C <sub>15</sub> H <sub>26</sub> O	38.651	222.37	69	41, 43, 93, 107, 161	3.70	GC-MS Co
38	1-Tridecanol	C <sub>13</sub> H <sub>28</sub> O	36.876	197.00	55	43, 69, 83, 97	1.75	GC-MS Co
10	Limonene	C <sub>10</sub> H <sub>16</sub>	22.200	136.24	68	39, 41, 53, 67, 93	1.52	GC-MS Co
4	Sabinene	C <sub>10</sub> H <sub>16</sub>	20.150	136.24	93	39, 41, 77, 79, 136	1.00	GC-MS
28	<i>α</i> -copaene	C <sub>15</sub> H <sub>24</sub>	34.101	204.36	105	41, 77, 81, 93, 119, 161	0.95	GC-MS
29	<i>α</i> -gurjunene	C <sub>15</sub> H <sub>24</sub>	34.376	204.36	189	41,55,91,105,119,161,161,204	0.56	GC-MS
47	Cyclodecene	C <sub>12</sub> H <sub>22</sub>	41.251	166.00	67	41, 54, 81, 95	0.54	GC-MS

Key

M<sup>+</sup> = Molecular weight

RT = Retention time (min.)

GC-MS = identification based on comparison of mass spectra in NIST/NBS and Wiley libraries only

GC-MS Co = identification based on comparison of mass spectra in NIST/NBS and Wiley libraries followed by a comparison with retention time identical to authentic compounds



## 4

**Attractive and repellent host odours guide ticks to their respective feeding sites<sup>1</sup>**

**Abstract:** We have studied on-host behaviour of adults of the brown ear tick (*Rhipicephalus appendiculatus* Neumann, 1901) and the red-legged tick (*Rhipicephalus evertsi* Neumann, 1897), which prefer to feed mainly inside the ears and the anal regions of bovids respectively. Both species were found to be relatively successful in orienting toward and locating their respective feeding sites from different parts of the host body. Our observations suggested the operation of both avoidance (closer to the feeding site of the other) and attraction (closer to its own feeding site) responses of the ticks. In the laboratory, odour trapped from cattle ears attracted *R. appendiculatus* but repelled *R. evertsi*, whereas that from the anal region had an opposite effect. This odour-based 'push-pull' pair of stimuli may largely account for efficient orientation behaviour of the two tick species to their respective feeding sites. We propose that such concurrent deployment of repulsive and attractive cues may be quite widespread among arthropods and related organisms that specialise on specific hosts or microenvironments in the performance of their biological functions.

**Key words:** *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, feeding sites, cattle, repellent and attractive odours, push-pull

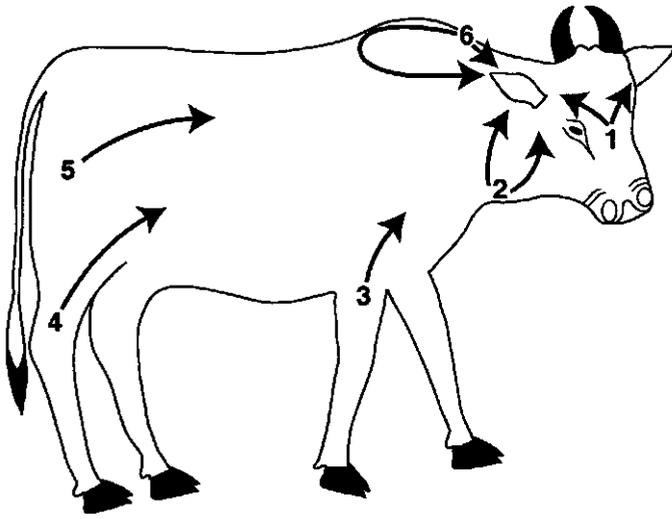
**Introduction**

Blood-feeding arthropods have evolved a variety of relationships with their mammalian hosts. Wide variations occur in host specificity, duration and multiplicity of contacts, and in host-location behaviour (Gibson and Torr, 1999). On host, related species may also demonstrate predilection for feeding at different sites. Although the signals used by some blood-feeders to locate their preferred hosts have been a subject of considerable research, only casual attention has been given to feeding site location behaviour of relevant arthropods. In this study, we report on-host behaviour of adults of two sympatric tick species, the brown ear tick (*Rhipicephalus appendiculatus* Neumann, 1901) and the red-legged tick (*R. evertsi* Neumann, 1897), which prefer to feed mainly inside the ears and the anal regions of bovids respectively (Walker, 1974).

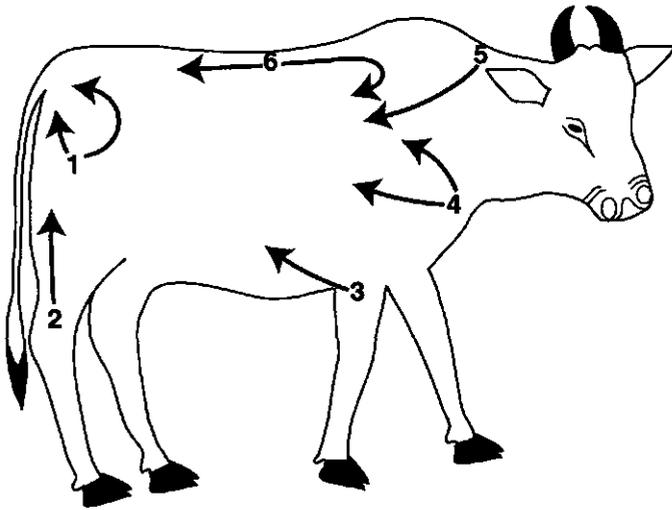
**Material and methods**

All procedures requiring experimental animals were approved by ICIPE's Institutional Animal Care and Use Committee and were performed in compliance with guidelines published by Kenya Veterinary Association and Kenya Laboratory Animal Technician Association.

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a



b

Fig. 4.1. General orientations of *R. appendiculatus* (a) and *R. evertsi* (b) that initiated movements from different release points (1–6).

**Ticks**

The two tick species used (*R. appendiculatus* and *R. evertsi*) were obtained from colonies at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. Rearing conditions and management were as described previously (Bailey, 1960; Irvin and Brocklesby, 1970).

**On-host observation studies**

On-host behaviour studies used one of two Friesian steers (body wt. 217–470 kg), with no prior exposure to ticks, held in a crush facility at ICIPE. The responses of ticks and their navigation patterns were monitored from six different body locations (Fig. 4.1a and b) of Friesian steers representing varying distance from preferred feeding sites and possible ar-

eas of alightment by the ticks from their questing positions on the vegetation (Browning, 1976). One tick at a time of mixed age and either sex was introduced at one of six sites and observed for up to 8 hours. *Rhipicephalus appendiculatus* were observed from 07.00 to 18.00 hours and *R. evertsi*, which is active at night, between 18.00 and 06.00 hours. All observations were made under dry weather with day temperatures in the range 24–28 °C and night temperatures in the range 20–24 °C. Each observation was replicated 20 times.

### Odour trapping

These were carried out using adsorbent sachets (4 x 2.5 cm) made up of stainless mesh-wire (250 mesh) containing either activated charcoal (0.5 g, 0.2 µg mesh; Chromopack, Middelburg, The Netherlands) or reverse-phase C<sub>18</sub>-bonded silica (0.2 g, 16–40 µm size; Sigma Aldrich Chemicals, Dorset, UK) similar to the prototype described previously (Gikonyo et al., 2002). The sachets were held on the inner side of ear pinna or the anal region with rubber bands for 12 h. The adsorbent from each sachet was transferred into a pasteur pipette and eluted with redistilled dichloromethane (4 ml, > 99.9%). Elutions from 60 trapping cycles were pooled, concentrated and stored at –20 °C until used for bioassays. For bioassays, an aliquot (2 mg) of the concentrate was taken up in dichloromethane (25 ml) to give ~80 ppm solution.

### Two-choice climbing assay

A set up consisting of an aluminium base (15 x 7 x 1.5 cm) with a pair of aluminium rods (26 cm long x 0.7 cm in diameter) 7cm apart covered with glass tubes (0.8 cm in diameter) was used (Fig. 3.1). A strip of filter paper (Whatmann No 7, 2 cm wide) was stapled to form a collar around the upper parts of each tube to provide the source of test odours. One collar on the pair of tubes was treated with test odour solution and the other with the solvent (dichloromethane) to serve as control. After the solvent was allowed to evaporate (10 min), these tubes were shielded with wider tubes (4.5 cm in diameter) from 4 cm above the aluminium base to facilitate relatively uniform vertical gradients of the test odours along the 3.7 cm gap between two tubes. Wet cotton wool plugs on the top of these tubes ensured relatively high humidity (>75%) within the columns. Ticks of mixed age and sex (5) were placed at the centre of the aluminium base and observed for 60 minutes. The apparatus was placed in a tray with shallow water, which prevented the dispersal of test ticks from the base. Initial comparison of the responses of the ticks in the set up with and without residual dichloromethane on one and both sides, showed no bias for either side and no effects of the residual solvent. Each assay was replicated 12 times. The number of ticks that climbed on treated and control columns were counted. Mean % attraction or repellency (–ve attraction) was calculated using the formula, percentage repellence (PR) = [(Nc – Nt)/(Nc + Nt)] x 100, where, Nc = the number of ticks that climbed on the glass rod and or above the filter paper collar strip on the control glass tube and Nt = the number of ticks that climbed on and or above the filter paper collar strip on the treated glass tube, respectively.

### Statistical analyses

On host data were analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedure for SAS for PC (SAS Institute, 1999–2000), after log (n+1) transformation. The means were compared by Student-Newman-Keuls test (Sokal and Rohlf, 1995) at P≥0.05. Two-choice assay data were analysed by Student's t-test.

**Table 4.1.** On host responses of ticks released at different body sites of Friesian steers

Body locations	% respondents	Reaction time (h)	Initial speed (cm/min)	% reach-ing feeding site	Time taken to reach feeding site (h)
<i>(a) R. appendiculatus</i>					
1.Forehead	100	0.13 ± 0.03a,b	2.00 ± 0.49a,b	95	3.74 ± 0.66a,b
2.Dewlap	80	0.58 ± 0.22 b,c	1.02 ± 0.25b,c,d	40	3.78 ± 0.95a,b
3.Fore leg (upper part)	45	0.84 ± 0.40c	0.93 ± 0.31c,d	15	(2.28 ± 0.56)
4.Rear leg (upper part)	50	0.72 ± 0.25c	0.80 ± 0.13d	20	(5.21 ± 1.41)
5.Escutcheon	85	0.06 ± 0.02a	2.12 ± 0.42a	65	5.15 ± 0.59a
6.Shoulder	85	0.24 ± 0.10 a,b	1.96 ± 0.43a,b	80	3.21 ± 0.52a,b
<i>(b) R. evertsi</i>					
1.Escutcheon	100	0.03 ± 0.01a	2.77 ± 0.21a	85	3.31 ± 0.53c
2.Rear leg (upper part)	100	0.09 ± 0.06a	1.79 ± 0.28b,c	90	5.71 ± 0.76b,c
3.Fore leg (upper part)	80	0.78 ± 0.39b	1.06 ± 0.13c,d	35	5.17 ± 1.02b,c
4.Dewlap	55	1.64 ± 0.66c	0.95 ± 0.22d	20	(9.24 ± 0.86)
5.Shoulder	70	0.08 ± 0.07a	3.04 ± 0.24a	60	6.51 ± 0.81a,b
6.Back	100	0.08 ± 0.06a	2.09 ± 0.40b	75	5.41 ± 0.71b,c

For a given column, means followed by the same letter are not significantly different from one another at  $P \geq 0.05$  (Student–Newman–Keuls test). Figures in parentheses in the last columns were not included in the statistical comparison because of the relatively low number of the ticks that arrived at the feeding sites.

## Results and Discussion

On–host observations of the ticks showed a typical sequence of behaviour involving: (i) a stationary phase at the release point, which was accompanied by outstretching of legs and adoption of a posture suggestive of scanning activity; (ii) random, seemingly exploratory movements; (iii) a clear directional movement resulting from bouts of strides and halts, interspersed with mis–turns and readjustments; and (iv) gradual arrestment closer to the feeding site followed by attachment. Interestingly, following initial random movements most of the respondents at each release point oriented toward their respective feeding sites (Fig. 4.1a and b), although, during the observation period, some appeared subsequently to lose their way. The proportion of ticks at different sites that initiated movement (respondents), the average time from placement on the steer to initial movements (reaction time), and initial speed were noted. The number of ticks that reached their feeding sites and the time between release and arrival were also recorded (Table 4.1a and b).

The results show relatively high rates of successful orientation of the ticks to their respective feeding sites and suggest the mediation of specific stimuli in the process. We first hypothesized that gradients of volatile odours from these sites may provide the appropriate orientation signals. Although this would account for lower responses (proportion of respondents, their reaction time and initial speed) of ticks deposited on locations further away from the sites (upper rear and fore legs for *R. appendiculatus*; upper fore leg and dewlap for *R. evertsi*), the behaviour of each species closer to the feeding site of the other suggested an additional effect (Table 4.1a and b). For *R. appendiculatus*, this is reflected in a relatively large proportion of the ticks that took off from the upper rear region within a

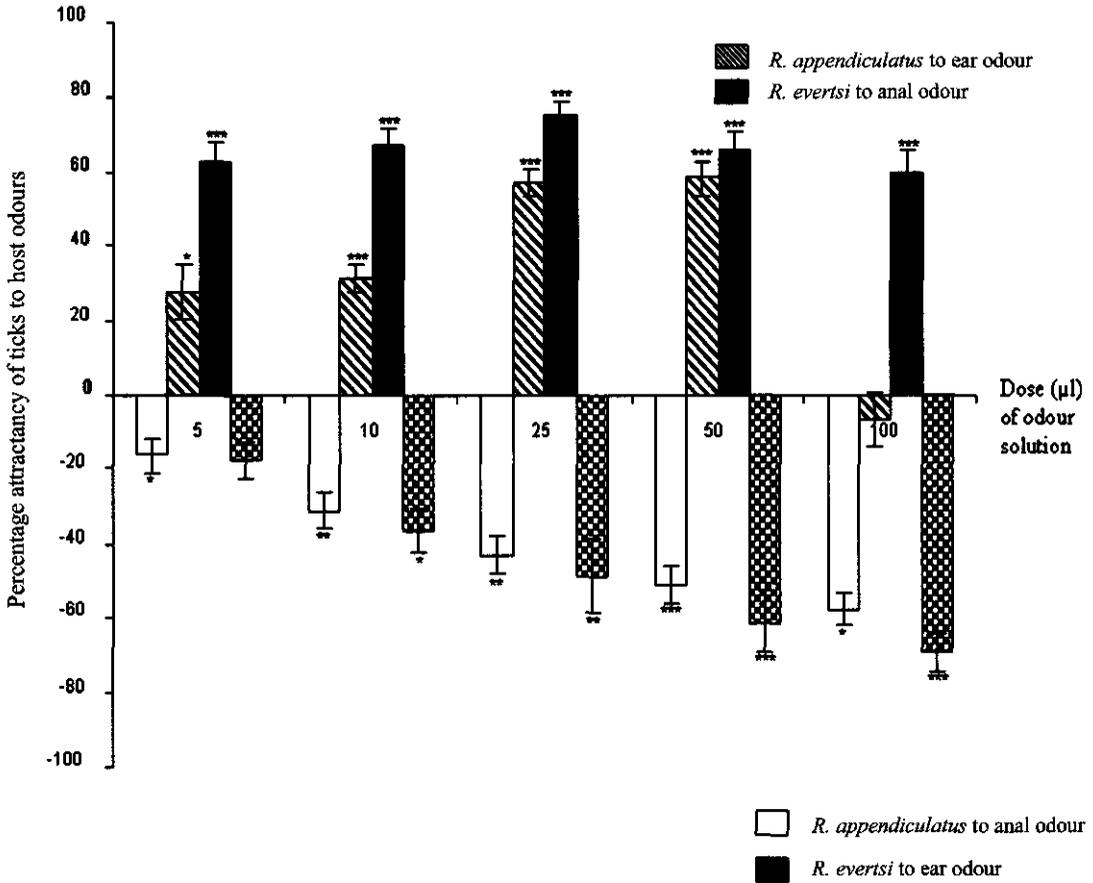


Fig. 4.2 Mean percentage attractancy or repellency of ear and anal volatiles to *R. appendiculatus* and *R. evertsi* in climbing bioassays (\*, \*\* and \*\*\* indicate statistical significance at  $P \leq 0.05$ , 0.01 and 0.001, respectively).

significantly short time ( $0.06 \pm 0.02$  h from escutcheon, compared to  $0.72 \pm 0.25$  h and  $0.84 \pm 0.40$  h from the upper rear and fore legs, respectively) at relatively high speed ( $2.12 \pm 0.42$  cm/min) comparable to conspecifics placed on the forehead close to the ears ( $2.00 \pm 0.49$  cm/min). Likewise, the reaction time and initial speed of *R. evertsi* deposited at the shoulder closer to the ear ( $0.08 \pm 0.07$  h and  $3.04 \pm 0.24$  cm/min, respectively) were not significantly different from those deposited at the escutcheon near the anal region ( $0.03 \pm 0.01$  h and  $2.77 \pm 0.21$  cm/min, respectively). Significantly, ticks of both species that were placed closer to the feeding site of other moved away and none were seen to navigate into the area during the observation period, indicating that local stimuli like temperature and/or humidity were unlikely to be primarily responsible and that the ticks were probably exhibiting an avoidance response at the sites. Accordingly, we modified our hypothesis to include the possibility of concurrent operation of both repellent and attractant effects ('push' and 'pull') in on-host orientation behaviours of the two tick species.

To verify this, we studied the responses of the ticks in the laboratory to odour collections from the ear and anal region of the steers, respectively. The bioassay design ex-

exploited the well-known predisposition of the ticks to climb up and aggregate on grass stems to await passing hosts (Browning, 1976; Chiera, 1985). A choice of two glass covered rods, one with a vertical concentration gradient of the test odour and the other with clean air was offered to groups of ticks. *R. appendiculatus* was attracted to the ear volatiles but repelled by anal volatiles (Fig. 4.2). On the other hand, *R. evertsi* was repelled by the ear volatiles, but attracted to the anal volatiles. Thus, the odour collections from the two sites have opposite effects on the two tick species and support our hypothesis on the operation of both repellent and attractant effects in the feeding site location behaviours of these ticks. However, at their respective feeding sites, other signals may also be involved in site selection process. Our observations show that closer to their respective feeding sites, the ticks are gradually arrested before finally attaching for feeding, suggesting the mediation of less volatile and/or chemotactile stimuli at these sites.

The concept of integrated use of the forces repulsion (or deterrence) and attraction (or stimulation), i.e., 'push-pull', was previously proposed as an efficient and sustainable way of diverting insect pests from a desired crop to a trap crop (Miller and Cowles, 1990). Recently, it was effectively deployed in reducing damage by stemborers to maize (*Zea mays* L.) in smallholder farms in Africa (Khan et al., 2001). The present study represents the first demonstration of the exploitation of the tactic in nature, and suggests that the phenomenon may be quite widespread among arthropods and related organisms, particularly where specialisation occurs in their interactions with their hosts or environmental niches in functions such as selection and location of hosts, feeding and oviposition.

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## 5

**Dual and no-choice assays on the effect of the essential oil of *Tagetes minuta* on *Rhipicephalus appendiculatus*<sup>1</sup>**

**Abstract:** The effects of the essential oil of *Tagetes minuta* on the climbing response of adult *Rhipicephalus appendiculatus* was studied in dual- and no-choice assays. Both assays showed a significant repellent effect of essential oil on climbing behaviour of adult *R. appendiculatus*. Although dose-dependent responses were observed in both assays, this was less clear in the dual-choice assay. Compared to the dual-choice assay, the no-choice assay underestimated the degree of repellency, particularly of low doses, implying that this assay may not be suitable for screening purposes. To achieve the same repellent effect, a higher dose is required in the no-choice assay. Reasons for the observed differences between the dual-choice and no-choice assay are discussed. The dual-choice assay appears to be a more sensitive assay than the no-choice assay.

**Key words:** *Rhipicephalus appendiculatus*, tick, behaviour, repellency, *Tagetes minuta*, essential oil, dual-choice assay, no-choice assay

**Introduction**

Ticks are increasingly becoming not only a big nuisance but a serious animal and human health risk (Tonbak et al., 2006; Vial et al., 2006; Salit, 2007; Jongejan, 2007). Among prophylactic measures used against them, botanical products (repellents, deterrents, toxicants, stimulants, arrestants and attractants) have, in the recent past, proven to offer a sustainable approach toward integrated tick control and management. In particular, plant-based repellents have been shown to protect vertebrates against tick bites (Weldon and Carroll, 2007) and have been recommended as an effective prophylactic measure against tick bites and/or tick-borne infection (Schreck et al., 1995; Okahl, 1996; Jaussaud et al., 2001; Jensenius et al., 2004; Roch et al., 2008). In the development of repellents, an effective and reliable bioassay is essential for rapid screening of candidate products (Dautel et al., 1999). The rationale for developing a reliable bioassay is to help screen large populations of candidate tick repellent botanicals accurately and identify effective ones for incorporation into tick control strategies (McMahon et al., 2003).

Worldwide, research laboratories have developed different assay apparatus to evaluate repellent/attractant properties of candidate plant products against livestock ticks (Dautel et al., 1999; Jaenson et al., 2006; Carroll et al., 2003; 2005; Garbouli et al., 2006). The assay methods employed vary a great deal but generally target the behaviour of ticks during questing for a host (Alekseev et al., 2000). Both choice and no-choice assays are widely used to study the behavioural responses of arthropods toward botanicals (repellents, deterrents, arrestants, stimulants and attractants) and synthetic products. They are also popular with studies involving other organisms, in particular insects (Howard et al., 1976;

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Mondy et al., 1998; Papachristos and Stamopoulos, 2002; Rodriguez-Saona et al., 2006). For instance, a dual-choice assay apparatus with the same scientific rationale as the one shown in Fig. 5.1a was used to test the ability of a termite to discriminate between two test chemicals and further showed that trail pheromones in *Reticulitermes virginicus* Banks, 1907, *R. flavipes* Kollar, 1837 and *R. tibialis* Banks in Banks and Snyder, 1920 were species specific (Howard et al., 1976).

Basically, three types of assay methods are commonly used for testing tick repellents. First, test substances are applied onto vertebrate hosts, which are subsequently exposed to hungry ticks and the percentage of feeding ticks and that of protection afforded are estimated (Solberg et al., 1995; Mwangi et al., 1995a). Secondly, test material is applied onto a horizontal or vertical walking path of ticks in the absence of any host cues and the percentage of ticks entering or passing the treated area is recorded and the protection percentage estimated (Mwangi et al., 1995a, b; Ndung'u et al., 1995). Thirdly, the test material is applied onto a horizontal or vertical walking path of ticks in the presence of host cues and the number of ticks entering or passing the treated area is recorded, from which the degree of protection afforded can be calculated (Alekseev et al., 2000). Using vertebrates as experimental hosts is unsuitable for routine tests with ticks because of the large number of animals required and because of the time- and cost-intensive procedures involved. On the other hand, assays without any host stimuli involved have the disadvantage that the behaviour-modifying activity of the tested material in the presence of host cues remains unknown (Schreck, 1977). Of particular interest too, is tick orientation behaviour under abiotic environmental parameters such as relative humidity, temperature and light as described by Okulova (1978) in the presence of candidate repellents/attractants and other host cues (Alekseev et al., 2000). Therefore, a suitable test system should be one that recognizes these conditions. For example, in the present study, the test system used took into account relative humidity and temperature, which have been described as attractive host-derived and environmental cues that determine the questing behaviour of ticks such as *Ixodes ricinus* L. (McLeod, 1935; Lees and Milne, 1951; Arthur, 1962; Alekseev et al., 2000). Furthermore, the test system examined the repellent activity of the essential oil of *Tagetes minuta* L. during critical behavioural steps of host finding.

Because dual-choice assays more closely represent the natural situation in the field, these are preferred to no-choice assays and are widely used (Ryan, 2002). For example, in the USA, a dual-choice filter paper assay was successfully used by Roe et al. (2006) to develop a botanical tick repellent (BioUD) against the American dog tick, *Dermacentor variabilis* Say. Similar to a dual-choice filter paper assay is a vertical assay, in which ticks are allowed to climb a vertical strip of filter paper whose central portion is treated with a repellent. This method was also used by Carroll et al. (2003) to compare the repellent properties of N,N-diethyl-3-methylbenzamide (DEET) and 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (AI3-37220) and to determine their relative effectiveness against host-seeking nymphs of the blacklegged tick, *Ixodes scapularis* Say and the lone star tick *Amblyomma americanum* L.

In South Africa, Nchu et al. (2004) compared three types of tick climbing repellent assay methods, that is, a no-choice assay, an avoidance assay and a dual-choice assay using essential oils from *T. minuta* and *Lippia javanica* (Burm.f.) Spreng. Although more ticks avoided the essential oil of *L. javanica* than that of *T. minuta*, there was no significant difference in the abilities of the three assays to test the repellency of the two oils against *Hyalomma marginatum rufipes* Koch ticks. In both no- and dual-choice assays, there was a significant increase ( $P < 0.05$ ) in repellency with increasing concentration for both *T. minuta* and *L. javanica* essential oils.

In Kenya, the dual-choice assay apparatus has been used to study the repellent effects of some botanicals against livestock ticks (Malonza et al., 1992; Mwangi et al., 1995a,b; Ndung'u et al., 1995). The assay apparatus was recently modified by inclusion of wider tubes to shield the inner climbing tubes, avoid diffusion of test materials laterally, and facilitate their more uniform gradients along the setup (Chapter 4). However, we realised that in the dual-choice assay, the choice of the first tick to climb the glass tube fitted with either an essential oil-treated or control filter paper collar may influence the final score of the test as the choice of one tick may affect that of the others. The no-choice assay was therefore proposed, as it does not have this effect and results from the essential oil treatment and control can be compared as independent data sets. In the present paper, we describe experiments to compare a dual-choice with a no-choice assay using the essential oil of *T. minuta* as the test substance to examine the behaviour of *R. appendiculatus* adult ticks.

## Materials and methods

### Legal framework of animal use

All procedures requiring experimental animals were approved by ICIPE's Institutional Animal Care and Use Committee and were performed in compliance with guidelines published by the Kenya Veterinary Association and the Kenya Laboratory Animal Technician Association (KVA and KLATA, 1989).

### Ticks

The tick species used (*R. appendiculatus*) was obtained from colonies at the International Livestock Research Institute (ILRI) and transferred to laboratories at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, for rearing and management. The rearing conditions and management were as described previously (Bailey, 1960; Irvin and Brocklesby, 1970).

### Extraction of *Tagetes minuta* essential oils

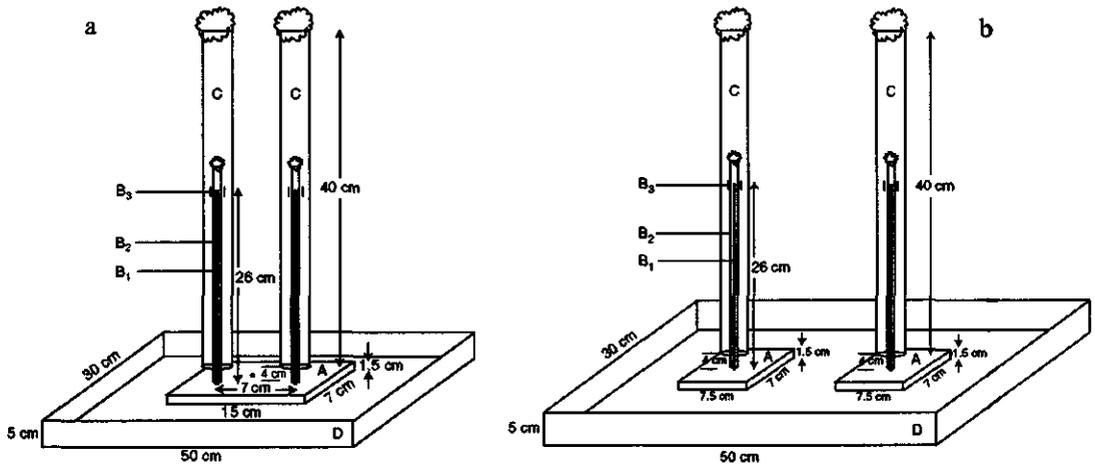
The fresh aerial parts of *T. minuta* were cut into small pieces and about 1 kg was hydrodistilled using a Clevenger-type distillation apparatus for 8 h (Sereshti and Samadi, 2007). Pure oil was collected into 2 ml- glass vials, sealed and stored at  $-20^{\circ}\text{C}$  until required for analysis and assay studies.

### The tick climbing assay methods

The two sets of tick climbing assay apparatus used included: (1) a double stranded dual-choice (Fig. 5.1a) and (2) a no-choice climbing assay apparatus (Fig. 5.1b).

### Double stranded dual-choice tick repellent climbing assay apparatus

A dual-choice tick climbing assay apparatus was used to test for tick repellency of essential oil of *T. minuta* (Fig. 5.1a). The assay apparatus exploited the behaviour of the ticks, *R. appendiculatus*, which climb up grass stems and settle for a period near the stem tip to wait for any passing potential hosts (Browning, 1976; Chiera, 1985), hence the design described previously (Chapter 4). This experiment was done according to the specification set up in the laboratory at ICIPE, Nairobi, Kenya. An aluminium base of area  $105\text{ cm}^2$  with two stands of 26 cm each in height and 7.0 cm apart were put in a basin of water, 1.5 cm deep (the water restricts the movement of the ticks to the aluminium base). The two sets of glass tubes were used, one of 4.5 cm (outer tube) and the other one 0.8 cm (smaller inner tube)



**Fig. 5.1.** Two bioassay devices to study the impact of putative repellents on tick climbing response (modified from Browning, 1976). Dual-choice climbing assay apparatus (a). For details, see Fig.3.1. For no-choice assay (b), the two aluminium rods ( $B_1$ ) on the aluminium base, A, were separated by water. The five ticks were introduced on each of the two aluminium bases at a distance of 3.5 cm from the bottom of an aluminium rod.

in diameter. A strip of filter paper (Whatmann No 7, 2 cm wide) was stapled to form a collar around the upper parts of each smaller inner tube at a distance of 20 cm from the aluminium base to provide the source of either test odours or pure solvent. One collar on the pair of the tubes was treated with test odour solution and the other one with the same amount of pure solvent (dichloromethane-DCM) alone to serve as control. After the solvent was allowed to evaporate (for about 10 min), these tubes were shielded with wider tubes (4.5 cm d) from 4 cm above the aluminium base to shield the inner ones and limit the diffusion of the test material laterally and facilitate relatively uniform vertical gradients of the odours along the 3.7 cm gap between two tubes. The upper ends of larger tubes were plugged with dry cotton wool. Wet cotton wool plugging the top of the smaller tubes ensured relatively high relative humidity (>75%) within the columns. The test materials and the solvent were dispensed by a calibrated Eppendorf pipette, equilibrated for 30 minutes and then five adult ticks of mixed ages and sexes were released at the centre of the aluminium base. Prior to each assay, ticks were kept at high relative humidity (RH) (>85% RH) for 24 h in containers with moist cotton wool.

All assays were conducted in a room of  $28 \pm 1$  °C and  $75 \pm 5\%$  RH. The room was continuously exhausted of air using a fan. The assays were left to run for 1 h, during which the number of ticks above the filter paper strip on the control glass tube ( $N_c$ ) and on the treated glass tube ( $N_t$ ) were counted and recorded after 15, 30, 45 and 60 minutes. After each test, the apparatus was thoroughly cleaned and dried at 100 °C. Initial comparison of the responses of ticks in the setup with and without residual dichloromethane on one and both sides, showed no bias for either side and no effects of the residual solvent. The repellent effect of the essential oil of *T. minuta* in dual choice assay was evaluated according to the formula adopted by Ndung'u et al. (1995) and Lwande et al. (1999) namely: percentage of repellency (PR) =  $[(N_c - N_t) / (N_c + N_t)] \times 100$ , where  $N_t$  and  $N_c$  represent the number of ticks that climbed on or passed the treated and control collar of filter papers on the glass tubes, respectively.

### **Single stranded no-choice tick repellent climbing assay apparatus**

Except for the experimental design, laboratory conditions and specifications of the single-stranded climbing apparatus were as described for the dual-choice assay (Fig. 5.1b). Two climbing rods were placed on separate bases at a distance of 27.5 cm within a tray (D) filled with tap water up to 1.5 cm deep. In each experiment, five newly-hatched and 24-hydrated adult ticks of mixed sexes were placed at a distance of 3.5 cm from the base of aluminium rod, B<sub>1</sub> (Fig. 5.1b). Here, the assays were also left to run for 1 h and the number of ticks above the filter paper strip on the control experiment glass tube (Nc) and on the treated experiment glass tube (Nt) were counted and recorded after 15, 30, 45 and 60 min.

The effect of the essential oil of *T. minuta* was evaluated by comparing the climbing response of ticks on the glass tube fitted with a filter paper collar treated with dichloromethane (control) with those climbing the glass tube fitted with a filter paper collar treated with the oil.

### **Choice of the type and dose(s) of the essential oils used**

In preliminary dose response assays with the essential oils of *T. minuta* and *Tithonia diversifolia* (Hemsl.) A. Gray, the former showed a higher repellent effect against adult *R. appendiculatus* than the latter (Chapter 4). The essential oil of *T. minuta* was therefore selected for comparison assays at the 0.025, 0.1, and 1 mg doses. In the no-choice assay a dose of 2.25 mg of oil was also tested.

### **Data management and analysis**

#### **Climbing assay data**

Data were entered into a Statistical Products and Service Solutions (SPSS version 11.1 for Windows) spreadsheet database and analysed. A one-way analysis of variance (ANOVA) and Univariate analysis of SPSS were used to compare means between doses and over time. The means were separated using Student-Newman Keuls test at  $P = 0.05$  (Zar, 1996). Using an independent samples t-test, the mean percentage of climbing ticks in the essential oil-treated experiment was compared with the ticks climbing the control glass tube whose collar of filter paper was treated with dichloromethane in the no-choice assay (Dixon and Massey, 1969).

### **Results**

#### **Evaluation of repellent property of *Tagetes minuta* essential oil using a dual-choice assay**

The results of the dose-dependent response of newly emerged adult *R. appendiculatus* to the essential oil of *T. minuta* in a dual-choice climbing assay apparatus are shown in Tables 5.1 and 5.2. For a given dose and time of observation, there were varying degrees of dose- and time-dependent responses, respectively. In the first 15 minutes, there was a significant difference between mean percentage of repellencies caused by different doses of essential of *T. minuta* ( $P = 0.006$ ). Thereafter, with the exception of 45th minute's observation ( $P = 0.036$ ), there was no significant difference between the mean percentage of repellencies caused by different doses of the essential oil of *T. minuta* ( $P > 0.05$ ). There was no significant difference between the mean percentage of repellencies caused by lower doses of essential oil of *T. minuta* over time ( $P > 0.05$ ). In the highest dose of essential oil of *T. minuta* (1 mg), there was a significant difference between the mean percentage of repellencies caused by the dose over time ( $P = 0.017$ ) (Student-Newman-Keuls test) (Table 5.1).

**Table 5.1.** The mean ( $\pm$  SE) percentage of repellency caused by *Tagetes minuta* essential oil over time and at different doses using the dual-choice assay (n = 5).

Essential oil doses (mg)	Time (min)				P-values
	15	30	45	60	
0.025	46.7 $\pm$ 15.07b1	66.7 $\pm$ 14.05a1	56.7 $\pm$ 15.24bc1	61.7 $\pm$ 13.94a1	0.795
0.1	51.7 $\pm$ 13.71b1	72.0 $\pm$ 12.27a1	72.0 $\pm$ 12.27b1	78.0 $\pm$ 12.09a1	0.483
1	100.0 $\pm$ 00a1	100.0 $\pm$ 00a1	100.0 $\pm$ 00ab1	88.0 $\pm$ 6.11a2	0.017
P - values	0.006	0.81	0.036	0.263	

Within a column, means with the same letter(s) and across a given row, means with the same number(s) after the letter(s) are not significantly different at P = 0.05 (Student-Newman-Keuls test), respectively.

**Table 5.2.** Overall mean ( $\pm$  SE) percentage of repellency of the essential oil of *Tagetes minuta* obtained using a dual-choice assay

Doses of essential oil of <i>Tagetes minuta</i> (mg)	Mean percentage of repellency
0.025	57.9 $\pm$ 7.11b
0.100	68.4 $\pm$ 6.26b
1.000	97.0 $\pm$ 1.69a
P-values	<0.05

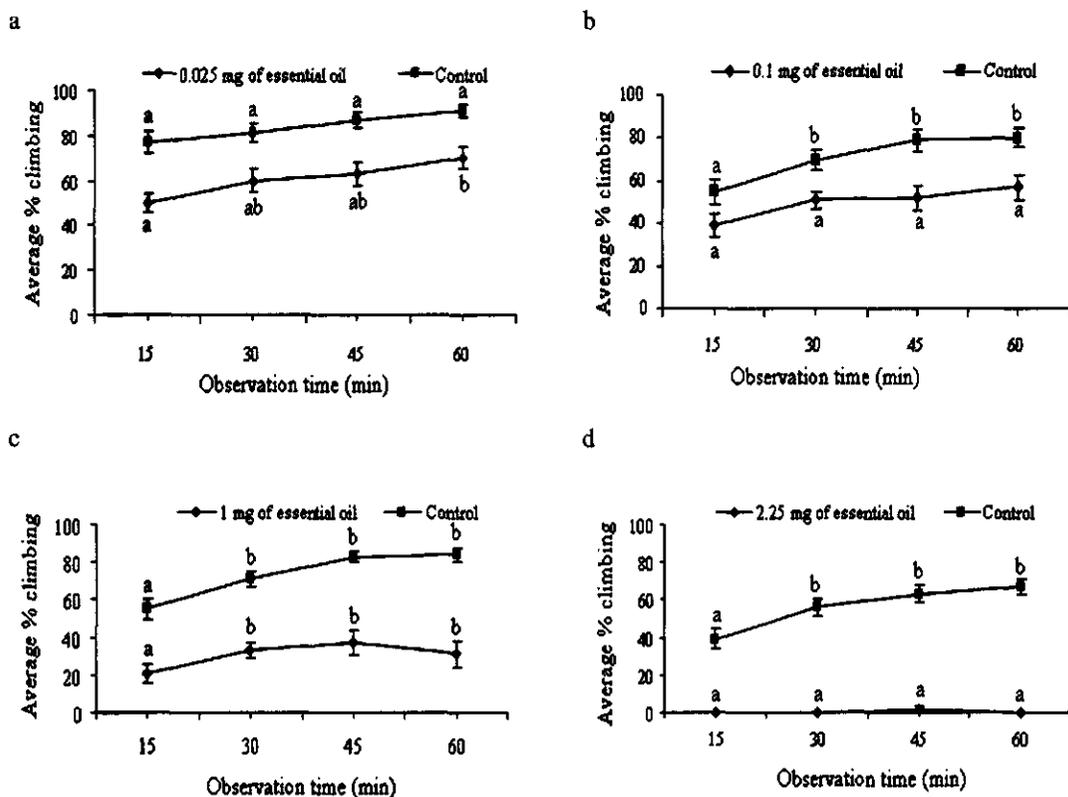
Within a given column, means ( $\pm$  SE) with the same letter(s) are not significantly different at P = 0.05 (Student-Newman-Keuls test).

This assay did not show a clear trend of time-dependent responses of adult *R. appendiculatus* to the essential oil of *T. minuta* oil. However, the assay shows a clear trend of dose-dependent tick climbing responses (Table 5.2).

#### Evaluation of repellent property of essential oil of *Tagetes minuta* using a no-choice assay

The results reporting the mean percentage of climbing ticks with respect to the doses of the essential oil of *T. minuta* and time of observation in the no-choice assay are shown in Fig. 5.2. These results suggest a time-dependent response in which the average number of ticks climbing up the glass tube in the presence of essential oil or dichloromethane is a function of the time taken for observation, i.e. the percentage of ticks climbing increased with time (Fig. 5.2). Using an independent samples t-test, the mean percentage of climbing ticks in the essential oil-treated experiment was compared with the ticks climbing the control glass tube whose collar of filter paper was treated with dichloromethane. At any one given time, significantly more ticks climbed up the control glass tube than the oil-treated tube for every observation made at all doses of essential oil (P<0.05)(Table 5.3).

The mean percentage of ticks climbing the glass tube fitted with an oil-treated filter paper collar decreases significantly with increasing concentration of the essential oil of *T. minuta* (P<0.05) (Fig. 5.2 and Table 5.3). At the highest dose of the essential oil, the repellent effect on *R. appendiculatus* was greatest (Table 5.3) and maintained for a longer period of time than with the other doses (Fig. 5.2d). However, at the lower doses (0.025 and 0.1 mg) there was an increasing number of ticks climbing up the essential oil-treated glass tube over time. The gap between the control and essential oil-treated experiments widened with increasing concentration of the essential oil over time for all doses except 0.025 mg. Thus the variables, time, dose and repellent effect of the essential oil are interac-



**Fig.5.2.** Mean climbing response (%) over time of different doses of essential oil of *Tagetes minuta* with adult *Rhipicephalus appendiculatus* using a no-choice assay. The figures from (a) to (d) represent doses of essential oil of *Tagetes minuta* exposed to newly emerged *Rhipicephalus appendiculatus* in a no-choice climbing assay (n = 5). For either control or essential oil treatment, the means ( $\pm$  SE) with the same letters are not significantly different from one another at  $P = 0.05$  (Student-Newman Keuls test).

tive. The trend of more ticks climbing over time was also noted in the controls (Fig. 5.2) and significantly higher than in the essential oil-treated experiment at all observation times for all the doses ( $P < 0.05$ ) (Table 5.3).

Between doses of the essential oil of *T. minuta*, mean percentages of ticks climbing the glass tube fitted with a treated collar of filter paper were significantly different from each other ( $P < 0.05$ ) (Table 5.3). With the 0.025 mg dose of essential oil, the mean percentage of ticks climbing the glass tube after the first 15 minutes was significantly different ( $P = 0.041$ ) from the subsequent mean percentage of ticks climbing the glass tube thereafter, between 30 and 60 minutes (Fig. 5.2). Within a 60-minute observation period, the mean percentages of ticks climbing the glass tube whose collar of filter paper is treated with the doses 0.1 mg, 1 mg and 2.25 mg of the essential oil of *T. minuta*, were not significantly different from one another at  $P = 0.087$ ,  $P = 0.279$  and  $P = 0.106$ , respectively (Fig. 5.2). While in the control more ticks climbed up the glass tube than in the essential oil-treated experiment, there were some significant differences in the percentage of ticks climbing up the glass tube between the observation periods ( $P < 0.05$ ) except for 0.025 mg dose ( $P = 0.067$ ).

**Table 5.3.** The mean ( $\pm$  SE) percentage of ticks climbing a glass tube fitted with a collar of filter paper treated with either the essential oil of *Tagetes minuta* or dichloromethane (control) in a no-choice assay

Doses of essential oil of <i>Tagetes minuta</i> (mg)	Mean percentage of ticks climbing a glass tube		
	Essential oil treatment	Control	P-values
0.025	60.7 $\pm$ 2.53a1	84.0 $\pm$ 2.03c2	<0.05
0.100	49.8 $\pm$ 2.59b1	71.0 $\pm$ 2.71b2	<0.05
1.000	30.5 $\pm$ 3.00c1	73.8 $\pm$ 2.39b2	<0.05
2.250	0.5 $\pm$ 0.35d1	56.8 $\pm$ 2.62a2	<0.05
P-values	<0.05	<0.05	

Within a given column, means ( $\pm$  SE) with the same letter(s) are not significantly different at  $P = 0.05$  (Student-Newman-Keuls test). In a given row, means ( $\pm$  SE) with the same number(s) after the letter(s) are not significantly different at  $P = 0.05$  (Student-Newman-Keuls test).

### Discussion and conclusion

The two assay methods investigated in this study showed a significant repellent effect of the essential oil of *T. minuta*, even though the oil was not tested in the presence of host-derived stimuli as suggested by Dautel (2004; Dautel and Cranna, 2006). The repellent effect of the essential oil of *T. minuta* in the presence of a live host was demonstrated, however, in Chapters 6, 7 and 8 and we conclude that this essential oil affects adult *R. appendiculatus* under various circumstances. Koschier and Sedy (2003) also showed a significant repellent effect of the essential oils from plants within the Lamiaceae family against *Thrips tabaci* Lindeman using the dual-and no-choice assays. The effect of the oil was less in the no-choice assay compared to the dual-choice assay, particularly with low doses, implying that this assay method may be less suitable for screening purposes (Ryan, 2002). This is because the no-choice assay may not be able to identify plants whose essential oils have low repellent activity and is therefore less discriminatory. In the no-choice assay, high values of the repellent effect comparable to those obtained in the dual-choice assay were only achieved with a high dose (2.25 mg) of the essential oil of *T. minuta*. In contrast to our predictions, therefore, using a no-choice tick climbing apparatus to study the effects of repellent of the oil did not increase treatment accuracy; instead, it was time consuming and the mean repellent effects were comparatively low.

Although the two experimental set-ups are two different designs, the mechanism by which these assays exert their influence on tick climbing behaviour, however, remains unknown. The advantage of the no-choice assay is that there is no interaction between the ticks climbing the essential oil-treated and dichloromethane-treated (control) glass tubes, as each is separately offered to the ticks (as an independent experiment). Because ticks in the treatment with the essential oil and in the controls are tested independently, the overall result of the assay should reflect the true effect of the oil on the ticks better than in the dual choice assay, where ticks responding to the oil and control may affect each other. We realise, that to obtain a better estimate of this behaviour, in the no-choice assay the behaviour of the ticks should ideally be examined on single individuals as potential aggregating effects may affect the end result (Sonenshine, 2006). Also, it seems better if the distance between the two climbing set-ups is much larger, or that tests are conducted in different rooms to exclude potential effects of treatment odour(s).

The equation used in this study to generate the data for the dual-choice assay does not, however, recognize (a) the varied interactions involved such as (1) influence of the

test odour from the treated filter paper collar and (2) test organisms that keep on climbing on the control and treated glass tubes and vice versa before making a final decision, (b) test organisms that show the test material to confer either true repellent effects or excito-repellent/irritant effects, (c) test organisms that drop off in water and drown and (d) non-responding test organisms, which were initially part of the entire interaction and original population ( $n = 5$ ). Although these factors are not considered in the equation, they influenced the number of ticks that climbed the glass tube fitted with filter paper collar treated with either dichloromethane or the essential oil of *T. minuta*. In a no-choice assay apparatus, the interaction (1) above is minimised while interaction (2) is completely removed. Nevertheless, factors b, c and d above equally affect the results obtained by both tick climbing assay apparatus. This could be the reason why the results showed the same pattern of increase in repellent effect with increasing concentration, as in the results obtained by Nchu et al. (2004; 2005). But this small difference emanating from interaction (2) above may not be sufficient to explain the low values obtained when a no-choice assay apparatus alone is used.

It is possible that from the experimental-design point of view in both assays, there might be an aggregation effect amongst interacting ticks due to pheromones (Sonenshine 2006). This pheromonal influence may be having an effect on the climbing behaviour and other intraspecific interactions of *R. appendiculatus* ticks in both dual-and no-choice assays (Sonenshine, 1985). This aggregation behaviour of *R. appendiculatus* has also been observed in the laboratory (Browning, 1976) and field (Chiera, 1985) and is attributed to pheromones (Sonenshine 2006). Possibly, the pheromonal effect may be suppressed by the essential oil in both assays. This suppression may be greater in the dual-choice assay than in the no-choice assay due to the fact that the two glass tubes fitted with treated filter paper collar in the former assay are close to one another. This pheromonal effect may be removed if ticks are observed one at a time and between observations, the assay apparatus are rinsed with 99.98% alcohol.

Although the dual-choice assay set up appears to be effective, it requires, during statistical analysis of data generated by the equation ( $PR = [(Nc - Nt)/(Nc + Nt)] \times 100$ ), a statistical model that includes all the existing variations and factors mentioned in the paragraphs above in order that the absolute repellency is estimated with respect to these variations and factors. However, in a dual-choice assay, data are pooled after employing the equation ( $PR = [(PR) = [(Nc - Nt)/(Nc + Nt)] \times 100$ ), and therefore, this does not put into consideration the time over which the observations were made. A no-choice set up generates data that gives more comprehensive information on the behaviour of the ticks in response to the essential oil of *T. minuta* over time and within doses than the dual-choice set up, especially about the interaction of the variables selected for analysis. However, the no-choice assay does not simulate a natural field situation of freedom of choice (Ryan, 2002), thus changing the behaviour of test organisms, a situation that makes it a less efficient testing device (Huang et al., 2003; Adebowale and Adedire, 2006).

In the no-choice assay, more ticks climbed the control rod than the essential oil-treated rod for all doses of the essential oil of *T. minuta* over time, and this difference was significant. This suggests a significant repellent effect of the essential oil on climbing behaviour of *R. appendiculatus*. Over time, the gap between the number of ticks climbing the control and the essential oil-treated glass tubes in the no-choice assay widened with increasing concentration of the essential oil of *T. minuta*. This implied significant dose-and time-dependent responses of *R. appendiculatus* adult ticks to the essential oil. Such consistent significant responses were not obtained with the dual-choice assay, except for dose responses. Thus, in the no-choice assay, the variables "time" and "repellency" were inter-

active. The average number of ticks affected by the repellent effect of the oil was a function of the time of exposure of ticks to the oil. In the first 15 minutes, the repellent effect was relatively high and thereafter this reduced with time. The reduction in repellency was greater with lower doses than with higher ones. The higher doses tended to maintain a higher repellent effect against ticks for a longer period of time than the lower ones. This trend of the results is comparable to that obtained by Dolan et al (2008) when testing essential oil (lemon, picaridin and nootkatone) against *I. scapularis* in vertical, finger and horizontal assays. However, these results were different from the results obtained with a dual-choice assay, implying that the two assays manifest different patterns of behavioural responses. The trend in which the average number of ticks climbing up the glass tube increased over time in the no-choice assay was also noted in the controls. Whether this trend reflects the natural tick climbing behaviour or not, is yet to be confirmed as the control material (dichloromethane) was not shown to cause any behavioural effects. It is possible that this behaviour was caused by increasing degrees of nutritional depletion, resulting in stronger behavioural responses.

It is concluded that both assay methods tested in this study provide baseline data, against which novel livestock tick repellents can be examined and selected for field-testing and subsequent development. The dual-choice assay proved a more sensitive assay than the no-choice assay.

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## **Part III**

### **Field evaluation of essential oils**



## 6

**The effect of essential oils of *Tagetes minuta* and *Tithonia diversifolia* on on-host behaviour of the brown ear tick *Rhipicephalus appendiculatus*<sup>1</sup>**

**Abstract:** On-host behaviour of *Rhipicephalus appendiculatus* was studied in the field 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:** *Rhipicephalus appendiculatus*, on-host orientation behaviour, predilection feeding site, cattle, repellent, *Tagetes minuta*, *Tithonia diversifolia*, essential oils

**Introduction**

Ticks and tick-borne diseases (TBDs) are a major constraint for the development of the 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* and transmitted by *Rhipicephalus appendiculatus*, is considered to be the most significant tick-borne disease of cattle. The parasite infects cattle as well as buffalo (*Syncerus caffer*) in 12 countries of the region and 25 million cattle are at risk. One million cattle are estimated to have died of ECF in 1989 alone (Mukhebi et al., 1992).

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 sub-Saharan Africa, in conjunction with control of animal movement, quarantine and slaughter

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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, illegal cattle movement, civil unrest, poor management and inadequate maintenance of dips, especially communal ones. The devastating extent of recurring prolonged drought 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 be-

haviour, 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 (Chapter 4). 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-dichlorophenol, 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.

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 bovinds, 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 *T. minuta* and *T. diversifolia* on this behaviour in natural field sites in Bungoma District, western Kenya.

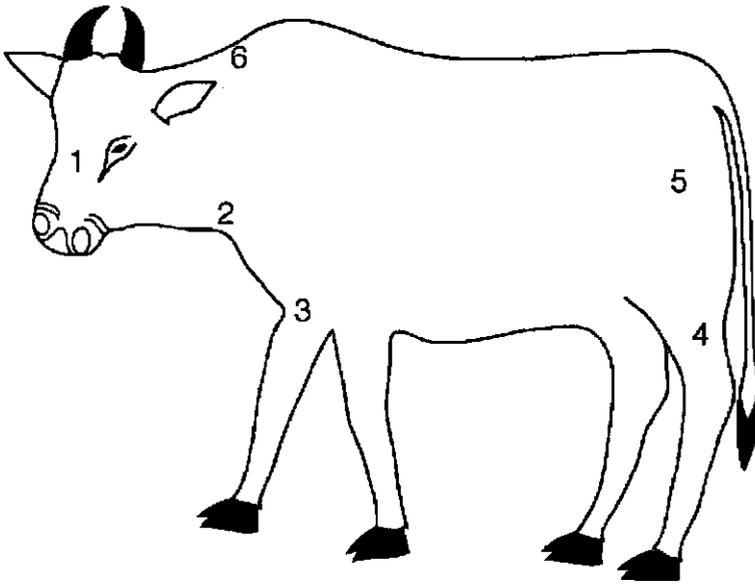
## Material and methods

### The study site

The experiments were conducted under outdoor conditions at the study site in Bungoma District, western Kenya, which lies between latitude 0°25'N and 0°53'N and longitude 34°21'E and 35°04'E (Fig. 2.1). The altitude ranges from 1200–3500 m above sea level. The mean annual temperature ranges from 21 to 22 °C with variations in mean maximum and minimum temperature ranging from 25 to 32 °C and 11 to 13 °C, respectively. Rainfall in the district has a bimodal pattern and varies on average from 1000–2000 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



**Fig. 6.1.** Sites on the host animal (bovid) used for the study of on-host navigation behaviour of *R. appendiculatus* towards 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.

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. The animals had not been exposed to acaricides or other chemicals. The animals were held in a crutch facility built at Mwibale. 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 (WW) from preferred feeding sites and possible areas of alightment by the ticks from their questing positions on the vegetation (Browning, 1976) (Fig. 6.1). 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 and 24:00 hours (Fig. 6.1). All observations were made during dry weather with day temperatures in the range of 24–28 °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.

### 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 (Sereshiti 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<sup>3</sup>–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) (Fig. 6.1). 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 District Veterinary Officer (DVO) of Bungoma District, western Kenya. The importance, seriousness and risk-free nature of the project were further explained to the Bukusu community by the DVO 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  $P = 0.05$  (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 (Chapter 4), 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 6.1 and 6.2).

Both release points and treatments (the essential oils of *T. minuta* and *T. diversi-*

**Table 6.1.** 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 <sup>1</sup>	No. of ticks that dropped off	Percentage of non-responding ticks <sup>2</sup>
1. Forehead	Control	10	7	2	1	0
	Tm	6	0	4	10	0
	Td	6	1	1	11	5
2. Dewlap	Control	6	7	2	0	25
	Tm	2	0	6	12	0
	Td	8	0	6	6	0
3. Foreleg	Control	4	3	9	3	5
	Tm	1	0	7	5	35
	Td	5	0	4	5	45
4. Rear leg	Control	2	1	6	0	55
	Tm	0	0	4	4	60
	Td	4	0	4	0	60
5. Escutcheon	Control	5	9	4	0	10
	Tm	2	0	11	7	0
	Td	5	0	12	3	0
6. Shoulder	Control	5	10	3	1	5
	Tm	1	0	8	11	0
	Td	4	0	4	12	0

<sup>1</sup>NRP—Neighbourhood of Release Points (body locations).

<sup>2</sup>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.

*folia* 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) = 8.972, 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 6.1). 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 by the treatment with the petroleum jelly (Table 6.1). 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 6.1).

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 6.2). 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 6.2). 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 6.2).

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 6.2).

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 6.2).

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 those that 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., 2007b).

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 Chapter 4 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.

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*. However, 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* A.DC. 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; Garbouli 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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## 7

**Essential oils of *Tagetes minuta* and *Tithonia diversifolia* affect orientation to cattle and attachment site preference of the brown ear tick, *Rhipicephalus appendiculatus*<sup>1</sup>**

**Abstract:** The effects of essential oils of *Tagetes minuta* and *Tithonia diversifolia* on orientation behaviour and attachment site preference of newly hatched adult *Rhipicephalus appendiculatus* on cattle were evaluated. Host animals were treated at the ear pinna (by smearing the oil directly on the ear or suspending a tube containing the oil on the ear pinna) and legs + tail in semi-field plots. The legs + tail sites of the essential oil application showed the lowest mean percentage of ticks observed on the body of the host ( $16.5 \pm 1.9\%$  and  $26.0 \pm 2.8\%$ ) and the highest mean percentage reduction of attaching ticks ( $76.5 \pm 3.9\%$  and  $67.0 \pm 0.8\%$ ) for the essential oils of *T. minuta* and *T. diversifolia*, respectively. The control animals had the highest mean percentage of ticks observed ( $93.0 \pm 2.1\%$ ). The ear tube resulted in the highest mean percentage of ticks on the host ( $47.5 \pm 5.1\%$  and  $55.8 \pm 5.1\%$ ) and a lowest mean percentage reduction of attaching ticks ( $44.8 \pm 5.1\%$  and  $36.5 \pm 7.4\%$ ) for the essential oils of *T. minuta* and *T. diversifolia*, respectively. For both the essential oils, legs + tail sites of essential oil application, followed by ear smear and then ear tube, had significant effects on orientation to the host and attachment site preference of adult *R. appendiculatus* on the host, in that order. As treatment of legs + tail is tedious, time consuming, and requires more essential oil than the other two sites, we recommend the ear smear site for treating hosts with essential oil. Better performance of the two essential oils might be obtained by increasing their concentration and by formulating them in a carrier material that stabilizes the active ingredients.

**Key words:** host-seeking *Rhipicephalus appendiculatus*, behaviour, semi-field, essential oils, *Tagetes minuta*, *Tithonia diversifolia*, repellents, cattle

**Introduction**

Ticks and tick-borne diseases continue to be a major constraint to livestock production and development, particularly in the tropics (McCosker, 1993; Slingenbergh et al., 2002; Moyo and Masika, 2008). The infestation of ticks on livestock and the resulting tick-borne diseases pose serious socio-economic problems (Jonsson et al., 1998; D'Haese et al., 1999; Makala et al., 2003; Peter et al., 2005; Muchenje et al., 2008).

The current conventional method of tick control relies mainly on the application of chemical acaricides. However, this method is associated with a number of problems including environmental pollution, chemical residues in food products as well as in wool,

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development of tick resistance and high costs of acaricides. The use of acaricides does not prevent the ticks already attached from transmitting the disease-causing pathogens. In addition, acaricide application only targets 5% of the total tick population in any given environment, while 95% of ticks, which are present on the vegetation, are left unaffected. Adult ticks can survive for more than a year without a blood meal, particularly in dense bush and rainforest (Lans, 2001). These ticks become a source for re-infestation of host animals during grazing. A method and product that continuously keep ticks away from the hosts are therefore desirable. Among tick control strategies under consideration to that effect are prophylactic measures that prevent contact between ticks and host animals, i.e., repellents. Repellents provide a practical means of protection against tick bites and along with vaccines, they may be a fundamental resource for minimizing the transmission of tick-borne diseases at an individual level (Staub et al., 2002).

Repellent compounds in various commercial formulations are available for tick bite prevention. There is concern however, about possible adverse effects of some of these compounds on human health and non-target species in the environment (Abdel-Rahman et al., 2001). For these reasons, the development of novel repellents can be of great value. Repellent effects of some plants and plant products on the brown ear tick *Rhipicephalus appendiculatus* have been demonstrated (Dipeolu and Ndungu, 1991; Malonza et al., 1992; Mwangi et al., 1995a, b; Lwande et al., 1999; Ndung'u et al., 1999). A 10% concentration of the essential oil of *Ocimum suave* in liquid paraffin was found to protect rabbits against the attachment larvae of *R. appendiculatus* and repelled more than 70% of adult *R. appendiculatus* for 5 days (Mwangi et al., 1995a; ICIPE, 1998/99). A neem oil concentration of 25% applied directly to the skin of hosts repelled all stages of *R. appendiculatus* and showed some antifeedant activities (ICIPE, 1997). *Tagetes minuta* and *Tithonia diversifolia* have been used traditionally to control livestock ticks in Central Kenya (Njoroge and Bussmann, 2006).

In the present study, an approach providing protection against *R. appendiculatus* bites on hosts was explored. The effects of essential oils of *T. minuta* and *T. diversifolia* on host location and attachment site preference of newly hatched adult *R. appendiculatus* on hosts treated on the ear (smearing oil on the ear or suspending a tube containing oil on the ear) and legs + tail are evaluated.

## Materials and methods

### Experimental host animals

A group of nine indigenous zebu cattle (*Bos indicus*) was purchased from livestock farmers in Bungoma District, western Kenya. Details of the area, such as the tick population, cattle diseases, climate and vegetation are described in Chapter 3 of this thesis. Theileriosis is endemic in the study area and there are large populations of alternative wild animal hosts for cattle ticks and diseases. The experimental cattle used had been reared under traditional management in Bungoma District. Some of the animals were purchased during a market day from the Bukusu livestock farmers as steers aged between 16½ to 18 months, while others were oxen used in the farms for ploughing. According to farmers' records, these animals had been immersed weekly in various types of acaricides used indiscriminately in the area for the control of ticks. Records at Bungoma District Veterinary Office (DVO) indicated that acaricides used included organochlorine compounds [lindane (0.5% to 25% v/v), dieldrin (0.55% w/v) and toxaphene (0.25-0.3% w/v)], organophosphate compounds [chlorpyrifos (0.02% v/v), chlorfenvinphos (0.05% v/v), coumaphos (0.1% v/v), dioxathion (0.075% w/v) etc.], carbamate acaricides [e.g., carbaryl (0.2% w/v), promacyl

(0.15% w/v)], pyrethrins (0.02% w/v), pyrethroids (permethrin, decamethrin, deltamethrin, cyhalothrin, cyfluthrin, flumethrin etc.) (0.02% - 0.05% w/v), and the commonly used formamidine (amitraz (0.025% w/v)].

### Experimental ticks

The tick species used (*Rhipicephalus appendiculatus*) was obtained from a colony reared in the insectary at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. This colony was initially obtained from the rearing unit at the International Livestock Research Institute (ILRI), Nairobi, Kenya. The colony at ILRI had been established from collections at Muguga in Kiambu District, Kenya and reared under laboratory conditions since 1952 using the methods described by Bailey (1960). At ICIPE, *R. appendiculatus* were bred on New Zealand white rabbits as described by Bailey (1960). Rearing conditions and management were as described previously (Bailey, 1960; Irvin and Brocklesby, 1970). The newly hatched adult *R. appendiculatus* were transported in a coolbox on moist sand from ICIPE to Bungoma (a distance of about 500 km), and used within a 48-h period after hatching.

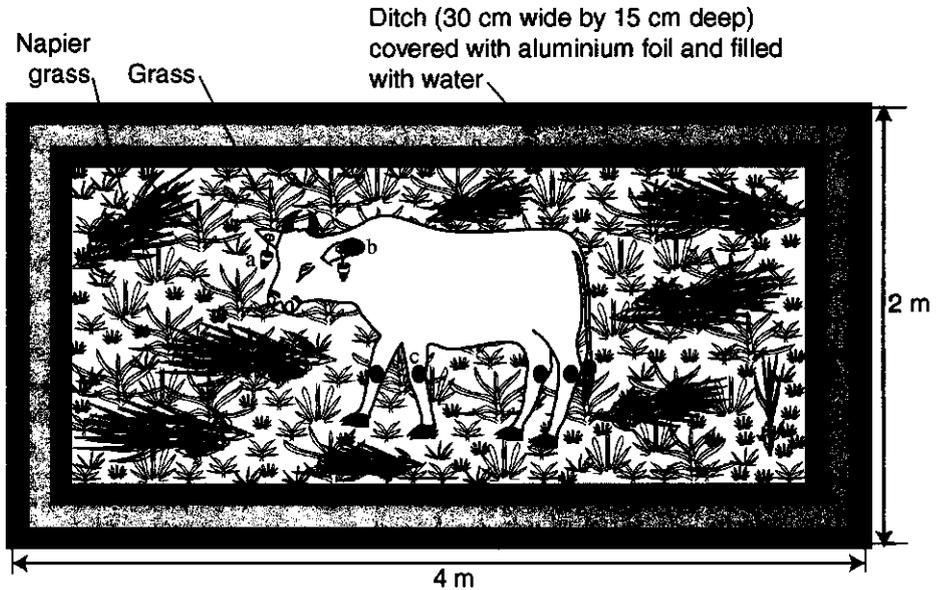
### Essential oils

Essential oils of *T. minuta* and *T. diversifolia* were obtained by the hydrodistillation method using 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 Limited). This carrier material contained no colour, fragrance or irritants and was used for control experiments. The essential oils were transported from ICIPE to Bungoma in a cool box on ice and stored in a freezer at  $-4^{\circ}\text{C}$  until required for use on hosts. After being taken out of the freezer, the oils were used within 12 hours. The formulation used in this study was adopted from the previous assays in the laboratory and field at ICIPE while evaluating the essential oil of *Ocimum suave* against *R. appendiculatus* (Mwangi et al., 1995a; ICIPE, 1998/99).

### Experimental pasture plots

Adult *R. appendiculatus* that had been starved for 24 h were released in a demarcated pasture plot measuring 4 m l x 2 m w, between 09:00 and 11:00 hours (Fig. 7.1). The release time coincided with the time when most livestock tick species leave their hideout to start hunting for suitable hosts for a blood meal (Norval et al., 1992). After this time, when it gets hotter, ticks hide under existing vegetation and/or in burrows to avoid desiccation and subsequent death. Within the demarcated experimental plots, any plants known to be used for protection against biting insects (Kokwaro, 1993) and livestock ticks (Malonza et al., 1992; Lwande et al., 1999; Ndung'u et al., 1995; 1999; Mwangi et al., 1995a; b) were removed to avoid interference with the results.

A ditch measuring 30 cm wide x 15 cm deep was dug round the demarcated plot (Fig. 7.1). The ditch was lined with aluminium foil and filled with water to prevent the ticks from leaving the plot during and after the experiment. The plot was then infested with 200 adult *R. appendiculatus*. Freshly collected, tick-free Napier grass was evenly spread over the entire plot as fodder, to make the experimental animal walk around the demarcated plot comfortably while feeding on this and some other grasses found within the plot. The treated host, having either known levels of tick infestation or no prior exposure to ticks, was allowed to stay in the plot for 3 h in order for ticks to attach to it and move to their preferred feeding sites. Three hours was, in preliminary observations, found to be enough time for the ticks to infest the treated animals and reach the preferred feeding site.



**Fig. 7.1.** Experimental plot in which 200 adult *Rhipicephalus appendiculatus* were released, indicating various experimental treatments: (a) tubes with petroleum jelly (control) or essential oils of *T. minuta* or *T. diversifolia*; (b) ears treated by smearing petroleum jelly (control) or essential oils of *T. minuta* or *T. diversifolia*; and (c) legs and tail treated by smearing with petroleum jelly (control) or essential oils of *T. minuta* or *T. diversifolia*.

After 3 h, ticks were recovered from the exposed animal and counted. The plot was then burnt down using paraffin in order to kill any ticks that had not climbed on the host, and thus avoid tick infestation in the study area. A new plot was used for each of the 27 replicates.

### Treatments

The nine host animals used were randomly divided into three groups of three, one for each of the treatment sites on the host: (a) Eppendorf tube with oil, suspended on the inner side of both ear pinnae of each animal (ear tube), (b) oil smeared on the inner side of both ear pinnae (ear smear), and (c) oil smeared on legs and tail (leg + tail smear) (Fig. 7.1). In each group, one animal was randomly selected to be treated with petroleum jelly (control), one was treated with the essential oil of *T. minuta* and the third host was treated with the essential oil of *T. diversifolia*, both essential oils diluted to 10% in petroleum jelly. Once used in this order, the hosts were not inter-changed until the end of the experimental period in order to avoid cross contamination.

There were three independent replicates for each treatment. Each replicate was conducted in its own plot. For each treatment site and within each treatment application, one animal was used to make observations three times successively. Between the replicates, the entire body of the animal was thoroughly cleaned using 99% alcohol. Thorough cleaning was meant to remove any residues of previous applications of petroleum jelly or essential oils and any odours that may have been produced by previously attached ticks, which could have affected the results of successive trials. This precaution was taken because it is

known that ticks themselves may play a role in guiding other ticks of the same species to preferred feeding sites, as has been observed in *Amblyomma* ticks (Schoni et al., 1984; Diehl et al., 1991; Norval et al., 1991) and *R. appendiculatus* (Sika, 1996). After cleaning, the host was allowed to stay in the open for about 30–45 min before being used again, allowing its fur to dry by evaporation.

#### Recovery of ticks and determination of the impact of treatment on tick attachment

Ticks were observed and recovered on the body of the host at five specific sites. The five sites were: 1) Head-eyes/nose/ears/horn-base (Hd), 2) Dewlap (Dp), 3) Forelegs (Fl), 4) Hind legs (Hl), and 5) Tail (T). The five sites were chosen as recovery sites following preliminary field observations of grazing animals that showed them to be the most preferred areas of not only alightment by the ticks from their questing positions on the vegetation (Browning, 1976) but also of temporary attachment (Wanzala et al., 2004). The following formula was used to estimate the percentage of reduction of adult *R. appendiculatus* found attached on the host, caused by each essential oil type:

$$\% \text{ reduction of ticks on host} = \frac{(\text{No. ticks released} - \text{No. attached in treatment}) - (\text{No. ticks released} - \text{No. attached in control})}{\text{No. ticks released}} \times 100$$

#### Statistical analyses

Data of the number of ticks observed on the body of hosts were analysed by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure for SAS system for PC (SAS<sup>®</sup> Institute, 2002-2003). The effect of essential oils delivered on hosts at the ear (smear or tube) and legs + tail and the mean percentage of reduction of attaching ticks were compared by Students-Newman-Keuls test (Sokal and Rohlf, 1995) at  $P = 0.05$ . The effects of the essential oils of *T. minuta* and *T. diversifolia* on the number of ticks that located the host and attached to it was examined by paired samples t-test statistics using the t-test procedure of the Statistical Products and Service Solutions (SPSS) version 15 for windows. A comparison of the three essential oil application sites was done using a one-way ANOVA.

#### Legal use of field experimental animals

All procedures requiring the use of experimental animals in the field were approved by District Veterinary Office of Bungoma District, western Kenya. The importance and risk-free nature of the project was further explained to the Bukusu community by the DVO and agricultural extension officers working within the study area. The field experiments were performed in compliance with guidelines published by the Kenya Veterinary Association and the 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 from whom we rented the experimental field plots.

**Table 7.1.** The mean ( $\pm$  SE) number and percentage of adult *Rhipicephalus appendiculatus* ticks observed on the host treated on the ear (smear and tube) and legs + tail with petroleum jelly (control) and the essential oils of either *T. minuta* (Tm) or *T. diversifolia* (Td) and % reduction in numbers on hosts of the 200 ticks released.

Animal code	Essential oil	Treatment site of the essential oil on the host	Mean tick counts on the host body after 3 h of host exposure ( $\pm$ SE)	Mean % of released ticks counted on the host after 3 h of host exposure ( $\pm$ SE)	% reduction in ticks on host after 3 h of host exposure
Cet	Control	Eartube	184.7 $\pm$ 4.67d	92.3 $\pm$ 2.33d	-
Ces	Control	Ear smear	172.3 $\pm$ 3.18d	86.2 $\pm$ 1.59d	-
Ctl	Control	Legs + tail smear	186.0 $\pm$ 4.16d	93.0 $\pm$ 2.08d	-
Tmet	Tm	Ear tube	95.0 $\pm$ 10.21bc	47.5 $\pm$ 5.11bc	44.8 $\pm$ 5.10bc
Tmes	Tm	Ear smear	50.3 $\pm$ 2.85a	25.2 $\pm$ 1.42a	61.0 $\pm$ 0.87ab
Tmtl	Tm	Legs + tail smear	33.0 $\pm$ 3.79a	16.5 $\pm$ 1.89a	76.5 $\pm$ 3.91a
Tdet	Td	Ear tube	111.7 $\pm$ 10.14c	55.8 $\pm$ 5.07c	36.5 $\pm$ 7.40c
Tdes	Td	Ear smear	83.3 $\pm$ 9.39b	41.7 $\pm$ 4.69b	44.5 $\pm$ 5.80bc
Tdtl	Td	Legs + tail smear	52.0 $\pm$ 5.69a	26.0 $\pm$ 2.84a	67.0 $\pm$ 0.76a

- not applicable, as the control values were used to calculate the mean % reduction in ticks on host in the equation described in the materials and methods.

For a given column, means followed by the same letter(s) are not significantly different from one another at  $P = 0.05$  (t-test, SAS).

## Results

Treatment of hosts had a significant effect on the percentage of the released ticks counted on their bodies ( $P < 0.05$ ) (Table 7.1). More ticks were found on the control hosts than on the animals treated with the essential oils of either *T. diversifolia* or *T. minuta* ( $P < 0.05$ ) (Table 7.1). The mean percentage of released ticks counted on the hosts treated with the essential oil of *T. minuta* was significantly lower than the mean percentage of released ticks counted on the hosts treated with the essential oil of *T. diversifolia* for corresponding treatment sites [ $t_{0.05}(8) = 3.438$ ,  $P = 0.009$ ].

The site of delivery of the essential oil on the hosts had a significant effect on the mean percentage of released ticks counted on their bodies ( $P < 0.05$ ) (Table 7.1). In a group of the hosts treated with the essential oil of *T. minuta*, the method of essential oil delivery using the ear tube had a significantly higher mean percentage of released ticks counted on the host than the ear and legs + tail smears ( $P < 0.05$ ). The legs + tail smear method had the lowest mean percentage of released ticks counted on the host. In the group of the hosts treated with the essential oil of *T. diversifolia*, the mean percentage of released ticks counted on the host were significantly different for the three methods of delivery of the essential oil on the hosts with the ear tube method having the highest percentage of ticks counted on the host while the legs + tail smear method having the lowest percentage of ticks counted on the host ( $P < 0.05$ ).

Treatment of hosts had a significant effect on the percentage of reduction of ticks on the host after 3 h of host exposure ( $P < 0.05$ ) (Table 7.1). There was a significant difference in percentage reduction of ticks on the hosts after 3 h exposure between the hosts treated with the essential oil of *T. minuta* and *T. diversifolia* for corresponding treatment sites [ $t_{0.05}(8) = 3.438$ ,  $P = 0.009$ ]. The percentage reduction of ticks on the hosts was significantly higher for animals treated with the essential oil of *T. minuta* than the essential oil of *T. diversifolia* ( $P < 0.05$ ). The control animals had the highest mean percentage of ticks (percentage of ticks released experimentally) ( $93.0 \pm 2.1\%$ ).

The site of treatment on the hosts had a significant effect on the percentage of reduction of ticks on the host ( $P < 0.05$ ). In a group of the hosts treated with the essential oil

of *T. minuta*, the ear tube treatment had a significantly lower mean percentage of reduction of ticks on the hosts than the legs + tail smear method but the ear smear treatment was not significantly different from the other two ( $P < 0.05$ ). In the group of the hosts treated with the essential oil of *T. diversifolia*, the ear tube and smear treatment sites had a significantly lower mean percentage of reduction of ticks on the host than the legs + tail smear treatment site ( $P < 0.05$ ). Corresponding values for mean percentage reduction of attaching ticks were significantly higher for the essential oil of *T. minuta*, however, than those for the essential oil of *T. diversifolia* ( $P < 0.05$ ).

Of the three application sites of essential oils, smearing of essential oils of either *T. minuta* or *T. diversifolia* on the legs and tail of the hosts caused a significantly greater repellent effects on orientation to the host and attachment site preference of adult *R. appendiculatus* on the host than treatment of the ear with the tube or smearing the essential oils on the ear (Tables 7.1). In both the groups of host animals treated with either essential oil of *T. minuta* or *T. diversifolia*, the ear smear site was second to that of smearing essential oils on the legs + tail, and the ear tube third, in terms of preventing ticks from attaching to the host at their preferred feeding site (Table 7.1). The three sites of application of essential oils had a significant effect on the mean number and percentage of adult *R. appendiculatus* observed on the hosts ( $P < 0.05$ ) (Table 7.1). The legs + tail and ear smear sites (with the exception of the sites treated with the essential oil of *T. diversifolia*) were not significantly different from one another but the former was significantly different from the ear tube method ( $P < 0.05$ ).

#### Site of tick attachment

The control hosts, treated with petroleum jelly only, were used as indicators for identifying preferential sites for attachment during the observation period and also as a reference point for comparing the behavioural effect of the two essential oils (Table 7.1). The consideration was based on the mean number of ticks observed at different sites on the body of the host mean (Table 7.2). The essential oil of *T. minuta* delivered by the tube on the ear as well as all methods used to deliver the essential oil of *T. diversifolia*, did not affect the choice of ticks for any of the five body sites (Dp, Fl, Hl, T and Hd) ( $P > 0.05$ ) (Table 7.2). This implies that using the three delivery methods, the essential oil of *T. diversifolia* affected the attachment of *R. appendiculatus* to the five body sites equally, whereas with the essential oil of *T. minuta*, there was a significant difference between the choice of ticks for body sites when using ear smear and legs + tail methods of delivering the essential oil ( $P < 0.05$ ) (Table 7.2). During the delivery of the essential oil of *T. minuta* using the ear smear method, *R. appendiculatus* preferred attaching at the hind legs and then the fore legs, followed by the remaining three sites which were equally selected. When the essential oil of *T. minuta* was delivered by the legs + tail method, the tail site was the least preferred for attachment by *R. appendiculatus* while the remaining four body sites were equally selected.

#### Comparison of the main repellent effects of the essential oils of *Tagetes minuta* and *Tithonia diversifolia*

The mean percentage of tick reduction on the host by the essential oil of *T. minuta* ( $60.8 \pm 4.9\%$ ) was higher than that by the essential oil of *T. diversifolia* ( $49.3 \pm 5.3\%$ ). The mean percentage of ticks observed (calculated as a percentage of the number of ticks released experimentally) on the host treated with the essential oil of *T. diversifolia* ( $41.2 \pm 4.8\%$ ) was higher than that of the essential oil of *T. minuta* ( $29.7 \pm 4.9\%$ ). This implies therefore that the essential oil of *T. minuta* had a greater repellent effect on adult *R. appendiculatus*

**Table 7.2.** The impact of treatment of the hosts with petroleum jelly (control) and the essential oils of *T. minuta* (Tm) and *T. diversifolia* (Td) on the site on the host where adult *Rhipicephalus appendiculatus* were observed to have attached (Dewlap, Dp; Forelegs, Fl; Hind legs, HI; Tail, T; and Head-eyes/nose/ears/horn-base, Hd) (n = 200).

Treatment	Site of application of essential oil on the host animal	Mean ( $\pm$ SE) number of ticks observed at different body sites						P-value
		Dp	Fl	HI	T	Hd		
Co	Ear tube	73.6 $\pm$ 9.87a3	41.0 $\pm$ 2.08b1	11.7 $\pm$ 6.17c1	15.0 $\pm$ 2.52c1	43.3 $\pm$ 3.28b2,3	P<0.05	
	Ear smear	63.3 $\pm$ 12.02a2,3	36.7 $\pm$ 12.24ab1	18.0 $\pm$ 6.25b1	16.0 $\pm$ 2.08b1	38.3 $\pm$ 2.85ab2,3	0.015	
	Legs + tail smear	77.7 $\pm$ 4.91a3	24.7 $\pm$ 5.90c1	22.0 $\pm$ 0.58c1	8.0 $\pm$ 3.79d1	53.7 $\pm$ 1.333b	P<0.05	
Tm	Ear tube	32.7 $\pm$ 1.76a1,2	26.0 $\pm$ 12.54a1	7.0 $\pm$ 1.53a1	3.7 $\pm$ 1.67a1	25.7 $\pm$ 15.68a1,2	0.172	
	Ear smear	3.3 $\pm$ 0.88c1	8.7 $\pm$ 1.86b1	26.7 $\pm$ 1.20a1	7.0 $\pm$ 0.58c1	4.7 $\pm$ 0.88c1	P<0.05	
	Legs + tail smear	7.0 $\pm$ 0.58ab1	6.3 $\pm$ 0.67ab1	5.7 $\pm$ 0.33ab1	4.3 $\pm$ 0.67b1	9.7 $\pm$ 1.76a1	0.026	
Td	Ear tube	56.0 $\pm$ 24.01a2,3	19.0 $\pm$ 5.69a1	19.7 $\pm$ 13.67a1	8.7 $\pm$ 6.67a1	8.3 $\pm$ 6.36a1	0.145	
	Ear smear	4.7 $\pm$ 1.20a1	19.0 $\pm$ 15.04a1	37.0 $\pm$ 21.50a1	18.3 $\pm$ 9.84a1	4.3 $\pm$ 1.20a1	0.393	
	Legs + tail smear	17.0 $\pm$ 9.29a1	5.3 $\pm$ 1.20a1	4.3 $\pm$ 0.33a1	2.7 $\pm$ 1.20a1	22.7 $\pm$ 5.84a1,2	0.062	
P-value	P<0.05	0.058	0.242	0.154	P<0.05			

In a given row, means followed by the same letter(s) are not significantly different from one another at P = 0.05 (Student-Newman-Keuls H-test).  
For a given column, means followed by the same number(s) after the letter(s) are not significantly different from one another at P = 0.05 (Student-Newman-Keuls H-test).

than the essential oil of *T. diversifolia*. The means were compared by paired samples t-test and the two differences were found significant,  $t_{0.05}(2) = 4.486$ ;  $P = 0.046$ .

### Discussion and conclusion

In this study, all three treatment sites on the host gave some degree of protection against host-seeking *R. appendiculatus*, but the essential oils of *T. minuta* and *T. diversifolia* varied considerably in their effects. *Rhipicephalus appendiculatus* were more affected by the essential oils of *T. minuta* and *T. diversifolia* when smeared round the legs + tail of the host than when applied on the ear by either smearing or suspending a tube containing the oil on the inner side of the ear. However, treatment of the legs + tail is tedious, time consuming, and requires more essential oil, and hence may be uneconomical for use by the target group, the rural livestock farmers. Suspending the tube on the ear had the least effect on host-seeking *R. appendiculatus*, and its difference with the legs + tail treatment was significant for both essential oils ( $P < 0.05$ ). The ear tube could possibly offer slow release of the odour, which would make it likely to affect ticks over a longer period of time than the other two sites. However, this has yet to be evaluated. Smearing the inner side of the ear of the host with the essential oil seems to offer the best compromise of ease of application and good repellency, and is therefore the technique that was used in Chapter 8.

In the control hosts (with the exception of the ear smear method), the dewlap was the most preferred site for *R. appendiculatus* attachment followed by head, forelegs, hind legs, and tail in that order. These observations, however, appear to contradict the known fact that *R. appendiculatus* ticks have a marked preference for attachment to the ears and head region of cattle (Yeoman and Walker, 1967). This contradiction may be due to the specific direction to which the odour coming from the ear initially flows in the highest concentration, as this would be more important to the 24 h-starved host-seeking ticks than tracing the actual source of the odour. This of course will depend on the positioning of the host ear and the direction of the breeze blowing around the entire ear of the host. Nevertheless, the odour intensity fluctuates considerably and gradually decreases after several hours compared to the source and this eventually results in the directional movement of ticks to the source (ear) (Sika, 1996; Wanzala et al., 2004).

A number of ticks that were observed on the legs of the host were found between the hooves. This part of the leg provides the first soft part of the host that the newly-hatched, host-seeking ticks come into contact with upon arrival on the host body. These soft parts act as stimulants for the ticks to start preparing to feed (Sika, 1996). This also accounts for the attachment of more ticks on the nose, eyelids and base of the horns, upon alighting on the host body from their questing positions on the vegetation. These parts are also soft and/or contain the soft waste residues of the host. By the time ticks were being located on the hosts, most of them were still scanning and therefore had not moved away from the entry site. However, the ticks that were removed from the host body while scanning, in the process of attaching, or attached, did not show any evidence of feeding; when crushed between white paper towels there was no blood apparent, nor had the females increased in size, a physical manifestation of feeding.

Due to the itching and irritation caused by tick movement and attachment on the host, a number of ticks are removed from the body of the host using tail, tongue and scratching with the mouth, more particularly on the forelegs and tail (Hadani et al., 1977). The hind legs are also used to scratch and remove ticks attached on the head. This may have reduced the number of ticks that were observed on the body of the host. From the previous studies (Sika, 1996; Wanzala et al., 2004), there is no doubt that the efficacy of

the essential oils was also affected by host-derived repellents (from the anal region) and attractants (from the ear) and other body emanations (Akinyi, 1991; Sika, 1996). Although it was hypothesized that the effects of host-derived repellents and attractants were masked by the essential oils, it is not yet known to what extent this happens. It is therefore possible that these host-derived repellents and attractants may be contributory factors to some of the observed discrepancies in the results, especially in the controls. The efficacy of essential oils was also influenced by several other factors, such as their chemical constituents, environmental conditions at the time of application, and the persistence and the spread of the active ingredients on the host body (Mwangi et al., 1995a).

This study has shown that the essential oils of *T. minuta* and *T. diversifolia* may provide a useful prophylactic means of reducing vector (tick)-host (livestock) contact, thereby reducing the number of tick bites and subsequent tick-borne infections, physical damage, and resulting secondary infections. Different formulations, particularly those that stabilize the active ingredients and perhaps higher concentrations than the one used (10%) should be tested in multi-locational field trials in order to determine whether these essential oils are appropriate for use on a large scale. The use of plant extracts with known effects on livestock ticks in this way may be a useful complementary or alternative tick control method to the heavy use of classical acaricides. This may decrease the quantity of toxic acaricide residues periodically released in the environment. In addition, the essential oils have the added advantage that they may provide a certain degree of control of flies and other ectoparasites that disturb and bite the target hosts (Toledo et al., 2003; Adedire and Akinneye, 2004; Taiwo and Makinde, 2005).

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## 8

## Essential oils of indigenous plants protect livestock from infestations with *Rhipicephalus appendiculatus* and other tick species in herds grazing in natural pastures in Kenya<sup>1</sup>

**Abstract:** The effects of essential oils of *Tagetes minuta* and *Tithonia diversifolia* on *Rhipicephalus appendiculatus* infesting livestock in Bungoma District, western Kenya, were studied. 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 the ear pinna, the preferred feeding site of *R. appendiculatus*. The 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 hosts treated with the essential oils was reduced by more than half that of the original population. By the 5th day post-treatment, more than 75 and 60% of adult *R. appendiculatus* and other tick species, respectively, were affected by the essential oils so that they 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*. The 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 the 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 such as *Amblyomma variegatum*, *R. evertsi* and *Boophilus* spp., although these tick species have different feeding sites. The results suggest the potential for essential oil formulations in reducing tick infestation and associated tick-borne diseases among the resource-limited livestock farming community in tropical Africa.

**Key words:** *Rhipicephalus appendiculatus*, ticks, repellents, essential oils, *Tagetes minuta*, *Tithonia diversifolia*, on-host field trials, Kenya, cattle

### 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 sub-tropical 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; Silva et al., 2007), biological (Samish et al., 2004), immunological (Willadsen and Kemp, 1988; Willadsen et al., 2006) 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

<sup>1</sup>This Chapter will be submitted for publication as: Wanzala, W., Takken, W., Mukabana, R.W. and Hassanali, A. Essential oils of indigenous plants protect livestock from infestations with *Rhipicephalus appendiculatus* and other tick species in herds grazing in natural pastures in Kenya.

Kaaya, 1996) The application of synthetic acaricides is still the main method for 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 (Chapter 1). 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).

Little data is 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 (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 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 moulting 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* prevented *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 et al., 1992; 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 pose 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 thesis, the repellent action of the essential oils of *T. minuta* and *T. 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 on the host from the ground and vegetation. In the study described in this chapter, 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.

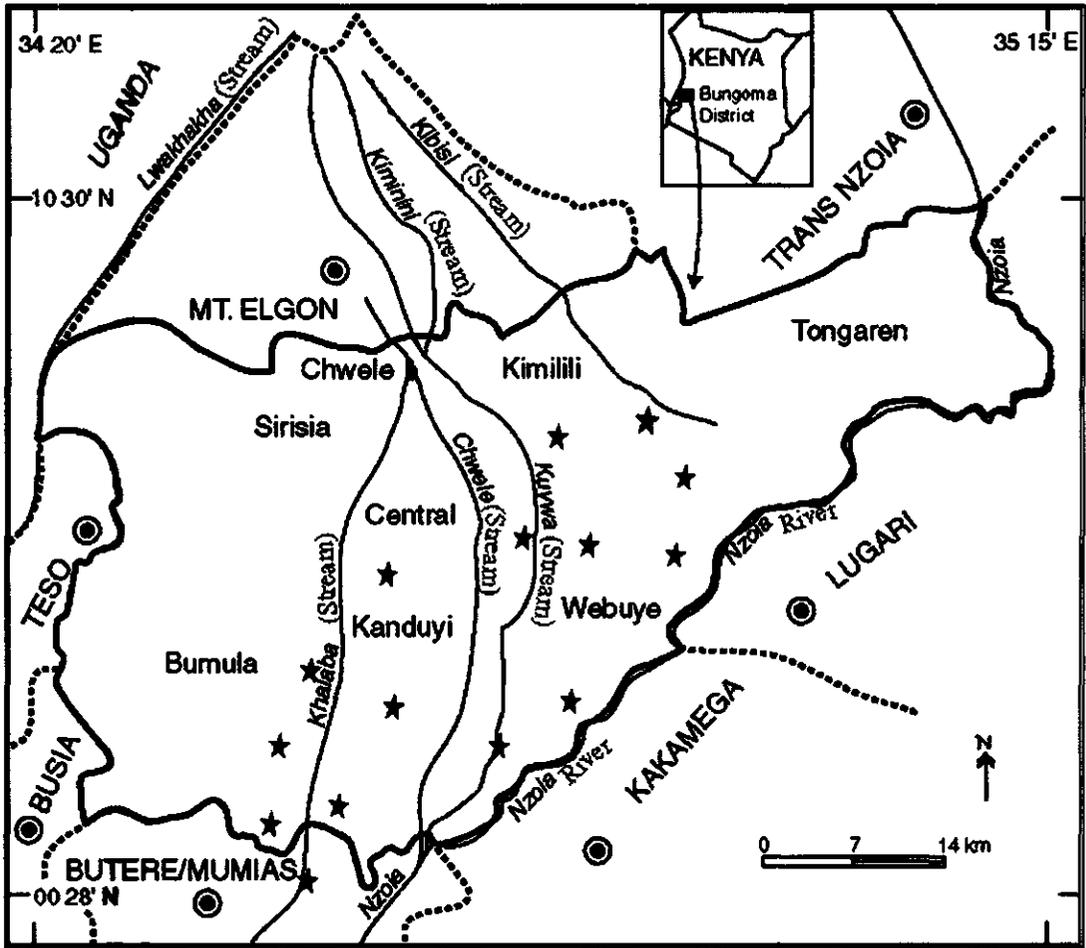


Fig. 8.1. The location of Bungoma District in Kenya. The field experiments were located on 15 different livestock farms (indicated with stars).

## Methods and materials

### Geography of experimental field site

The study was carried out in Bungoma District, western Kenya, situated between latitude  $00^{\circ}28'$  and  $10^{\circ}30'$  N and longitude  $34^{\circ}20'$  and  $35^{\circ}15'$  E (Fig. 8.1). The District occupies an area of 3 074 square km. on the southern slopes of Mt. Elgon, with an altitude ranging from 1 200 to 4 000 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-farm management units are home gardens, homesteads, live fences, coffee- and banana-groves and annual cropping fields (Backes, 2001). Average annual rainfall ranges from 1 600 to 2 000 mm, and mean annual temperatures in the southern areas are about 21 to 22 °C, whereas in the northern areas closer to the mountain, they are in the lower range of 5 to 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 Bun-

goma District Veterinary Office (DVO). The DVO receives reports from regulated and monitored local public markets within Bungoma District, 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 District were received from individual livestock farmers (DVO, pers. comm.). A preliminary survey within this area confirmed the DVO's report that the site was more heavily infested with livestock ticks than any other area within the District.

#### **Essential oils used in the study**

The essential oils used were extracted from *T. minuta* and *T. diversifolia* as described in Chapter 4 of this thesis. 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  $-4^{\circ}\text{C}$  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 behavioural 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 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 identified following the descriptions of Hoogstraal (1956a, b), Matthyse 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 analysed using Statistical Products and Service Solutions (SPSS version 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 analysed 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 ( $\text{Log}_{10}(\text{tick counts} + 1)$ ) (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 ani-

**Table 8.1.** The mean ( $\pm$  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 of *Tithonia diversifolia* in petroleum jelly of *Tagetes minuta*, and III = those hosts selected for treatment with 10% essential oil in petroleum jelly) (n = 45).

Tick species	Mean ( $\pm$ SE) tick counts <sup>1</sup>		
	I	II	III
<i>R. appendiculatus</i> (♂)	32.3 $\pm$ 18.19a	38.7 $\pm$ 18.47a	24.7 $\pm$ 14.59a
<i>R. appendiculatus</i> (♀)	31.3 $\pm$ 18.60a	23.2 $\pm$ 12.92a	24.1 $\pm$ 14.73a
Other tick species	32.2 $\pm$ 21.07a	38.7 $\pm$ 21.39a	21.9 $\pm$ 11.09a

<sup>1</sup>Values within a row were not significantly different (P = 0.05).

mals 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 District Veterinary Office (DVO) of Bungoma District, western Kenya. The importance of the project was further explained to the Bukusu community by the DVO and agricultural extension officers working within the study area through organized workshops and local sub-chiefs and chiefs' meetings in the entire District. 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.

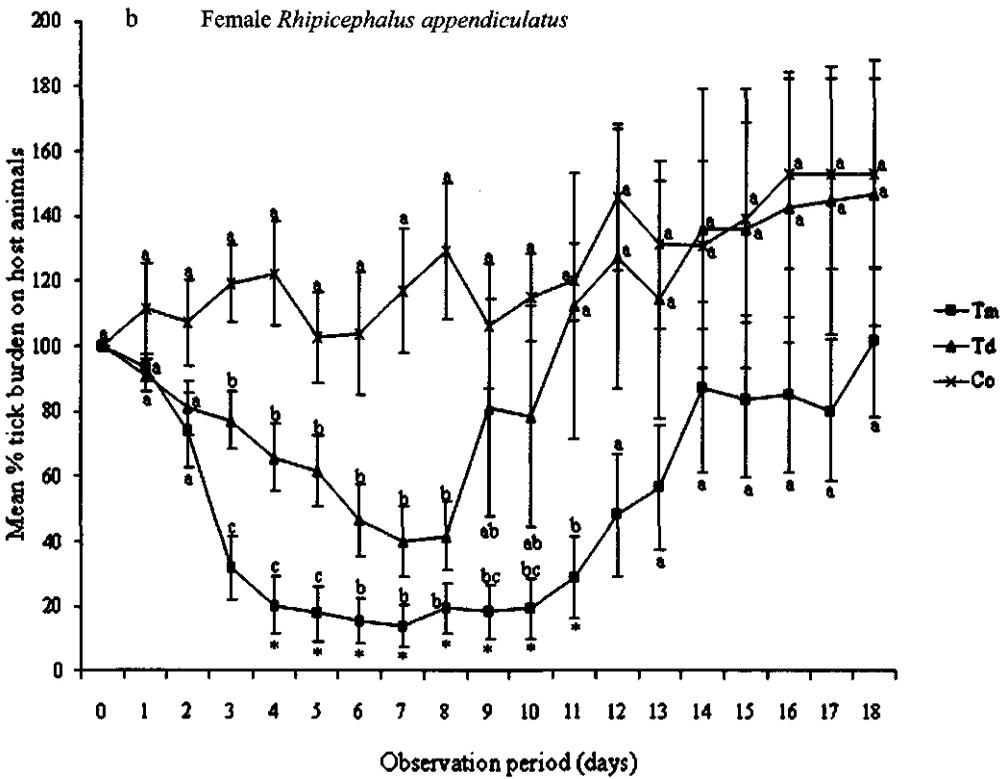
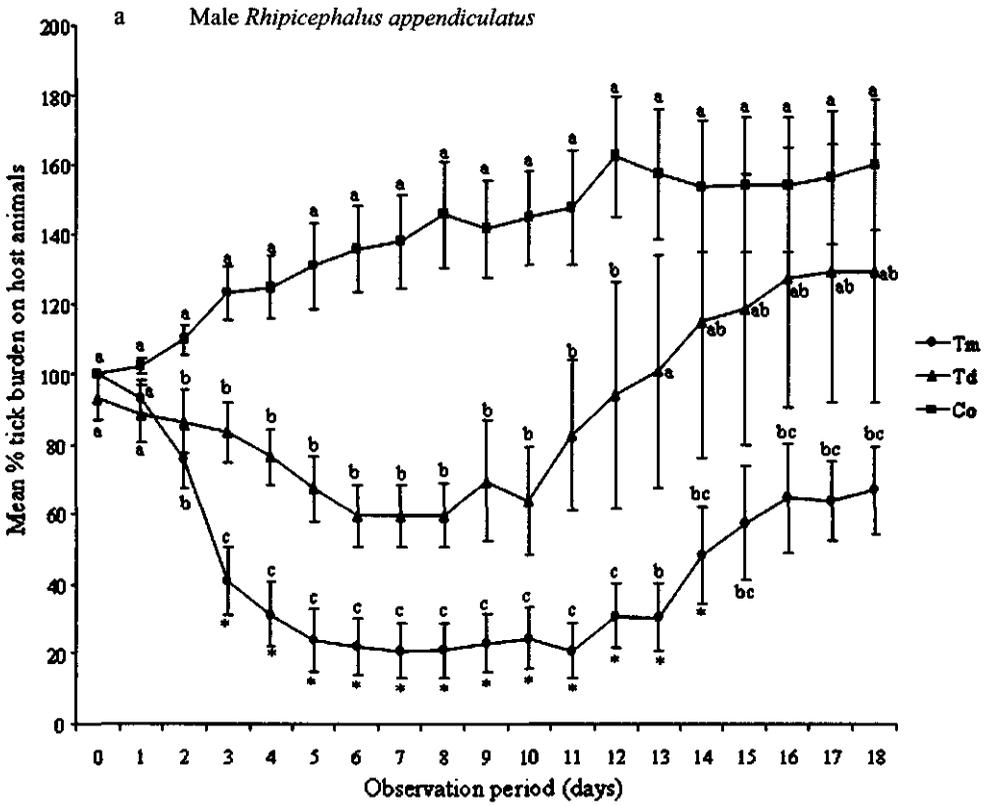
## Results

### 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 8.1). 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) (Figs. 8.2a and b). In the treatment with the essential oil of *T. minuta*, the tick infestation level significantly dropped up to 11–14 days post-treatment, after which it increased 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, were not significantly different (P > 0.05) (Figs. 8.2a and b). Between days 3 and 14 post-treatment, the mean tick infestation levels on hosts were significantly lower than the other days (P < 0.05) (Figs. 8.2a and b). For hosts treated with *T. diversifolia* and petro-



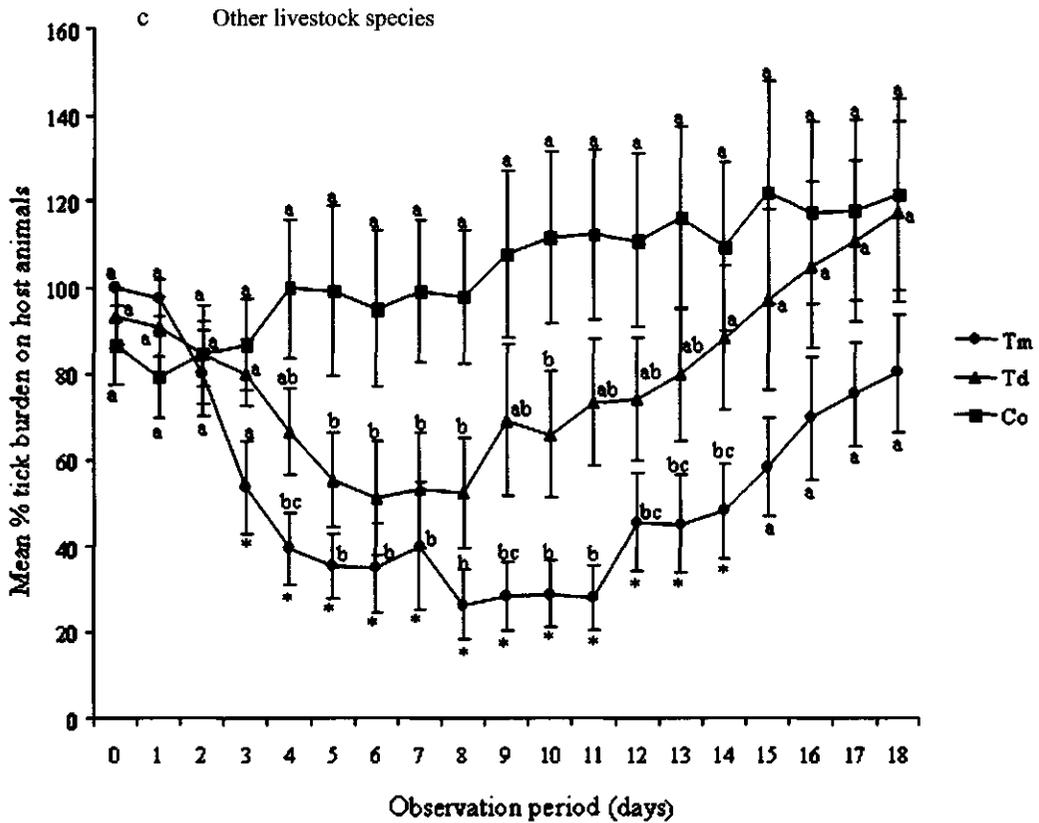


Fig. 8.2. mean ( $\pm$  SE) percentage of tick infestation on the hosts treated with essential oil of *Tagetes minuta* (Tm), *Tithonia diversifolia* (Td) or petroleum jelly (Co) (control) and monitored daily for 18 days. The effects of these treatments were evaluated on (a) male *Rhipicephalus appendiculatus*, (b) female *Rhipicephalus appendiculatus* and (c) other livestock tick species. On each day, values capped with the same letters are not significantly different at  $\alpha = 0.05$  (Student-Newman Keuls H-test). The asterisk (\*) on the standard error 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;  $n = 45$ ).

leum 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 the hosts treated with *T. diversifolia* for both male and female *R. appendiculatus* and other tick species but this drop was not significant ( $P > 0.05$ ) (Figs. 8.2a and 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. 8.2). In both essential oil treatments, there was a phase of reduction of tick infestation lasting 3–4 days with *T. minuta* and 6–7 days with *T. diversifolia*, and the effects of both essential oils reached an optimum between 3–5 days and 6–7 days post-treatment, respectively (Fig. 8.2). Tick re-infestation started between 11–12 days post-treatment in the hosts treated with *T. minuta*, whereas in the hosts treated with *T. diversifolia*, re-infestation started at 9 days post-

treatment. More ticks re-infested hosts treated with *T. diversifolia* than those treated with *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 *T. diversifolia* than those treated with *T. minuta*. Tick infestation levels following re-infestation were maintained at a lower level than they were initially for the hosts treated with *T. minuta* compared to those treated with *T. diversifolia*. In all the control treatments, there was a moderate but gradual increase in tick infestation of the animals (Fig. 8.2).

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* (Figs. 8.2a and b). Specifically, residual efficacy time of *T. minuta* was 8 days for female *R. appendiculatus* (Fig. 8.2b) and 12 days for male *R. appendiculatus* (Fig. 8.2a). *Tagetes 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 *T. diversifolia*, these values were 11 days and 6 days for males and females, respectively. In the treatment with *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 *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 *T. minuta* and *T. diversifolia*, respectively (Fig. 8.2c).

### Protection of host animals with essential oils of *Tagetes minuta* and *Tithonia 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 8.2). 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. 8.2c). The duration of protection from tick re-infestation on the 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 outlined in Chapters 4 and 6 and semi-field results summarized in Chapters 7 and 8 of this thesis, in which essential oils of *T. minuta* causes 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 at-

**Table 8.2.** 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 <i>Tagetes minuta</i>	Hosts treated with essential oil of <i>Tithonia diversifolia</i>
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 $\pm$ SE	12.53 $\pm$ 1.07	7.87 $\pm$ 0.61

tained 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 labour 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* than 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 in the field like *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 (W.W., pers. obs.). 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 it was recently established with some tsetse flies' 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 favour 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. One, a substantial proportion of female ticks may engorge faster than males and drop off the host or be preferentially rejected by the host. Alternatively, more male than female ticks may succeed in finding a host. 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 hosts, weather, grass status and 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 significantly succumb to repellent effects of the essential oils more than their female counterparts, we expected this sex ratio to change in favour 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 behaviour 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.

### Acknowledgements

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## 9

## General discussion and conclusion

Despite more than 150 years of research on tick control and management, livestock is still strongly affected by ticks and tick-borne diseases in Africa. The situation is not yet improving, as tick-borne zoonotic diseases are on the rise not only in livestock but also in humans (Tonbak et al., 2006; Vial et al., 2006; Jongejan, 2007). In addition, major tick control programmes have come to an end without achieving much success and climatic changes caused by global warming are posing new challenges (Jongejan, 2006; Uilenberg, 2006). New methods for controlling and managing ticks that go beyond the previous and current ones need therefore to be explored. Many previous studies have focused on control and management of ticks on the host without providing effective solutions to the chronic problems caused by ticks to the livestock industry. The work of this thesis has examined the strategic use of plant-derived essential oils as potential repellents for on-host control of the brown ear tick *Ripicephalus appendiculatus*. It was found that essential oils of *Tagetes minuta* and *Tithonia diversifolia*, two plants widely present in western Kenya, affect the behaviour of *R. appendiculatus* (and other tick species) and afford protection against tick infestation for a period of approximately 2 weeks under field conditions. Key results of this research have indicated how ethnobotanical repellents can be identified and tested, how they affect tick behaviour, and how they can be possibly used for livestock tick control and management. Several other questions need to be answered before the research presented in this thesis can be effectively applied: (1) How easy and sustainable is the integration of locally-derived repellents in tick control and management programmes by livestock farmers? (2) What are the advantages and disadvantages of using repellents to control and manage livestock ticks? (3) Can livestock ticks develop resistance to repellents as they have done to acaricides? (4) Can repellents provide a sustainable solution to the chronic problems caused by ticks to the livestock industry? This Chapter discusses these questions with relevance to the results reported in this thesis and where possible, gaps in knowledge are highlighted and recommendations are made that are expected to lead to improved control and management of livestock ticks.

### Identification of sustainable sources of tick repellents

Potential tick repellents can either be of natural origin from plants and animals or synthetic. Synthetic repellents tend to be more effective and/or longer lasting than 'natural' repellents (Collins et al., 1993; Fradin and Dax, 2002). However, some plant-derived repellents are either comparable to or somewhat better than synthetic repellents (Fradin and Dax, 2002; Jaenson et al., 2005; 2006).

Of the synthetic repellents, N,N-diethyl-3-methylbenzamide (DEET) is widely used and remains the principal arthropod repellent today (Gupta and Rutledge, 1989; Carroll et al., 2004; Katz et al., 2008). Other important commercially available synthetic repellents in use include: 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-, 1-methylpropylester, picaridin (Nentwig et al., 2002), Insect-Repellent 3535 (Thavara et al., 2001) and 1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine (Carroll et al., 2004). Each of these

new ingredients have some advantages over DEET and their efficacy appears to be good based on laboratory and field studies (Debboun et al., 2005). In many studies, these laboratory-derived compounds with known repellent efficacy and property are taken as standards against which candidate repellents are compared. For example in Chapter 4 of this thesis, the results of the laboratory assays of the repellent effects of the essential oils of *T. minuta* and *T. diversifolia* against adult *R. appendiculatus* at their highest dose of 1 mg were comparable to that of DEET. These comparisons give a measure of the repellent ability of the candidate substance(s) and provide an opportunity of decision making as whether to continue developing the candidate substance(s) as potential repellent(s).

The focus of this study was on the evaluation of the effects of the plant-derived chemical repellents on livestock ticks (*R. appendiculatus*). Globally, more than 150 natural repellents are in use, with the most common ones being citronella, eucalyptus, lemon leaves, peppermint, lavender, cedar, canola, rosemary, pennyroyal, and cajeput. Although any of these repellents might be of interest in protection of livestock against tick infestation, these are all “new” products to the Bukusu community and introducing these essential oils in the community would not be better than the failed and abandoned top-down approach in agricultural research and development (Whyte, 1981). In this study, a bottom-up approach was adopted in order to involve the local community in decision-making, based on traditional knowledge, rather than conceding this role to conventionally-trained experts (Warry, 1992; Etkin, 1993).

Chapter 2 of this thesis describes how the source plants for the essential oils were identified, while in Chapter 3, an explanation is given on how the plants were selected from a group of 154 plants described in Chapter 2 and evaluated for their repellent effects against adult *R. appendiculatus*. The selection of eight plants for essential oil extraction and laboratory testing was based on: (1) informants’ knowledge on how they use the plants for livestock tick control and (2) a non-experimental validation procedure (Lans, 2001) (Chapter 3). The non-experimental method allows for the separation of plants used for cultural reasons from those with anti-tick properties (Browner et al., 1988; Heinrich et al., 1992; Lans et al., 2007), thus facilitating the focus of the research subject.

Developing scientifically acceptable and standardized tests for screening the bioactivity of candidate compounds is important for developing effective products. A standard screening technique to compare behavioural effects of candidate repellents with those of known repellent compounds is lacking. Laboratories may develop their own methods for testing the repellent effects of candidate substances based on the context in which the term “repellency” is understood (Schreck, 1977). Repellents may keep the arthropods away from their target host animals (true repellents) (Metcalf and Metcalf, 1982), or elicit a behavioural response that causes them to move away from the host animals after contact (excitorepellents or irritants) (Chou et al., 1997). True repellents are highly volatile compounds that are detected by the olfactory receptors of the arthropods and impede contact between them and their hosts. Recent research has demonstrated that in arthropods, repellents may act in the same way as true olfactory compounds and that repellent behaviour is a response to the excitation of the repellent-specific olfactory neuron (Davis, 1985; Syed and Leal, 2008; Logan et al., 2008).

The first step toward experimental validation of the Bukusu ethnoknowledge is described in Chapter 3 of this thesis. Eight plants were selected for essential oil extraction and laboratory testing, was based on: (1) informants’ knowledge on how they use the plants for livestock tick control (Chapter 2) and (2) a non-experimental validation procedure (Lans, 2001; Heinrich and Gibbons, 2001; Lans et al., 2006) (Chapter 3). The essential oils were screened for their repellent effects against adult *R. appendiculatus* and

the results compared to a commonly used commercial arthropod repellent, DEET (Ndung'u et al., 1995). The essential oils of the two plants, *T. minuta* and *T. diversifolia*, having relatively strong repellent effects compared to the oils of the other plants, were selected for an in-depth scientific study. The results from these assays demonstrated repellent effects of the essential oils of the plants, thus providing justification for their use for tick control and management.

In Chapter 5, the repellent effects of the essential oil of *T. minuta* on the climbing response behaviour of adult *R. appendiculatus* in dual- and no-choice assays were compared. Although the dual-choice assay proved a more sensitive assay than the no-choice assay, the design of neither assay was, however, ideal for approximating the repellent effect of a candidate substance. A study of other methods used for testing behavioural effects of repellent substances on tick species revealed that the methods varied greatly in their experimental design, mode of exposure of ticks to the test materials, time between treatment and exposure of ticks to the test materials and the number of experimental ticks exposed to the test material (Schreck, 1977; Hadani et al., 1977; Mathewson et al., 1981; Staub et al., 2002; Garboui, 2008). Also, the equations used to calculate the degree of repellency were varied making a comparison between results obtained by different methods difficult. In addition, the potential role of aggregation pheromones affecting tick behaviour may have affected the climbing assay as well (Sonenshine, 1985; Sonenshine et al., 1982; 1992). Furthermore, the no-choice assay set-up does not simulate a natural field situation of freedom of choice while the equation used to generate the data in the dual-choice assay, has several limitations (Chapter 5). An experimental design that would allow the observation of one tick at a time without being affected by all the factors outlined in Chapter 5 is recommended.

#### Aspects of tick behaviour affecting development of on-host tick control device

Understanding all aspects of the behavioural biology of arthropods in the presence of their hosts is the basis for the development of effective control and management strategies of any pest, not only ticks. Various aspects of tick biology that characterise its life cycle have been studied (Sonenshine, 1991; Dipeolu, 1990; Amoo, 1992). Unfortunately, few of these studies address the question of how the knowledge gained may be used in tick control and management strategies, and none of them addresses the question of how some ticks (e.g., *R. appendiculatus* and *R. evertsi*) locate specific feeding sites while on the host animal. Factors that determine vector-host interactions and initiate tick-feeding processes are critical to pathogen transmission, yet, this appears to be the least well understood aspect of tick behavioural biology. Knowledge on species-specific tick behaviour, such as differences in preferred on-host feeding sites between closely related tick species (Ioffe-Uspensky et al., 1997; Chapter 4), contributes to the development of anti-tick interventions targeted at a specific tick species. This can improve the efficacy of tick control by avoiding expenditures for the control of non-target species (Amoo, 1992). In Chapter 4 of this thesis, on-host behavioural biology of two sympatric tick species (*R. appendiculatus* and *R. evertsi*) was studied in order to understand and compare the navigation behaviours of the two tick species to their respective feeding sites on the host.

To locate the feeding site on hosts, adult ticks, *R. appendiculatus* and *R. evertsi* exploit the concept of integrated use of repulsive forces (away from the preferred feeding site) and attractive forces close to the preferred feeding site to provide the appropriate orientation signals and movement (Chapter 4). Interestingly, the two species have evolved to feed far away from one another on the same host with *R. appendiculatus* preferring to feed at the ear while *R. evertsi* prefers to feed at the anal region (Sika, 1996; Wanzala et al.,

them expensive. The plant-derived repellents may be disadvantaged as people may prefer growing food crops to source plants of repellents on their limited arable lands. In addition, exposure of repellents to ambient conditions (solar radiation, temperature fluctuations, rainfall, humidity etc.) may influence their effectiveness a great deal (Mwangi et al., 1995; Chapter 8).

#### **Development of tick resistance to repellents**

The development of arthropod resistance against repellent compounds such as pyrethroids has been reported (Pennetier et al., 2007). Ticks have developed resistance to the repellent effect of permethrin, which acts as a true repellent as well as an excitorepellent/irritant (Metcalf and Metcalf, 1982; Hibbard and Bjostad, 1989) and contact acaricide (ISW-TBE, 2008; Bayer Healthcare, 2008). While DEET remains the most broad-spectrum and effective arthropod repellent developed to date (Katz et al., 2008), recent reports also show that the chief malaria-carrying mosquito, *Anopheles albimanus*, in the United States is becoming resistant to it (Ngan, 2008). This is an indication that ticks could also develop resistance to this compound. If the use of repellents is widespread and effectively organised, selection of a gene conferring resistance to repellents might be favoured rapidly, leading to a high degree of resistance. However, if not all farmers would use repellents, or if a proportion of each herd is left untreated, it might be possible to postpone or even avoid the development of resistance, certainly if ticks also feed on other host animals such as wildlife. Furthermore, as these essential oils are composed of many compounds acting together in synergism to exert the repellent effect (Knight and Norton, 1989), the development of resistance may be avoided.

#### **Repellents as a sustainable solution to the chronic problems caused by ticks to the livestock industry**

Repellents have been in use for the control of arthropods for many years (Dethier, 1956). They have become a popular method for obtaining protection from biting arthropods. Studies have shown, however, that the risk of contracting an infection transmitted by a given vector when using an effective repellent against that vector goes down significantly (Schwantes et al., 2008). Repellents work in a unique way, different from any other methods used for vector control and management. True repellents rarely kill the target vector arthropods, instead, the vectors are just kept at bay (Metcalf and Metcalf, 1982). In exerting their effects, repellents interfere with mating and oviposition responses as well as feeding (Hocking, 1963). Not all target arthropod species are equally affected by a given repellent and within a given species, not all organisms are affected by the same dose of a recommended repellent. Repellents also do not select between the pathogen(s)-infected and non-infected host-seeking arthropods. Repellents may not be able to prevent blood uptake from the host or injection of saliva into the host by the feeding vector. In either way, there may be transmission of pathogens from the host to the vector and vice versa. However, this may only be possible for instances of pathogen transmission that take a short period of time during the vector-host contact but may not be possible when the vector-host contact period is quite long as a prerequisite for transmission to take place. In the case of *R. appendiculatus* and cattle, it takes between 24 and 72 h of tick feeding before pathogens are transmitted to the host animal (Ochanda et al., 1988; Konnai et al., 2007b). Ticks may not be able to withstand the effects of an effective repellent for this period of time. Though some of the above factors may appear to undermine the role of repellents in the control and management of vectors and vector-borne diseases, the fact that repellents significantly minimise the risk of contracting a disease (Strickman et al., 2001), suggest that it is worth-

while investing in repellent research and development.

Results from Chapters 3–8 (with the exception of Chapter 4) and previous studies (Bond, 2007) indicate that it is possible to keep ticks away from their appropriate hosts using repellents. However, if tick repellents are to be considered as an option for providing protection against on-host tick infestation, the following three questions need to be addressed adequately: (1) As repellents neither kill nor cause any harmful effects to the ticks, will the ticks starve to death or find an alternative host in the presence of the repellents? (2) If an alternative host will be sought, what kind of host will it be? (3) In the event of the development of a new parasitic relationship, what will be its possible nature and how about the involvement of the zoonotic disease transmissions? These questions should be resolved as part of a long-term strategy of repellents for prevention of tick infestation in livestock. However, repellents may be the main tool available to provide prophylactic measures against infestations of *R. appendiculatus* and the resulting tick-borne diseases (Gupta and Rutledge, 1994). There is a need, therefore, to have a coordinated effort among various research groups associated with the science of arthropod repellents to investigate (1) the mode of action of repellents, (2) doses of repellents needed to generate threshold level responses and (3) controlled release delivery mechanisms. This may help in understanding the mechanisms by which repellents work under varying conditions, thus leading to the development of better products and to their wider application. It is envisaged that this approach in turn may revolutionize the development of repellents for protection at an individual level and their use in vector control (Hocking, 1963). The availability of formulations that stabilise the active ingredients in a controlled release strategy of the repellent odour so that they remain effective for a longer period of time, may be a way toward developing effective repellents (Won-Ja et al., 2007).

#### **The interception of tick orientation towards predilection feeding sites and subsequent attachment as a means for on-host tick control**

The *in-vivo* and *in-vitro* studies in this thesis have shown that two sympatric tick species, *R. appendiculatus* and *R. evertsi*, show marked preferences for different feeding sites on the same host animal (Sika, 1996; Chapter 4). These studies further demonstrated that the feeding site preference behaviour of the two tick species was mediated by host semiochemicals emanating from the predilection feeding sites (Sika, 1996; Chapter 4). Our on-host observations and laboratory assays in Chapter 4 suggested the operation of both avoidance (closer to the feeding site of the other) and attraction (closer to its own feeding site) responses of *R. appendiculatus* and *R. evertsi*. This pair of host attractive (from feeding sites) and repulsive (from non-feeding sites) odours may be the major chemo-orientation mechanism utilised by the two tick species to locate predilection feeding sites in a “push-pull” mode, although non-olfactory cues may also play a role (Waladde et al., 1979; Sika, 1996; Chapter 4). This push-pull mode seemed to ensure that the ticks were unlikely to move in the wrong direction on the host and enhanced the probability of orienting toward their respective predilection feeding sites. While the stimulus from one site acted as a strong kairomone (attractant) to the tick species that fed at that site, it acted as an allomone (repellent) against the other tick species, which did not feed there (Sika, 1996; Chapter 4). The mechanisms involved in predilection feeding site location are not yet clearly understood and probably, the full explanation of this push-pull mechanism may await the characterisation of the chemical composition of host odour in order to understand its origin, molecular structure, and mode of action.

From the quantitative data presented by Cumming (1998), *R. appendiculatus* preferred bovid hosts to any other hosts, for unexplained reasons. As outlined in Chapter 1,

host identification and selection is dependent on physical parameters such as host movement (host-derived vibrations), host breath, and contact, and environmental parameters such as light, temperature and humidity (Amoo, 1992). Although Waladde (1987) stated that stimuli emitted by the hosts could be sensed by the ticks through thermoreceptors and olfactory receptors, host specificity and feeding site location are as yet unexplained factors. Therefore, whether or not chemical cues emitted by the host are involved in host selection, has not yet been fully understood, unlike for mosquitoes, where host-specific semiochemicals have been identified as behaviour-mediating cues (Takken and Knols, 1999). Furthermore, the role of the parasites in both infected tick vectors and hosts in host selection by the ticks is also not yet clearly understood. There is a need, therefore, to undertake behavioural studies on the interaction between ticks and host-volatiles in order to understand the specific chemical cues involved in host preference (Cumming, 1998).

My own unpublished data and that of others (A. Hassanali, pers. comm.) provided evidence for some host-attractive chemical cues for predilection feeding site location originating from micro-organisms (bacteria) living inside and around the host ears, and not from the host itself. This probably explains why Cumming (2000) found that host distributions do not limit the tick species ranges of most African ticks and that the host *per se*, does not explain the evolutionary history of African ticks. This is a complex sequence of beneficial and non-beneficial evolutionary relationships that warrant further investigations in order to understand how these might be used for the development of on-host tick control and management strategies.

The possibility of intercepting tick orientation pathways toward predilection feeding sites on the host was shown in Chapters 6 and 7. The interception of these chemo-orientation pathways on the host animals may provide an individual-based tick control and management option that best fits the resource-limited livestock farming system of tropical Africa. This was the focus of the current study, which culminated in Chapter 8 with a field test in collaboration with local livestock farmers. The results of the present study have provided a basis for the development of an on-host tick control and management strategy using plant-based allomones and host-based semiochemicals at an individual level. Devices loaded with tick repellent formulations may be strategically deployed on the host and replaced after 11–14 days for the essential oil of *T. minuta* and 8 days for the essential oil of *T. diversifolia*. The results of the field study described in Chapter 8, suggest that behavioural manipulations of tick–host interactions may represent an effective and environmentally acceptable strategy for tick control and management at an individual level.

## Conclusions

I have endeavoured to give specific conclusions derived from the analysis of the results presented in this thesis in accordance with the objectives stated at the beginning of the studies.

My first objective, to document and evaluate potential anti-tick ethnopractices used by the Bukusu community in Kenya, resulted in the establishment of a list of 154 plants used in many different ways as repellents. Of these, I selected the eight most likely candidates for experimentation, based on a non-experimental validation procedure (Chapter 2).

Using these eight plants as a starting point, my second objective was to extract their essential oils, determine their chemical compositions, and evaluate their effects on adult *R. appendiculatus*. The essential oils of *T. minuta* and *T. diversifolia* turned out to be the most promising of the eight in terms of their effects on ticks, and were further used in the experiments on tick behaviour (Chapter 3). The essential oil of *T. minuta* had a higher

proportion of monoterpene compounds than the essential oil of *T. diversifolia*, which had a higher proportion of sesquiterpenes than the essential oil of *T. minuta*.

Objective 3 was to study the effects of the essential oils on host selection, on-host navigation and attachment behaviour of *R. appendiculatus* (Chapters 6 and 7). We showed that essential oils of *T. minuta* and *T. diversifolia* caused a disruption of orientation, walking and attachment behaviour of *R. appendiculatus* on the host. For both essential oils, the legs + tail sites of application, followed by ear smear and then ear tube, had significant effects on orientation to the host and attachment site preference of *R. appendiculatus*, in that order. The ear smear method is recommended for treating hosts with essential oils, but concentrations and formulations of the oils may need to be improved in order to stabilise their active ingredients.

My fourth objective was to evaluate a "push" tactic with the repellent ethnobotanicals for on-host control of *R. appendiculatus* in the field using the ear smear site for treating host animals with essential oils as recommended in Chapter 7. Within 1–4 days post-treatment, the number of ticks on animals treated with the essential oils was pushed off the host body by more than half the original population. By the 5th day post-treatment, more than 75 and 60% of adult *R. appendiculatus* and other tick species, respectively, had dropped off the host. A stronger pushing effect was shown by the essential oil of *T. minuta* than the essential oil of *T. diversifolia*. The results suggest the potential for the essential oils to be incorporated in the on-host "push" and "push-pull" strategy for the control of *R. appendiculatus* and associated tick-borne diseases among the resource-limited livestock farming community in tropical Africa.

In addition to the four major objectives of my study, two other aspects of tick behaviour were studied. The first one was to compare on-host behaviour of two sympatric tick species, *R. appendiculatus* and *R. evertsi* (Chapter 4). Each species of tick showed specific behavioural sequences in the navigation to their preferred feeding sites. Finally, we compared a dual-choice with a no-choice assay, using the essential oil of *T. minuta* as the test substance on the climbing response behaviour of *R. appendiculatus* (Chapter 5). The dual-choice assay proves a more sensitive assay than the no-choice assay, although the no-choice assay provides greater residual effects.



**Appendix 1.**  
**Enumeration of documented plants, which have effects on livestock ticks from the survey studies in the Bukusu community (Bungoma District, western Kenya) and the neighbourhood.**

Scientific name	Local name(s)	Family name	Growth Habit or life form	Frequency of mention for use	Plant part(s) used (application form)	Plant uses	References
<i>Euphorbia heterochroma</i> (Pax.)	Kumutua (1)	Euphorbiaceae	Succulent shrub	✓ ✓ ✓	Sap/stem (used to make suspension)	T	Kaaya et al., 1995
						B	Watt and Breyer-Brandwijk, 1962
						A	Philippe and Sura Faucon, 2003 Schmidt, 2003b
<i>Euphorbia tirucalli</i> (L.)	Kumusongofwa	Euphorbiaceae	Perennial thornless cactus-like tree	✓ ✓ ✓ ✓	Whole plant (used to make suspension)	I	ICIPE Annual Report, 1998/99 Regassa, 2000
						T	Hines and Eckman, 1993 Schmidt, 2003b Clevinger, 2003
						B	ICIPE Annual Report, 1998/99 Kaaya et al., 1995 Regassa, 2000 Watt and Breyer-Brandwijk, 1962 Getahun, 1976 Duke, 1983a Hines and Eckman, 1993 Bowen and Hollinger, 2002
<i>Euphorbia candellabrum</i> (Kotschy.) (Trémaut) var. <i>candellabrum</i>	Kumutua (2)	Euphorbiaceae	Tree	✓ ✓ ✓	Leaf/stem/sap (used to make suspension)	C	Hines and Eckman, 1993
						T	Kaaya et al., 1995 Regassa, 2000
						A	ICIPE Annual Report, 1998/99
<i>Margaritaria discoideus</i> (Webster./Baillon)	Erionoi (Teso) Atego (Luo) Kamenyabazi (Luganda) Muremampango (Rumyankore)	Euphorbiaceae	Tree	✓	Whole plant (used to make suspension), wood smoke repels mosquitoes and snakes	B	Hines and Eckman, 1993 Schmidt, 2003b
						I	ICIPE Annual Report, 1998/99 Schmidt, 2003b
						T	Regassa, 2000
						A	Kaaya et al., 1995
						B	UW/MG, 2003 Schmidt, 2003b
C	Hines and Eckman, 1993 Schmidt, 2003b						

<i>Bridelia scleroneura</i> (Mull. Ag.)	Kumunyekerwe	Euphorbiaceae	Tree/shrub	✓	Stem/root and or bark	I	Hines and Eckman, 1993
						B	Anonym, 2001c Schmidt, 2003b MTDP, 2003
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
						F	Persson, 1986
						C	Persson, 1986
<i>Bridelia micrantha</i> (Hochst. / Baillon)	Kumulondam-wombe or Kumukhulang'wa	Euphorbiaceae	Small tree/ herb	✓ ✓ ✓ ✓ ✓	Leaf/root/bark (used to make suspen- sion)	I	Hines and Eckman, 1993 Anonym, 2001d
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
						F	Tredgold, 1990 Hines and Eckman, 1993 Zemedu and Tadesse, 2001
						B	Rulangaranga, 1989 Hines and Eckman, 1993 Abo and Ashidi, 1999 Izelle, 2002
						C	Hines and Eckman, 1993
<i>Erythrococca bongensis</i> (Pax.)	Lupiniapiria	Euphorbiaceae	Tree	✓ ✓	Stem/root/leaf	B	Kayonga and Habiyaeremye, 1987 Maundu et al., 2001 Schmidt, 2003b
						T	Regassa, 2000 Kaaya et al., 1995 ICIPE Annual Report, 1998/99
						F	Anonym, 1968
						S	Tredgold, 1990
						B	Kayonga and Habiyaeremye, 1987 Maundu et al., 2001 Schmidt, 2003b
<i>Macaranga Kilimandscharica</i> (Pax.)	Kapibema (Sebei)	Euphorbiaceae	Tree	✓	-	B	Deweets, 1995 Maundu et al., 2001
						T	ICIPE Annual Report, 1998/99 Kaaya et al., 1995 Regassa, 2000 Schmidt, 2003b
						C	Deweets, 1995

<i>Neoboutonia melleri</i> (Müll.Arg. Prain)	Kumulongo	Euphorbiaceae	Tree/shrub	✓ ✓ ✓	Stem/root (used to make suspension)	B	Schmidt, 2003b
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
<i>Croton sylvaticus</i> (Hochst. ex Krauss)	Kumuchwichi	Euphorbiaceae	Tree	✓	Seed	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
						B	Watt and Breyer-Brandwijk, 1962 Mwangi et al., 1998 Schmidt, 2003b
<i>Croton macrostachyus</i> (Hochst. ex Delile.)	Kumuchwichi or Kumutotoa or Kumukunusia or Kumutoboso (Saboti)	Euphorbiaceae	Tree	✓ ✓	Leaf/bark/root (suspension)	I	Fernandes et al., 1985 Stockbauer, 2003
						F	Fernandes et al., 1985
						B	Getahun, 1976 Toyang et al., 1995 Fernandes et al., 1985
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
						C	Fernandes et al., 1985 Hines and Eckman, 1993
<i>Croton megalocarpus</i> (Hutch.)	Kumukunusia kumukeni or Kumutotoa kumukeni	Euphorbiaceae	Tree	✓ ✓	Leaf/bark/root (suspension)	B	Harjula, 1980 Politz and Leketley, 1988 Minja, 1989 Gachathi, 1989 ICRAF, 1992 Hines and Eckman, 1993 Kokwaro, 1993 Masinde, 1996 ITDG and IIRR, 1996 Minja, 1994 Schmidt, 2003b
						F	Hines and Eckman, 1993
						C	Hines and Eckman, 1993
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000

<i>Ricinus communis</i> (L.)	Kumuroborobo or Kumubono	Euphorbiaceae	Tree/shrub	✓ ✓ ✓ ✓	Seed/stem/root (used to make suspension)	A	van Rijn and Tanigoshi, 1999 Mitchell and Ahmad, 2006						
						B	Getahun, 1976 Duke and Wain, 1981 Core, 1981. Fernandes et al., 1985 Toyang et al., 1995 UWMC, 2003 Schmidt, 2003b						
						I	Mahgoub and Ahmed, 1996						
						P	Fernandes et al., 1985						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						B	Irvine, 1961 Marin, 1999 Schmidt, 2003b						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						B	Schmidt, 2003b						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						I	Anonymous, 2003a Lister, 2002						
<i>Drypetes gerrardii</i> (Hutch.)	Kumwilima	Euphorbiaceae	Tree	✓ ✓	-	B	Schmidt, 2003b						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						I	Anonymous, 2003a Lister, 2002						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						F (Fruits)	Zemedu and Tadesse, 2001						
						B	Samuelsson et al., 1992 Schmidt, 2003b						
						B	Schmidt, 2003b						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						B	Schmidt, 2003b						
<i>Flueggea virosa</i> (Roxb. ex Willd) Voigt.	Lubwili	Euphorbiaceae	Shrub	✓ ✓	Whole plant	I	Anonymous, 2003a Lister, 2002						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						F (Fruits)	Zemedu and Tadesse, 2001						
						B	Samuelsson et al., 1992 Schmidt, 2003b						
						B	Schmidt, 2003b						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000						
						B	Schmidt, 2003b						
						<i>Sapium ellipticum</i> (Krauss/ Pax.)	Kumuchaso	Euphorbiaceae	Tree	✓ ✓ ✓	Root/bark	B	Schmidt, 2003b
												T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000												
B	Schmidt, 2003b												
<i>Phyllanthus ovalifolius</i> (Forsk)	Kumsekese	Euphorbiaceae	Many stemmed shrub	✓ ✓	-							T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
												B	Schmidt, 2003b

<i>Phyllanthus muellerianus</i> (O. Kuntze/Exell.)	Kumutikaa	Euphorbiaceae	Tree	✓ ✓	-	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
							Schmidt, 2003b
<i>Clusia richardiana</i> (Moll.) Arg.)	Nabilikhe or Luyebeye or Lulako	Euphorbiaceae	Shrub	✓	-	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
							Muhammad et al., 1999 Schmidt, 2003b Abourashed et al., 2003
<i>Clusia abyssinica</i> (Jaub. and Spach.) var. <i>abyssinica</i>	Nabilikhe or Luyebeye or Lulako	Euphorbiaceae	Shrub	✓	-	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
							Duke, 2002 Schmidt, 2003b
<i>Clusia molitis</i> (Pax.)	Nabilikhe or Luyebeye or Lulako	Euphorbiaceae	Shrub	✓	-	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
							Schmidt, 2003b
<i>Manihot esculenta</i> (Crantz.)	Kumwoko	Euphorbiaceae	Perennial woody shrub	✓ ✓ ✓	Root/bark/stem	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
							Schmidt, 2003b
<i>Acalypha racemosa</i> (Wall. ex Baill.)	-	Euphorbiaceae	Shrub	✓ ✓	Root/bark/stem	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
							Schmidt, 2003b
<i>Ekebergia capensis</i> (Sparrm.) (Dog Plum)	Kumusilisisi (1)	Meliaceae	Evergreen or semi-deciduous, medium-sized to large tree.	✓ ✓ ✓	Root/bark/fruits/flowers/leaf/stem	T, B, C, F	World Agroforestry Centre, 2003a
							Schmidt, 2003b
<i>Ekebergia rueppelliana</i> (Fresen.) A. Rich.)	Kumusilisisi (2)	Meliaceae	Evergreen or semi-deciduous tree	✓ ✓ ✓	Root/bark/fruits/flowers	T, C, F, B	World Agroforestry Centre, 2003b





<i>Ximena americana</i> (L.) var. <i>americana</i>	Kumutuli	Oliaceae	Woody shrub or small tree	✓ ✓ ✓	Leaf/bark/root/fruit	P, B, C, F B	Hines and Eckman, 1993 Anonymous, 2003b
<i>Sesbania sesban</i> (L.) Merrill. Var. <i>nubica</i> (Chiov.)	Chisubasubi (1)	Papilionaceae	Tree	✓ ✓ ✓	Leaf/stem (used to make suspension)	T	Matzigkeit, 1990
<i>Kotschyia africana</i> (Endl.) Var. <i>bequaertii</i> (De Wild.) Verdc.	Chisubasubi (2)	Papilionaceae	Tree	✓	Parts of the plant	T	Matzigkeit, 1990
<i>Sesbania macrantha</i> (Phil. and Hutch.)	Chisubasubi (3)	Papilionaceae	Tree	✓ ✓ ✓	Leaf/stem (used to make suspension)	T	Matzigkeit, 1990
<i>Indigofera emarginella</i> (Fresen.)	Lasisakwe	Papilionaceae	Shrub	✓ ✓ ✓	Root/stem	T	Matzigkeit, 1990
<i>Erythrina abyssinica</i> (Lam ex DC.) Subsp. <i>abyssinica</i>	Kumukurum or Kumurembe	Papilionaceae	Perennial shrub/tree	✓ ✓	Leaf/bark (used to make hanging bouquet/dusting/fumigation)	T	Matzigkeit, 1990 Mansingh and Williams, 1998
						B	Rulangaranga, 1989 Hines and Eckman, 1993 ITDG and IIRR, 1996
						C	Rulangaranga, 1989 Hines and Eckman, 1993
<i>Tephrosia vogelii</i> (Hook F.)	Kumufila or Esumu	Papilionaceae	Shrub	✓	Leaf/stem/fruit/bud/ bark/seed/root (whole plant)	A, T	Lemmens and Fryer, 1917 Cremllyn, 1978 Matzigkeit, 1990 Toyang et al., 1995 Kambewa et al., 1997 Kambewa et al., 1997
						I	Niang, 1987
						Af	Bertram, 1988
						B	Niang, 1987 Getahun, 1976
						P	Matzigkeit, 1990 Cremllyn, 1978
<i>Rhynchosia resinosa</i> (A.Rich.) Baker	Kurnustuli	Papilionaceae	Tree	✓	Leaf/stem/root/bark	T, B	Matzigkeit, 1990 William, 1999
<i>Macuna poggei</i> Taub. var. <i>pesa</i> (De Wild) Verdc.	Kurnuchuli or Kurnustuli	Papilionaceae	Perennial climbing herb or shrub	✓	-	T	Matzigkeit, 1990
<i>Macuna poggei</i> Taubert var. <i>poggei</i> (De Wild) Verdc.	Kurnuchuli or Kurnustuli	Papilionaceae	Perennial climbing herb or shrub	✓	-	T	Matzigkeit, 1990

<i>Acacia macrothyrsa</i> Harms.	Kumukholondo	Mimosaceae	Shrub/tree	✓ ✓ ✓	Root/leaf/bark (used to make hanging bouquet/ suspension)	B, C, T, F	Marin, 1999 Le Houérou, 2003
<i>Albizia coriaria</i> Welw. ex Oliver.	Kumupeli Ober (Luo)	Mimosaceae	Perennial tree	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	Stem/root/bark/leaf	T, B	Kokwaro, 1993 Bekalo et al., 1996 Schmidt, 2003d
<i>Albizia grandibracteata</i> Taub.	Kumufunjiamwe/ Kumulongo/ Kumuniayuni	Mimosaceae	Perennial tree	✓	-	T, N	-
<i>Entada abyssinica</i> A.Rich.	Kumuseribe	Mimosaceae	Perennial tree	✓ ✓	Root/bud/leaf/bark (used to make suspen- sion)	T, C, F, B	Hines and Eckman, 1993 Toyang et al., 1995 Freiburghaus et al., 1998 Duke, 2002
<i>Acacia sieberiana</i> (DC.) (Paperback acacia)	Kumulemba	Mimosaceae	Perennial Tree	✓ ✓ ✓ ✓	-	F, C, B, T	Anonymous, 2001a
<i>Acacia nilotica</i> (L.) Will ex Del.	Kumulemba	Mimosaceae	Tree	✓ ✓ ✓ ✓	Bark/roots	B, I, F	Duke, 1983b Anonymous, 2001d
<i>Pithecolobium/Bauhinia thon- ningii</i> (Schumacher.) Milne- Redh.	Kumulalama	Caesalpini- aceae	Perennial Tree/shrub	✓ ✓	Parts of the plant-barks/ leaf/root	T, B, C, F	Ogundaini, 1999 Oni, 2001 Anonymous, 2001b Le Houerou, 2003
<i>Senna siamea</i> (Lam.) Irwin & Barneby.	Mbekoraisi or Kumuraisi	Caesalpini- aceae	Tree	✓ ✓ ✓ ✓	Root/stem/leaf (used to make suspen- sion)	T, I, R	Adoyo et al., 1997 Mansingh and Williams, 1998 Anonymous, 2001d
<i>Senna singuana</i> (Dec.) Lock.	Kumusiamosi	Caesalpini- aceae	Tree	✓ ✓ ✓	Fruit/root/leaf/bark (used to make suspen- sion)	B, T, I	Adoyo et al., 1997 Mansingh and Williams, 1998
<i>Tamarindus indica</i> L.	Kumukhua	Caesalpini- aceae	Medium to large tree reaching 30 m in height.	✓ ✓ ✓ ✓	Root/bark/leaf (used to make suspen- sion)	I, B	Imbabi et al., 1992 El-Siddig et al., 1999 Rao et al., 1999 Gurumurthy and Sreenivasa, 2000
<i>Senna didymobotrya</i> (Fresn.) Irwin & Barneby	Kumupinipinu	Caesalpini- aceae	Tree	✓	Root/bark/leaf (used to make suspen- sion)	B T, B	Zemede and Mesfin, 2001 Fernandes et al., 1985 Mansingh and Williams, 1998 UW/MG, 2003

<i>Tylosema fassoglensis</i> (Kotschy ex Schweinf 1868). Torre & Hillc. 1955	Kumukayu	Caesalpiniaceae	Perennial herb/shrub	✓	-	T, F	Anonymous, 2001b
<i>Grewia trichocarpa</i> (A. Rich.)	Kumusitaiti or Lusitaiti	Tiliaceae	Tree	✓	Root	N, -	N
<i>Grewia bicolor</i> Juss.	Kumusitaiti or Lusitaiti	Tiliaceae	Tree	✓	Root/bark	B	<a href="http://www.pbs.org/wnet/africa/explore/sahel/sahel_vegetation_10.html">http://www.pbs.org/wnet/africa/explore/sahel/sahel_vegetation_10.html</a> as retrieved on 19 August, 2004.
<i>Vernonia amygdalina</i> (Del.)	Kumulusia or Kumwirurusia	Compositae	Shrub or small tree	✓ ✓ ✓ ✓	Whole plant	T, F, B, I	Getahun, 1976 Toyang et al., 1995 Adoyo et al., 1997 Zemede and Tadesse, 2001 Adoyo et al., 1997
<i>Vernonia lasiopus</i> O. Hoffm.	Namatuma	Compositae	Shrub	✓ ✓	Whole plant	T, F	
<i>Vernonia grantii</i> Oliv.	Endwasi	Compositae	Shrub	✓ ✓	Whole plant	T	Adoyo et al., 1997
<i>Tithonia diversifolia</i> (Hemsl.) A. Gray	Kamang'ulie or Kamaua or Kiming'ulie	Compositae	Herb	✓ ✓ ✓ ✓	Whole plant/paste	I, P, T, B, F	Adoyo et al., 1997
<i>Bidens pilosa</i> L.	Engwesi Ingwesi (Wanga)	Asteraceae	Weak annual herb	✓ ✓ ✓ ✓ ✓	Whole plant Aerial parts	I, R, Af., B, F	Anonymous, 2001d Taylor, 2002
<i>Microglossa pyrifolia</i> (Lam.) O. Kuntze	Enguu Inguu (Wanga)	Asteraceae	Shrub	✓ ✓ ✓	Whole plant-leaf-dust. (used to make also suspension)	T, B	Bekalo et al., 1996
<i>Chrysanthemum cinerariifolium</i> (Trevit.) Vis.	Pyrethrum?	Asteraceae	Herb	✓ ✓ ✓	Flower/leaf/stem/aerial parts (used to make suspension/dust)	T, P, A, B, I	Cremllyn, 1978
<i>Togetes minuta</i> L.	Nanjaka Nanzaka (Wanga)	Asteraceae	Herb/weed/forb	✓ ✓ ✓ ✓	Leaf/flower-dust, suspension and hanging bouquet (acquotes)	T, P, A, B, I	Getahun, 1976 Berger, 1994 Anonymous, 2001d
<i>Solanecio mannii</i> (Hook. F.) C. Jeffrey	Nandebe Bukusu) Mwathathia (Kikuyu) Omukhupafula (Banyala) Kumukhuyu	Asteraceae	Shrub	✓ ✓ ✓ ✓ ✓	Whole plant	A, C	University of Nairobi Herbarium
<i>Ficus sycamoros</i> L.	Kumutoto kumutotoa	Moraceae	Tree	✓ ✓ ✓ ✓	Leaf/fruit/bark/latex	T, B, F, C	Puyvelde (van) et al., 1985 Hines and Eckman, 1993 Regassaa, 2000
<i>Ficus vasta</i> Forskål	Kumutoto kumutotoa	Moraceae	Tree	✓ ✓ ✓ ✓	Root/fruit/bud/latex (used to make suspension)	T, F, C	Puyvelde (van) et al., 1985 Regassaa, 2000

<i>Ficus amadiensis</i> De Wild.	Moraceae	Tree	✓ ✓	Root/fruit/bark/latex	T, C	Puyvelde (van) et al., 1985 Regassaa, 2000
<i>Ficus thoningii</i> BL.	Moraceae	Tree	✓	Root/fruit/bark/latex	T, F, B, C	Puyvelde (van) et al., 1985 Hines and Eckman, 1993 Regassaa, 2000 Stockbauer, 2003
<i>Ficus glumosa</i> Del.	Moraceae	Tree	✓	Root/fruit/bark/latex	-	-
<i>Ficus sur</i> Forsk. Broom cluster fig	Moraceae	Tree	✓ ✓	Root/fruit/bark/latex (used to make sus- pension)	T, F, C	Puyvelde (van) et al., 1985 Regassaa, 2000
<i>Garcinia buchananii</i> Bak.	Guttiferae	Tree	✓ ✓ ✓ ✓ ✓ ✓	Twigs/bark/root/ fruit (used to make sus- pension)	Ps, B	Smith, 1969 Duke-Elder and MacFaul 1972 Schmidt, 2003
<i>Psorospermum febrifugum</i> Spach. var. <i>febrifugum</i>	Guttiferae	Tree	✓	Fruit/bark/stem/leaf	B	Aubréville, 1936 Dalziel 1937 Irvine 1961 Cassady et al., 1990 Schmidt, 2003c
<i>Harungana madagascariensis</i> Lam. ex Poir.	Guttiferae	Tree	✓	Leaf/bark/stem	B	Woodland, 1997 Irvine, 1961 Schmidt, 2003c The World Bank Group, 2003 Erah et al., 2003 Getahun, 1976
<i>Maesa lanceolata</i> Forsk. var <i>golungensis</i>	Myrsinaceae	Tree	✓ ✓	-	T, B	Marzigkeit, 1990 Mwangi, 1996 IDTG and IRR, 1996, Cremlyn, 1978
<i>Nicotiana tabacum</i> L.	Solanaceae	Herb	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	Leaf/stem-dust (used to make sus- pension smoke and hanging bouquet also)	P, A, I, B, C	Dipeolu and Ndungu, 1991 Juliette de Ba Levy, 1991 Stoll, 1988 Berget, 1994 Adoyo et al., 1997 Mansingh and Williams, 1998
<i>Capsicum annum</i> L.	Solanaceae	Shrub	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	Fruit/leaf/stem suspension, dust, smoking and hang- ing bouquet	P, I, T, B, F	Stoll, 1988 Berger, 1994 Adoyo et al., 1997 Mansingh and Williams, 1998 Regassaa, 2000







<i>Prunus africana</i> (Hook.f.) Kalkman.	Kumuturu/ Kum-wilima	Rosaceae	Tree	✓ ✓ ✓	Bark/leaf/root	P, I, B	Cunningham and Mbenkum, 1993 Schippmann, 2001 World Agroforestry Centre, 2003b USDA, ARS, National Genetic Resources Program, 2004
<i>Strychnos innocua</i> Delile	Kumulende	Loganiaceae	Tree	✓ ✓ ✓ ✓	Powder of dried leaves/ seeds (storage pests- weevils)	P, F (fodder) B	Assefa and Williamson, 2001 Hines and Eckman, 1993
<i>Strychnos spinosa</i> Lam.	Kumukhubwe	Loganiaceae	Tree	✓	Leaf/bark/fruit	C, F	Volfová and Patočka, 2003 Stedman, Wilcox & Follett Co., 1942 Wehmeyer, 1966 Fox and Norwood-Young, 1982 Taylor, 1986 Phillipson, 1995 Saka and Musomthi, 1994 Mizrahi et al., 2002 Kristensen, 2003
<i>Clerodendrum myricoides</i> 'Ugandense' (Hochst.) R. Br. ex Vatke	Nangoso	Lamiaceae	Shrub	✓ ✓	Whole plant	B	<a href="http://members.lycos.co.uk/ethiopianplants/shimasha.html">http://members.lycos.co.uk/ethiopianplants/shimasha.html</a> Getaahun, 1976 Jembere et al., 1995
<i>Tinnea aethiopica</i> Hook. f.	Lubilikhe	Lamiaceae	Shrub	✓ ✓ ✓	Leaf	T	Jembere et al., 1995
<i>Plectranthus barbatus</i> Andr.	Ling'ulie or Kamang'ulie	Lamiaceae	Shrub	✓	-	T	Jembere et al., 1995
<i>Plectranthus lactiflorus</i> (Vatke) Agnew	-	Lamiaceae	Shrub	✓	-	T	Jembere et al., 1995
<i>Leonotis nepetifolia</i> (L.) R. Br.	Susuni	Lamiaceae	Shrub	✓	-	T	Jembere et al., 1995
<i>Hostundia opposita</i> Vahl.	Bifwofwo	Lamiaceae	Herb	✓ ✓ ✓	-	T	Jembere et al., 1995
<i>Ocimum kiltimandscharicum</i> Baker ex Gürke.	Bifwofwo	Lamiaceae	Herb	✓ ✓	-	T C	Jembere et al., 1995
<i>Leucas calostachys</i> Oliv. Var. <i>fascicularis</i> (Baker) Sebald.	Nasusi	Lamiaceae	Herb	✓	-	T	Jembere et al., 1995
<i>Olea capensis</i> L.	Kumutamaywa	Oleaceae	Tree	✓	Leaf/bark/fruit	F, C B	Hines and Eckman, 1993 Mutangah, 1996 Stockbauer, 2003 Hines and Eckman, 1993

<i>Olea europaea</i> L. ssp. <i>africana</i>	Kumunyubuti	Oleaceae	Woody perennial tree	✓	Bark/stem/leaves	C	Palgrave, 1988 Hines and Eckman, 1993
						F	Hines and Eckman, 1993
<i>Maytenus senegalensis</i> (Lam.) Exell.	Kumwayakhafu	Celastraceae	Tree	✓ ✓	Fruit/leaf/stem/ bark/root	B	Botanical South Africa, 2003 Viabile Herbal solutions, 2003
						B	El Tahir et al., 1998 El and Satti, 1999 Hussain et al., 1999 Bhatt et al., 2001 Kumar, 2003
<i>Maytenus heterophylla</i> (Eckl. & Zehy) N.K.B. Robson.	Kumuwawa No.1	Celastraceae	Tree	✓	Fruit/leaf/stem/ bark/root	F, B	Working papers FGR/18E, 2001
						B	N
<i>Maytenus arbutifolius</i> Wilczek.	Kumuwawa No.2	Celastraceae	Tree	✓	Fruit/leaf/stem/ bark/root	B	N
						P, T, I	Berger, 1994
<i>Terminalia mollis</i> Laws.	Kumukhonge	Combretaceae	Tree	✓ ✓	Stem/root/bark	P, T	Berger, 1994
						P, B, C, T, I	Berger, 1994 Stockbauer, 2003
<i>Combretum molle</i> R.Br ex G. Don. (Velvet Bush-willow)	Kumukimila	Combretaceae	Tree	✓ ✓	Stem/root/bark	P, T	Berger, 1994
						P, T	Berger, 1994
<i>Combretum collinum</i> Fres.	Kumulaha	Combretaceae	Tree	✓ ✓	Resin/root/bark/leaf	P, T	Berger, 1994
						P, T	Berger, 1994
<i>Combretum bindarianum</i> Kotschy.	Kumulaha	Combretaceae	Tree	✓ ✓	Resin/root/bark/leaf	P, T	Berger, 1994
						P, T	Berger, 1994
<i>Combretum elgonense</i> Exell.	Kumulaha kumu- kalukha	Combretaceae	Tree	✓	Resin/root/bark/leaf	P, T	Berger, 1994
						P, T	Berger, 1994
<i>Euclea divinorum</i> Hiern.	Kumuchanjaasi	Ebenaceae	Tree	✓ ✓ ✓ ✓ ✓	Root/leaf/bark	B,	Homer et al., 1990 Homer et al., 1992 Dagne et al., 1993 Hines and Eckman, 1993 van Grinsven et al., 1999 Lukwa et al., 2001
						C	Hines and Eckman, 1993
<i>Carisa edulis</i> Vahl.	Sirwa	Apocynaceae	Shrub	✓ ✓	Leaf/fruit/root	F	Zemede and Mesfin, 2001
						B	Ibrahim et al., 1999
<i>Gynandropsis gyanandra</i> (L.) Briq.	Chisaka (Chisaka- Luluya, Ejobyo-Luganda and Akeyo-Luo)	Cappariaceae	Herb	✓	Whole plant	A	Malonza et al., 1992 Dipeolu et al., 1992 Torto and Hassanali, 1997 Lwande et al., 1999
						B	UWAMG, 2003
						T	Ndungu et al., 1995 Ndungu et al., 1999

Gardenia ternifolia Schum. & Thonn.	Siuna	Rubiaceae	Shrub	✓ ✓ ✓ ✓ ✓ ✓	Root/fruit	F	Zemedede and Tadessa, 2001
<i>Pavetta oliveriana</i> Hiern.	Kumukokhakokhe	Rubiaceae	Tree/shrub	✓	Root/leaf	-	N
<i>Pavetta crassipes</i> (K. Schum)	Kumusindusianjofu/ Kumupepenambusi kumukhasi	Rubiaceae	Tree	✓	Root/leaf	F, B	Sanon et al., 2003.
<i>Steganotaenia araliaceae</i> Hochst.	Kumutomolo (1) or Kumupepenambusi kumusecha	Umbelliferae	Tree	✓	Leaf/stem/bark	B	Rukangira, 2003
<i>Annona senegalensis</i> Pers. ssp. <i>Senegalensis</i>	Kumufwora	Annonaceae	Tree	✓	Leaf/bark/root	I, F	Stockbauer, 2003
<i>Annona chrysophylla</i> Boj.	Kumufwora	Annonaceae	Tree	✓	Leaf/bark/root	I, B, F	Stockbauer, 2003
<i>Syzygium guineense</i> (Willd.) Dc. ssp. <i>guineense</i>	Kumusitole kumusecha	Mrytaeae	Tree	✓	Bark/root/fruit	C, F, B	Hines and Eckman, 1993
<i>Syzygium cordatum</i> Krauss.	Kumusemwa	Mrytaeae	Medium-large tree.	✓	Stem/root/bark/fruit	F	Trade Winds Fruit, 2003
<i>Syzygium cumini</i> (L.)							
<i>Dolichos kilimandscharicus</i> Taubert Subsp. <i>parviflorus</i> Verde.	Nandwasi	Fabaceae	Herb/shrub	✓ ✓ ✓ ✓	Root/stem/leaves (used to make concoction)	B, P (cattle)	University of Nairobi Herbarium Library/records on voucher specimen.
<i>Mondia whytei</i> (Hook f.) Skeels.	Kumukombela	Periplocaceae	Herb	✓	Powdered root	P	Golob et al., 1999
<i>Teclea nobilis</i> Del.	Kumutare	Rutaceae	Tree	✓	Root/stem?	B	Okwemba, 2002
<i>Cupressus lusitanica</i> Mill.	Kumusayiprasi	Cupressaceae	Tree	✓ ✓ ✓ ✓ ✓	Root/stem/bark/leaf	C, B	Hines and Eckman, 1993 Marin, 1999
<i>Juniperus procera</i> Hochst. ex Endlicher.	Kumutarakwa (1)	Cupressaceae	Tropical African timber tree with fragrant wood	✓ ✓ ✓ ✓	Leaf/stem	C, I	Personal communication with herbalists
					Leaf/root/bark	I, B	Getahun, 1976
						I	Anonymous, 2001d Hines and Eckman, 1993

<i>Podocarpus falcatus</i> Mind. (Fern Pine)	Kumutarakwa (2)	<u>Podocarpaceae</u>	Shrub	✓	Leaf/root/bark	I	Stockbauer, 2003
<i>Podocarpus latifolius</i> (Thunb.) R.Br. ex Mirb.	Kumutarakwa (3)	<u>Podocarpaceae</u>	Tree	✓ ✓	Stem/root/leaf	C	Stockbauer, 2003
<i>Pittosporum viridiflorum</i> Sims. ssp. <i>viridiflorum</i> ssp. <i>quartiniianum</i> (Cuf) Cuf.	Nambaa (1)	Pittosporaceae	Shrub	✓ ✓	Stem bark /root/leaf	F, B	Coetzee et al., 1999 Berger, 2002 Sec, 2002
<i>Harrisonia abyssinica</i> Oliv.	Sibondwe	Simaroubaceae	Tree	✓ ✓	Leaves/roots/barks/ stem	B	Fabry et al., 1996 Watt and Breyer-Brandwijk, 1962 Johns et al., 1990 Kokwaro, 1993 Bally, 1937
<i>Withania somnifera</i> (W. ashwagandha)(L.) Dunal	Nambaa (2)	Solanaceae	A perennial herb	✓ ✓	Stem/root/leaf	B	Pande, 1999 Singh and Kumar, 1998 McIntyre, 2002
<i>Zea mays</i> L.	Kamayindi	Sapindaceae	Grass	✓ ✓ ✓	Leaf/stem	B	Lukwa et al., 2001d
<i>Sorghum bicolor</i> (L.) Moench.	Kamayemba	Gramineae	Grass	✓	Whole plant (particularly grains, leaf and stem).	I, F, B	Grieve, 1931 Watt and Breyer-Brandwijk, 1962 Duke and Wain, 1981 Morton, 1981 Perry, 1980 Duke, 1983c Anonymous, 2001d
<i>Saccharum officinarum</i> L.	Kimiba (Kumwiba)	Gramineae	Grass	✓	Leaf/stem/juice	N	-
<i>Ipomoea batatas</i> (L.) LAM	Kamabwoni (Chindabii Chekamabwoni)	Convolvulaceae	Herb	✓ ✓ ✓	Leaf/stem/sap	B	Cevallos-Casals and Cisneros-Zevallos, 2002
<i>Hibiscus cadyphyllus</i> Cav.	Kumwarakumba	Malvaceae	Shrub	✓	Leaf/stem/aerial parts/root	F, N	Puckhaber et al., 2002
<i>Jacaranda mimosifolia</i> D. Don	Chakaranda	Bignoniaceae	Tree	✓ ✓	Leaf/root/stem	-	-
<i>Albizia coriaria</i> Welw. ex Oliv.	Ober (Luo)		Tree	✓	Bark/root	B	Bekalo et al., 1996 Kokwaro, 1993

## Other suggested non-botanical anti-tick agents (works best in mixed form)

Bar soap (toxic)								
Fish (odours) (repellent)								
Cattle urine + sisal (Toxic and repellent)								
Cattle cowdung (repellent)								

## Key

- ✓ Refers to *a single* mention of plant(s) for its or their use, respectively, in tick control and or control of other livestock pests
- A Plants that were found in literature to demonstrate *acaricidal* activities/compounds
- B Plants that were found in literature to have *bioactive* compounds
- P Plants that were found in literature to demonstrate *pesticidal* activities/compounds
- I Plants found in literature to be resistant to attack by *insects* or found to demonstrate *insecticidal* activities compounds
- T *Taxonomic* affinity to plant species (at genus and family levels) known to possess bioactive, insecticidal, acaricidal activities/compounds
- N *No* leading bioactive, insecticidal and or acaricidal information from literature
- F Human *food* plants, unless otherwise stated
- Af Plants with *antifeedant* activities
- C Plants with *cultural* implication and applications.  
Information not available for documentation
- S *Sericiculture* - the cultivation of silk worms for the production of natural silk fiber.
- Ps *Poisonous* plants



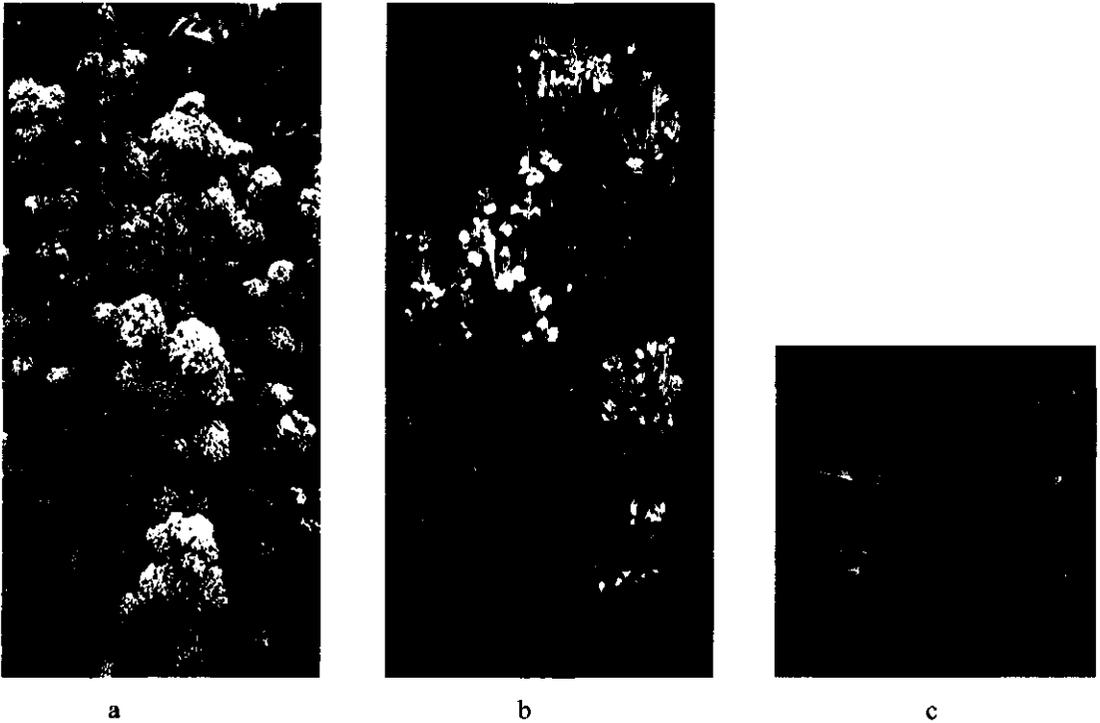
## Appendix 2.

### Background information on two selected plants for an in-depth scientific study

#### *Tagetes minuta* L.

##### *Origin and morphology of Tagetes minuta*

*Tagetes minuta* (Asteraceae) is an erect annual herb, growing to a height of 1-2 m (Fig.A2.1). The plant resembles the garden marigold (*T. erecta* L). Wild marigold, as it is commonly known, is a problematic weed of pastures and numerous crops in East and South Africa, South America, and Australia (Soule, 1993). The undersurface of the leaves bear a number of small, punctate, multicellular glands, orangish in color, which exude a licorice-like aroma when ruptured. Glands may also be found on the stems and involucre



**Fig. A2.1.** *Tagetes minuta* L. (Asteraceae) plant showing (a) the young and (b) the mature aerial parts. **Flowers** are hermaphrodite (having both male and female organs) and are in erect clusters at the ends of the branches. **Flower heads** are on short stalks. **Leaves** are slightly glossy green, and are pinnately dissected into 4 to 6 pairs of pinnae. **Leaf margins** are finely serrate. **Heads** numerous, usually in flat-topped cymes, four or five fused involucre cylindrical bracts surround each head (b), 8-12 mm high, apex 3-5-toothed yellow-orange ray florets usually 3 per head, rays 1-2 mm long; disk florets usually 3-5 per head, and 10 to 15 yellow-orange disk florets per capitula, corollas are 2.5 mm long; longer pappus scales 2-3 mm long, the others are 1 mm long. The dark brown **achenes** (c) are 10 to 12 mm long, with a pappus of 1 to 4 tiny scales and 0 to 2 retrose serrulate awns, which are 1 to 3 mm long (Adopted from Soule, 1993; Wagner et al., 1999).

bracts. Four or five fused involucre bracts surround each head. The seeds also have an unpleasant odour and can reduce the value of grain harvests when they are mixed in.

The genus *Tagetes* belongs to the Asteraceae family and comprises 56 species, 27 of them annuals and 29 perennials. *Tagetes* spp. are grown all over the world as multi-purpose plants (Soule, 1993; Vasudevan et al., 1997). Of these, *T. minuta*, *T. erecta*, *T. patula* and *T. tenuifolia* are the most common (Vasudevan et al., 1997) with *T. minuta* being the most studied species. *Tagetes minuta* is a suitable essential oil-bearing species and therefore cultivated in several countries such as Egypt, France, Morocco, Brazil, USA and India for its applications in the perfumery, flavouring and pharmaceutical industries (Chalchat et al., 1995).

#### ***Ethnobotanic uses of Tagetes minuta***

*Tagetes minuta* has a long history of human interest and is used as beverage, condiment, ornamental, medicinal decoction and in ritual practices (Rees, 1817; Morton, 1981; Soule, 1993). The plant's leaves, stems, and flowers are used. In recent years, there has been an increasing interest in the use of *T. minuta* by indigenous people in India (Anjaria, 1989). Wild marigold is sometimes an alternative host for *Sclerotinia sclerotiorum*, a fungal pathogen that can infect a variety of crops (Soule, 1993).

The New World peoples have been using *T. minuta* as a beverage, a medicinal tea, and a condiment since pre-Columbian era (Rees, 1817). The local names of *T. minuta* vary by region, ethnicity and/or by dialects. A beverage is prepared from *T. minuta* by steeping a "half-handful" of the dried plant in hot water for 3-5 minutes. The beverage may be consumed warm or cooled, and may be sweetened to individual taste (Neher, 1968). For medicinal use, a decoction made by steeping a "double handful" of the dried plant in boiling water for 3 to 5 minutes is used as a remedy for the common cold; including upper and lower respiratory tract inflammations, and for digestive system complaints; stomach upset, diarrhea, and "liver" ailments. In many native regions of the world, the plant is used in popular as anthelmintic, diuretic, antispasmodic and to treat stomach and intestinal diseases (Amat, 1983). The plant's decoction is consumed warm, and may be sweetened to individual taste (Cavanilles, 1802; Parodi, 1959; Neher, 1968). *Tagetes minuta* is used as a condiment in Chile and Argentina. It is popular in rice dishes and as a flavouring in stews. In northern Chile *suico* (a preparation of *T. minuta* that enhances flavour of food) is so highly prized that many people collect wild plants to dry a sufficient supply to last the winter (Soule, 1993). Leaf infusions and extracts from *Tagetes* spp. have been used in folk medicine to treat intestinal and stomach diseases and some of them have been found to have bioactivity against Gram positive and Gram negative microorganisms (Tereschuk et al., 1997; Broussalis et al., 1999). *Tagetes minuta* is often referred to as a weed in many parts of the world including South America (Cabrera, 1971). Many Latin American farmers who do not practise industrialized agriculture will leave plants of *T. minuta* in their fields. This second crop is beneficial in several ways: first, rapid growth of *T. minuta* quickly shades out other plant species that may be of less use to the farmer, second, it can be harvested for personal use, or for sale in city markets; and third, it has been reported to aid in the retention of humidity in the field (Jimenez-Osornio, 1991). *Tagetes minuta* is commercially grown and harvested for its essential oils, which are used in the flavour and perfume industry as "*Tagetes* Oil" (Lawrence, 1993). The oil is also a major component in most major food products, including cola beverages, alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, puddings, condiments, and relishes (Leung, 1980). Brazil is one major producer of *T. minuta* for *Tagetes* Oil (Craveiro et al., 1988). World-wide production of the oil was around 1.5 tonnes in 1984 (Lawrence, 1985a;b).

In Kenya, an infusion of *T. minuta* is used for the treatment of snake bites in the Luo and Kamba communities (Owuor and Kisangau, 2006) and protection against mosquito bites in the tribes of western Kenya (Seyoum et al., 2002).

### **Ecology of *Tagetes minuta***

*Tagetes minuta* is native to temperate grasslands and montane regions of southern South America (McVaugh, 1943), including Argentina (Espinar, 1967), Chile (Reiche, 1903), Bolivia (Perkins, 1912), Peru and in the Chaco region of Paraguay (Herrera, 1941). Starting with the Spanish conquest of South America, it was introduced into Europe (Jordano and Ocana, 1955), Asia (Cherpanov, 1981), Africa (Hillard, 1977), Madagascar (Humbert, 1923), India (Rao et al., 1988), Australia (Webb, 1948) and Hawaii (Hosaka and Thistle, 1954). *Tagetes minuta* is now a widespread weed in Africa, South Europe, South Asia and Australia (Cabrera, 1971). In Kenya, *T. minuta* is naturalized primarily in arable lands as a weed ever since its introduction from South America in the 1920s (Stadler et al., 1998). *Tagetes minuta* is often found growing in disturbed areas during early successional stages. This affinity for disturbed sites has allowed the species to colonize many areas around the world (Soule, 1993). This noxious, rapidly growing aromatic herb forms a dense ground cover at higher elevations (850–2750m). The seeds cling to the hair of animals and are dispersed by both domesticated and wild animals. Towards the end of its growing season, the aerial parts of *T. minuta* dry up and may easily be destroyed by fire, but new colonies are formed rapidly in the following season from seeds deposited in soil.

### **Chemotypes of *Tagetes minuta***

The composition of the essential oils of many species of *Tagetes* has been reported previously (Machado et al., 1994; Lawrence, 2000; Stojanova et al., 2000) and showed significant differences in their composition (Lawrence, 1985b; Graven et al., 1991; Zygadlo et al., 1993; De Feo et al., 1998; Senatore and De Feo, 1999). The *Tagetes* species can be unambiguously differentiated by the chemical composition of their essential oils (Ross et al., 1981; Héthelyi et al., 1987; 1989; Croes et al., 1989). The GC and GC-MS analysis of *T. minuta* oils indicates that plant organ, stage of growth, soil, climate, chemotypes, sunlight, latitude and methods of extraction all affect the composition of the oil (Héthelyi et al., 1987; Lawrence and Reynolds, 1992; Chalchat et al., 1995; Daghero et al., 1999; Gil et al., 2000). *Tagetes minuta* is rich in secondary compounds, including acyclic, monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, thiophenes, and aromatics (Rodriguez and Mabry, 1977). There is evidence that the secondary compounds in *Tagetes* are effective deterrents of numerous organisms, including: - fungi (Chan et al., 1975), including those pathogenic to humans (Camm et al., 1975), bacteria (Grover and Rao, 1978), round worms in general (Loewe, 1974), trematodes (Graham et al., 1980), nematodes (Grainge and Ahmed, 1988), and numerous insect pests through several different mechanisms (Jacobsen, 1990; Saxena and Koul, 1982; Maradufu et al., 1978; Saxena and Srivastava, 1973). Many closely-related plant secondary compounds have demonstrated medicinal value in humans (Kennewell, 1990; Korolkovas and Burekhalter, 1976).

In Argentina, dihydrotagetone,  $\alpha$ -phellandrene, limonene, *o*-cymene, as well as the isomers of  $\beta$ -ocimene, tagetone and tagetenone, were the major constituents of *T. minuta* essential oil (Gil et al., 2000). In Saudi Arabia, GC and GC-MS analyses confirmed the presence of tagetone (11.52%), 5-octyn-4-one,2,7-dimethyl (11.52%), Propanedinitrile,dicyclohexyl- (10.45%) and 2-pinen-4-one (8.03%) to be the main components with lesser amounts of 1-acetoxy-*p*-menth-3-one (0.17%) and 9-octacenamide(*Z*) (0.48%) (EL-Deeb et al., 2004). In India, the freshly distilled *T. minuta* oil contained ocimene 54.97%,

and dihydrotagetone 32.58% as major constituents (Singh et al., 1992), while in other studies (*Z*)-tagetone, (*Z*)- $\beta$ -ocimene, dihydrotagetone, (*Z*)- and (*E*)-ocimenone were found as major constituents (Chalchat et al., 1995; Bansal et al., 1999). In Egypt, the main components of the *T. minuta* essential oil were monoterpenes of which *trans*- and *cis*-tagetone were present in 52.3 % - 64.2 % (Mohamed et al., 2002). In Iran, the GC and GC-MS analysis revealed the main components to be  $\alpha$ -terpineol (20.8%), (*Z*)- $\beta$ -ocimene (17.7%), dihydrotagetone (13.7%), (*E*)-ocimenone (13.3%), (*Z*)-tagetone (8.4%) and (*Z*)-ocimenone (6.1%) (Moghaddam et al., 2007). In other studies, out of one hundred and four chemical components, the major constituents of *T. minuta* essential oil were tagetone, *E/Z*-ocimenone, *E/Z*-ocimene, germacrene, limonene, *trans*-anethole and dihydrotagetone (Héthelyi et al., 1986c). From these analyses, it could be seen that the composition of the oils varied according to a number of factors: the harvesting location (Cravello et al., 1988; Chalchat et al., 1995), growth stage (Héthelyi et al., 1986b), plant parts (Héthelyi et al., 1986a; Chalchat et al., 1995), soil type and nutrient status (Graven et al., 1991) and chemotypes (Gil et al., 2000) etc.

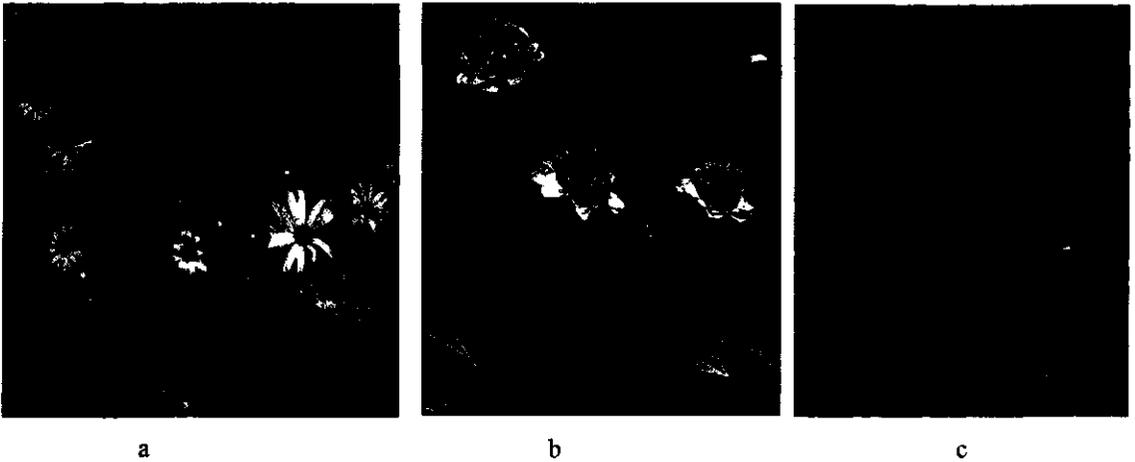
#### ***The biological properties of the essential oil of Tagetes minuta***

Validation of some of the folkloric claims has shown that *T. minuta* contains compounds that have a wide range of bioactive properties. *Z*- $\beta$ -ocimene and dihydrotagetone, which are constituent compounds of the essential oil of *T. minuta*, were found to be antiviral, active against carnation ring spot (CaRSV) and carnation vein mottle viruses (CaVMV) (Okioga and Rajamannan, 1997; Singh et al., 2002). The essential oil of *T. minuta* generally affect a variety of microbial organisms (Ross et al., 1981; Héthelyi et al., 1989 Souza et al., 2000; Senatore et al., 2004). Allelopathic activities of *Tagetes* spp, particularly those against nematodes (*Meloidogyne* spp), have been reviewed (Akhtar and Malik, 2000; Korkalis-Burelle and Rodriguez-Kabana, 2006). Nematocidal activity of *T. minuta* roots is attributed to thienyls while the biocidal components of the essential oil from flowers and leaves are terpenoids (Vasudevan et al., 1997). Dihydrotagetone and *Z*- $\beta$ -ocimene isolated from *T. minuta* oil showed strong nematocidal activity against eggs and juveniles of *Meloidogyne incognita* with dihydrotagetone showing a higher level of toxicity than *Z*- $\beta$ -ocimene (Adekunle et al., 2007). Some evidence suggests that the secondary compounds of *T. minuta* are inhibitory to parasitic root nematodes and other microbes (Soule, 1993). While carotenoid pigments from *Tagetes* are useful in food coloring, the volatile oils of *T. minuta* are used as flavour components in food products and as perfumes, and have a suppressive biological activity against some insects and pathogens (Vasudevan et al., 1997).

#### ***Tithonia diversifolia* (Hemsley) A. Gray**

##### ***Origin and morphology of Tithonia diversifolia***

*Tithonia diversifolia* is a member of the sunflower family, Asteraceae. *Tithonia* was named for Tithonus, a legendary Trojan loved by the dawn goddess Eos, who turned him into a grasshopper. *Tithonia diversifolia* is native of Mexico and Central America and is cultivated for its beautiful flowers and enormous size. The genus occurs throughout Middle America and the West Indies and has become naturalized around the tropics. Depending on the geographical location of the plant, it may be either annual or perennial, 2-3 m in height with upright and sometimes ligneous stalks in the form of woody shrubs (Fig. A2.2). The plant is mostly leafless on its lower parts. The leaf arrangement is helically alternate. The typical leaf is 15-30 cm long and has a minute roughness (scabridity) on the upper surface. The whitish petiole (acuminate) is fringed halfway with blade tissue at the terminus of



**Fig. A2.2.** *Tithonia diversifolia* (Hemsley) A. Gray (Asteraceae) (Mexican sunflower/wild sunflower). (a) shows floral parts in early stages with buds and (b) shows floral parts in late stages. **Leaves:** mostly subovate, acute, serrate, 10-40 cm long, long (2-10 cm) petiolate, simple or mostly 3-7-lobed with acute serrate lobes, somewhat glandular, slightly grayish beneath, puberulent on both surfaces; petiole slightly bilobed at base. It is pubescent on the lower side, which appears greyish. Leaf veins are parallel. **Peduncles (b):** are 10-30 cm long; heads large and showy, 5-15 cm wide; involucre of bracts in 4 series; ray-florets about 12-15, with yellow ligules to 6 cm long. **Flowers:** similar in appearance to the common sunflower but smaller(a). The flower disc is about 3 cm diameter with yellow petals, 4-6 cm long. Each mature stem may bear several flowers at the top of the branches. **Seeds (b) and (c):** wind, water and animals easily disperse the light seeds. **Achenes:** cuneate, 4-angled, appressed-puberulent, 5 mm long, 2-awned

which three whitish midveins vascularize a mitten-like blade with three or five prominent lobes (Kokwaro, 1994). Occasionally, new shoots possess unlobed leaves.

The plant's flowers are a favourite of bees and are formed by an orange-yellow inflorescence composed of many small flowers crowded together. Around the perimeter, 11-13 ray florets (ligulate flowers) frame 200-300 tubular disk florets, which smell like a daisy (Fig. A2.2c). After pollination, the inferior ovary of each disk floret develops as a hairy, gray, flattened, dry, one-seeded fruit (an achene) hidden by papery, brown-tipped bracts that, at maturity, are arranged into a hemispherical mound (Kokwaro, 1994).

#### ***Ethnobotanic use of Tithonia diversifolia***

By etymological description, the Kenyan common ethnic names include among others, *maruru*, *maua* and *amalulu* (for Luhya), *maua makech* (for Luo) and *amaua amaroro* (for Kisii), all implying that the plant is bitter to the taste. The African farmers have many uses for the plant, the most popular use being as an organic fertilizer for vegetable crops in either compost or a tea form (Jama et al., 2000). Traditionally, Kenyans use it for ornamentals, livestock feeds, wind breaks, environmental conservation (for both soil and water conservation), honey production, curing of fire-cured tobacco, fuel wood (dry stumps), live fences, boundary demarcation and as a medicine in the form of leaves' infusion for constipation, stomach pains, indigestion, sore throat and liver pains and diarrhoea in livestock (Kokwaro, 1993).

Adoyo et al. (1997) reported that farmers working with the Kenya Woodfuel and Agroforestry Programme (KWAP) identified *T. diversifolia* as a potential insecticide to control termite infestations in farms and homes. One farmer's experiment with tea from either fresh leaves or the ash of *T. diversifolia*, *Cassia siamea* and *C. spectabilis* applied to

affected trees, provided protection from termites for up to 45 days. Another farmer, who had a problem with underground termites, made a solution based on fermented extracts of *T. diversifolia* and *Melia azedarach*, which controlled the pests when the concoction was made and poured into the termite mound. After two years of research, farm results showed the most effective treatment to be a solution made from *T. diversifolia*, *Vernonia amygdalina* and *Agave sisalana* (Adoyo et al., 1997). Not only did this solution control termites, but it also contributed to soil fertility (Nagarajah and Nizar, 1982; Wanjau et al., 1997). By using such local resources, one avoids the need to purchase the hazardous synthetic chemical pesticides. Moreover, farmers were reported as being enthusiastic about the use of *Tithonia* green manure and its effectiveness (Adoyo et al., 1997).

In Nigeria, there are oral reports among herbal medicine practitioners linking *T. diversifolia* with the treatment of menstrual pain (Owoyele et al., 2004). In addition, *T. diversifolia* extracts are used in the treatment of wounds (Rüngeler et al., 1998) and diabetes mellitus (Takanashi, 1998). And recently, in western Kenya, *T. diversifolia* has been discovered as a very important organic fertilizer (Wanjiru, 2003; SACRED Africa, 2007), enhancing the availability of Phosphorus to crops, which led to its recommendation for biomass transfer technologies in Kenya (George et al., 2002 a;b).

In Mexico, the place of origin of the plant, it is used to treat sprains, bone fractures, bruises and contusions. People in Mexico grow the plant in their gardens and use it for relieving dermatological problems (Heinrich, 1998a). In the Lowland Mixe, it is used orally to treat malaria and other forms of fever and topically to treat hematomas and muscular cramps (Heinrich et al., 1992; Heinrich, 1996; Heinrich et al., 1998b). It is also used as a liniment in Yucatan (Batchelder, 2001). These medicinal uses may result from the similarity of the flower heads of this species to the ones of European arnica (*Arnica montana* L.). This assumption is corroborated by its popular names: *Arnica de la montana* and *arnica*. Berlin and Berlin (1996) listed *T. diversifolia* as an important remedy for gastrointestinal complaints and it is cited as an anti-inflammatory and as treatment for wounds and skin eruptions.

In southern China, people use *T. diversifolia* to treat skin diseases (such as athlete's foot), night sweats, as a diuretic, hepatitis, jaundice and cystitis. In Taiwan, the plant is sold in herbal medicine markets as an infusion to improve liver function while in Thailand and Japan, it is highly regarded as an ornamental plant.

#### **Ecology of *Tithonia diversifolia***

In Kenya, *T. diversifolia* is found growing in Western and Central Provinces as well as coastal regions and parts of the Rift Valley, between latitude: 1°0'N and longitude: 38°0'E. It is a bushy perennial weed and a valuable green manure (Thijssen et al., 1993; Wanjau et al., 1997). It was introduced in Kenya from Central America as an ornamental and escaped from cultivation and now grows as a wild plant on the fields, in hedges, along roadsides and on wasteland/disturbed areas, not only in Kenya, but also elsewhere in the world (Akobundu and Agyakwa, 1987; Smith, 1991; Space and Flynn, 2000). Although sometimes cultivated (Kendall and van Houten, 1997), *T. diversifolia*, is now a pantropically distributed weed.

#### **Chemotypes of *Tithonia diversifolia***

The chemical composition of essential oils from *T. diversifolia* has been previously described (Lamaty et al., 1991; Kuo and Chen, 1997; Elufioye and Agbedahunsi, 2004a; Moronkola et al., 2007). For instance, Moronkola et al. (2007) found leaf oil to be comprised of an abundance of  $\alpha$ -pinene (32.9%),  $\beta$ -caryophyllene (20.8%), germacrene D (12.6%),  $\beta$ -

pinene (10.9%) and 1, 8-cineole (9.1%). Germacrene D (20.3%),  $\beta$ -caryophyllene (20.1%) and bicyclogermacrene (8.0%) characterized the oil of the flower while a number of aliphatic fatty acids and a diterpenoid compound, sandaracopimaradiene, that were present in the flower, could not be detected in the leaf oil (Moronkola et al., 2007).

***The biological properties of the essential oil from Tithonia diversifolia***

The validation of some of the folkloric claims have shown that *T. diversifolia* contains compounds that have a wide range of bioactive properties, namely, cytotoxic (Wu et al., 2001), anti-malarial (Elufioye and Agbedahunsi, 2004b), anti-inflammatory (Rüngeler et al., 1998; Owoyele et al., 2004), potential cancer chemopreventive (Gu et al., 2002), anti-amoebic (Tona et al., 1998), antiviral activity against human immunodeficiency virus type-1 (Cos et al., 2002), anti-diarrhoeal (Tona et al., 1999), anti-amoebic and spasmolytic activities (Tona et al., 1998, 2000), and analgesic properties (Owoyele et al., 2004). Tagitinin C, an anti-plasmodial sesquiterpene lactone, has been isolated from the aerial parts of *T. diversifolia* for development (Goffin et al., 2002; 2003). (-)-Germacrene D, a chemical constituent of the essential oil of *T. diversifolia*, increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens* (Mozuraitis et al., 2002). Compounds isolated from the aerial parts of *T. diversifolia* showed cytotoxic activity against HL-60 leukemia cells with IC<sub>50</sub> values ranging from 0.13 to 13.0  $\mu$ M (Kuroda et al., 2007). *Tithonia diversifolia* has also been reported as a potential candidate for bioinsecticide preparations against *Callosobruchus maculatus* (Coleoptera: Bruchidae) (Adedire and Akinneye, 2004).



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## Samenvatting

Dit proefschrift beschrijft de effecten van verscheidene etnobotanische plantenextracten op het gedrag van de teek *Rhipicephalus appendiculatus* (bruine oortek), de belangrijkste overdrager van East Coast fever (ECF) op rundvee in Centraal- en Oost-Afrika. Gebruik makend van participatieve onderzoeksmethoden, de traditionele kennis over tekenbestrijding onder de bevolking van de Bukusustam uit West Kenia werd verzameld met het doel te achterhalen welke plantensoorten gebruikt worden om teken, die een bedreiging voor de veestapel vormen, te bestrijden. Dit resulteerde in een lijst van meer dan 150 plantensoorten vallende onder 10 genera en 51 plantenfamilies. Een selectie van 8 plantensoorten uit deze lijst werd gebruikt om etherische oliën te maken, welke vervolgens in het laboratorium gebruikt werden in gedragsexperimenten met teken. Uit deze eerste experimenten kwamen de planten *Tagetes minuta* (geelgroen afrikaantje) en *Tithonia diversifolia* (Mexicaanse zonnebloem) naar voren als het meest geschikt om verder getest te worden.

Door middel van twee-keuze-experimenten in het laboratorium werd vastgesteld dat etherische oliën afkomstig van *T. minuta* of *T. diversifolia* een dosisafhankelijke, afstotende werking op het klijngedrag van de teek hebben. Op stieren bleek dat *R. appendiculatus* een voorkeur hebben om zich aan de binnenzijde van de oren vast te hechten. Behandeling van de oren van stieren met het extract van zowel *T. minuta* als *T. diversifolia* resulteerde in een significante afname van tekenbeten in het oor. Deze afname werd ook in een veldexperiment gevonden, waar de extracten naast een afname van *R. appendiculatus* ook voor een significante afname van andere tekensoorten op de runderen zorgden. Tevens bleek uit veldexperimenten dat *T. minuta* een sterkere werking op alle aangetroffen tekensoorten heeft dan *T. diversifolia*.

De in dit proefschrift beschreven resultaten tonen de potentie van etherische oliën van planten voor de bestrijding van *R. appendiculatus* en andere tekensoorten, bijvoorbeeld met een "push-pull" methode, en daarmee gepaard gaande door teken overdraagbare ziektes in veehouderijsystemen in tropisch Afrika.



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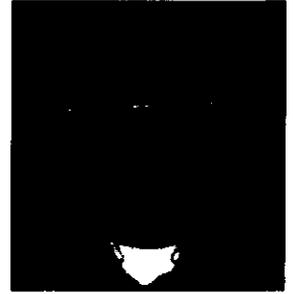
To my parents, Mr. Charles Wanzala Osundwa and Mrs. Phanice Masakhwe Wanzala, thank you most sincerely for starting the PhD project in my early life. Both of you were determined to see every child in the family receive quality education. You went through hard times looking for school fees but the course was worthwhile. *Mama*, you were a no nonsense woman to joke around with concerning education issues and the whole village new about it! Thank you *mama*, I can now understand why you had to cane me once for running away from school. The combined efforts of parenting and school administration have now yielded some good fruits, the thesis you are now reading!

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## Curriculum vitae

Wycliffe Wanzala<sup>1</sup> was born on August 24 1969 in Mumias District, western Kenya. He started formal education at Namamba Primary School (Musamba village, Matungu, Mumias District) where he attained a Certificate of Primary Education (C.P.E.) in 1983. He proceeded to Lubinu Boys Secondary School (Mumias District) for his ordinary level of education ('O' level) and obtained a Kenya Certificate of Education (K.C.E.) in 1987 with Division 1. In 1988, he joined Nakuru High School (Nakuru District) for his advanced level of education ('A' level) and obtained a Kenya Advanced Certificate of Education in 1989. In 1990 he joined the Faculty of Science, College of Biological and Physical Sciences, Chiromo Campus, University of Nairobi and obtained a BSc degree (Zoology, Botany and Meteorology with a BSc dissertation in Applied Entomology) in 1994. In 1993/95, he worked as a laboratory technician in the Zoology Department, University of Nairobi under Prof. Richard W. Mwangi and in 1995/97 he was promoted to the position of Research Assistant/Co-ordinator (in Applied Entomology) in the Faculty of Science for a project between the University of Nairobi and Kenya Agricultural Research Institute (KARI). In 1995, he entered the MSc class for Medical Parasitology following the award of a scholarship by the German Academic Exchange Service (DAAD) and graduated in 1999. Between 1996 and 1997 he worked on his MSc thesis on veterinary public health, immunology and epidemiology of human and bovine taenioses at the National Veterinary Research Centre (NVRC), Muguga, KARI. On 1<sup>st</sup> June, 2000, he joined the Behavioural and Chemical Ecology Department (BCED) of the International Centre of Insect Physiology and Ecology (ICIPE) as an Intern researcher in preparation for his PhD programme and studied on-host tick behavioural ecology. Between March and August, 2001 he was a visiting scientist at the Department of International Animal Health, Freie Universität, Berlin, Germany. From January 2002-current he became the Principal Investigator (PI) of an ethnoveterinary project (control and management of livestock ticks) funded by the International Foundation for Science (IFS), Sweden at ICIPE and the University of Nairobi, Kenya. In 2004 he was awarded a sandwich PhD fellowship by the executive board of Wageningen UR, the Netherlands and in the same year, 2004, he became the founding Director of The Scholarship Network Centre, Nairobi and Community Research Associate at Matungu Rural Herbal and Research Centre, Kenya. In March 2005 he was appointed Honorary Lecturer, School of Biological Sciences, University of Nairobi, Kenya. In July, 2005, ICIPE offered him a postgraduate research training fellowship award under the African Regional Postgraduate Programme in Insect Science (*ARPPIS*). In August, 2005, he was appointed a Part-time Lecturer at the Department of Natural Sciences, Faculty of Science, The Catholic University of Eastern Africa (CUEA), Kenya. In August, 2006, he was appointed Part-time Lecturer, Christ the Teacher Institute for Education, St. Mary's University of Minnesota, USA, Nairobi Campus, Kenya. On 1<sup>st</sup> September, 2008 he returned to Wageningen UR to complete his PhD studies following the award of the second sandwich PhD fellowship by the executive board of Wageningen UR. After defending his PhD thesis, Wanzala will go back to Kenya to continue with his research on ethnoknowledge and its application to the control and management of livestock ticks and other economically important parasites affecting humans. He will also be teaching at the university, CUEA/Christ the Teacher Institute for Education, St. Mary's University of Minnesota, USA, Nairobi Campus. His research work will be based at the School of Biological Sciences, University of Nairobi.



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## List of publications

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### Submitted

- Alwala, O. J., **Wanzala, W.** and Ndiege, I. O. Characterization and repellent effect evaluation of essential oil of *Mangifera indica* L. leaves from Kenya. *South African Journal of Botany*.

### Papers to be submitted

- Wanzala, W.**, Takken, W., Pala, A.O., Mukabana, R.W., Hassanali, A., Traditional knowledge on tick control within the Bukusu community in Bungoma District, western Kenya.
- Wanzala, W.**, Takken, W., Mukabana, R.W. and Hassanali, A., Repellent activity of essential oils of indigenous plants against the brown ear tick *Rhipicephalus appendiculatus*.
- Wanzala, W.**, Takken, W., Mukabana, R.W., Van Lenteren, J.C. and Hassanali, A., The effect of the essential oils of *Tagetes minuta* and *Tithonia diversifolia* on on-host behaviour of the brown ear tick *Rhipicephalus appendiculatus*
- Wanzala, W.**, Mukabana, R.W., Hassanali, A. and Takken, W. Essential oils of *Tagetes minuta* and *Tithonia diversifolia* affect the orientation behaviour of the tick, *Rhipicephalus appendiculatus*, on cattle.
- Wanzala, W.**, Takken, W., Mukabana, R.W. and Hassanali, A. Essential oils of indigenous plants protect livestock from infestations with *Rhipicephalus appendiculatus* and other tick species in herds grazing in natural pastures in Kenya.

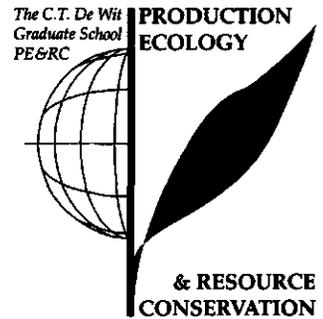
### Papers presented at conferences/workshops

- Midiwo, J.O., Mang'uro, A.L., **Wanzala, W.**, Kiprono, P.C. and Kraus, W (1997) Anti-feedant principles from the Myrsinaceae and *Polygonum senegalense*. Workshop on funding of Agricultural Research: Experiences and Future Perspectives. Kenya Agricultural Research Institute, Headquarters, Nairobi, Kenya.
- Onyango-Abuje, J.A., **Wanzala, W.** and Lumumba P. (1997) Evaluation of an Antigen-ELISA for the diagnosis of bovine cysticercosis. National Veterinary Research Centre, Annual Scientific Seminar, Muguga, KARI, Kenya, 27th-28th, November, 1997.
- Wanzala, W.** Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H. and Harrison L.J.S. (2000) A comparison of meat inspection method and an Antigen-ELISA test in the diagnosis of bovine cysticercosis in Kenyan cattle. 4th International Conference of the Federation of African Immunological Societies, Yaounde, Cameroon, 14th-18th February 2000.

- Wanzala, W., Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H. and Harrison L.J.S. (2000)** Distribution of *Cysticercus bovis* in bovine carcasses of naturally and artificially infected Kenyan cattle and its implication for routine meat inspection method. 21st African Health Sciences Congress Kenya Medical Research Institute Headquarters, Mbagathi Road, Nairobi, Kenya, 24th to 28th April 2000.
- Wanzala, W., Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H. and Harrison L.J.S. (2000)** Distribution of *Cysticercus bovis* in bovine carcasses of naturally and artificially infected Kenyan cattle and its implication for routine meat inspection method. International Conference on: Sustainable Animal Agriculture and Crisis Mitigation in Livestock Dependent Systems in Southern Africa-Lilongwe, Malawi on 30th. October to 1st November 2000.
- Wanzala, W., Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H., Harrison, L.J.S. (2001)** Serological diagnosis of bovine cysticercosis by detecting live *Cysticercus bovis* using a monoclonal antibody-based antigen-ELISA. Poster paper presentation at the International Conference in Italy in August, 2001-scholarship awarded for it from WAAVP African Foundation in South Africa.
- Wanzala W., Gule S. and Hassanali A. (2002)** Attractive and Repellent host odours guide ticks to their respective feeding sites. International Centre of Insect Physiology and Ecology (ICIPE), Scholars Workshop, Annual Governing Council General Meeting, International Centre of Insect Physiology and Ecology (ICIPE) Headquarters, Nairobi, Kenya.
- Wanzala W., Hassanali, A., Ochanda, H. and Kabaru, J. M. (2002)** African ethnopractitioners and HIV/AIDS in rural areas- an experience from western province, Kenya. Presented in the conference on: Young Women and HIV/AIDS in Eastern and Southern Africa, November, 2002.
- Wanzala, W., Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H., Harrison, L.J.S. (2003)** Serological diagnosis of bovine cysticercosis by detecting live *Cysticercus bovis* using a monoclonal antibody-based antigen-ELISA. Paper presented during the Fifth International Conference of African Immunological Societies at Victoria Falls, Zimbabwe, 27th April to 2nd May 2003.
- Wanzala W., Hassanali, A., Ochanda, H. and Kabaru, J. M. (2004)** Anti-tick ethnoknowledge: unutilized resource of the rural poor livestock farmers. Scholars Workshop, International Centre of Insect Physiology and Ecology (ICIPE), Annual Governing Council General Meeting, ICIPE Headquarters, Nairobi, Kenya.
- Wanzala W., Hassanali, A., Ochanda, H. and Kabaru, J. M. (2004)** Anti-tick ethnoknowledge: a potential resource to revolutionize the rural livestock industry in tick-prone areas. A paper presented at the International Conference on: Medicinal Herbs and Herbal Products-Livelihood and Trade Options India Habitat Centre, New Delhi, India, 26th-27th March 2004.
- Wanzala, W., Hassanali, A., Mukabana, R.W., Takken, W., van Lenteren, J.C. (2008)** Anti-tick ethnoveterinary knowledge in Bungoma District, western Kenya. International Seminar on: Medicinal Plants and Herbal Products. Held 7th to 9th March, 2008. Sri Venkateswara University, Tirupati, A.P, India. [http://ismphabstracts.blogspot.com/2008/04/oral-papers\\_22.html](http://ismphabstracts.blogspot.com/2008/04/oral-papers_22.html)

## **PE&RC PhD Education Certificate**

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



### **Review of Literature (5.6 ECTS)**

- Traditional knowledge of tick control and management in livestock Holdings with focus on the use of Ethnobotanicals (2005)

### **Writing of Project Proposal (7 ECTS)**

- An evaluation of anti-tick ethnobotanicals and their strategic application in tick control and management (2005)

### **Post-Graduate Courses (4.8 ECTS)**

- Basic and advanced statistics; PE&RC (2005)
- Science workshop for teaching science subjects in universities; Catholic University of Eastern Africa, Kenya (2006)
- Preparing and writing competitive and winning research proposals; International Foundation Science (IFS) and Kenya Forestry Research Institute (KEFRI), Kenya (2003)

### **Competence Strengthening / Skills Courses (1.5 ECTS)**

- Ecological aspects of bio-interactions; Entomology (2005)
- Ecological methods 1; Ecology Group (2005)
- Analysis and prevention of health risks in tropical countries; Entomology (2005)

### **Deficiency, Refresh, Brush-up Courses (1.9 ECTS)**

- Communication, planning and research; Social Science (2005)
- PhD Competent assessment; PE&RC (2005)
- Make a move career-week; Wageningen UR (2005)

### **Discussion Groups / Local Seminars and Other Scientific Meetings (7 ECTS)**

- Weekly meetings at the Behavioural and Chemical Ecology Department (BCED); International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya (2001-2007)
- Vector Group meetings; Laboratory of Entomology, Wageningen University and Research Centre, Wageningen, the Netherlands (2005 and 2008-2009)
- PhD Lunch discussion meetings; Laboratory of Entomology, Wageningen University and Research Centre, Wageningen, the Netherlands (2005 and 2008-2009)

### **PE&RC Annual Meetings, Seminars and the PE&RC Weekend (2.7 ECTS)**

- PE&RC Introduction weekend (2005)
- HEATOX Symposium on food toxicants: threats and opportunities (2005)
- Excursion Oostvaardersplassen and Veluwezoom (2005)
- Seminar: recent developments in *Phytophthora ramorum* research in California: nurseries as hotspots of diversity (2005)
- Debate: how to manage changes in the rural areas? (2005)
- Symposium: perennial ryegrass in the dairy cows (2005)
- Symposium for SUSPROT Group (2005)

### **International Symposia, Workshops and Conferences (7 ECTS)**

- IFS and OPCW International workshop: "Chemistry in nature- natural resources: chemical, biological and environmental aspects"; Nairobi, Kenya (2006)
- Annual Governing Council General Meeting and scientific workshop for poster presentation by PhD students; ICIPE Headquarters, Nairobi, Kenya (2004, 2005, 2006)
- 5<sup>th</sup> International conference on ticks and tick-borne pathogens; Université de Neuchatel, Switzerland (2005)
- Chemical communication: from gene to ecosystem; Wageningen, the Netherlands (2005)

### **Courses in Which the PhD Candidate Has Worked as a Teacher**

- Medical Helminthology / Protozoology; University of Nairobi; 24 days
- Ethnobotany and Phytochemistry / Developmental Biology / Immunology; Catholic University of Eastern Africa, Kenya; 52 days
- Biostatistics / Health Science for Education / Plant Taxonomy; St. Mary's University of Minnesota, USA / Nairobi Campus, Kenya; 84 days

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