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

Wycliffe Wanzala

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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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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[1] 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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[1] 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).

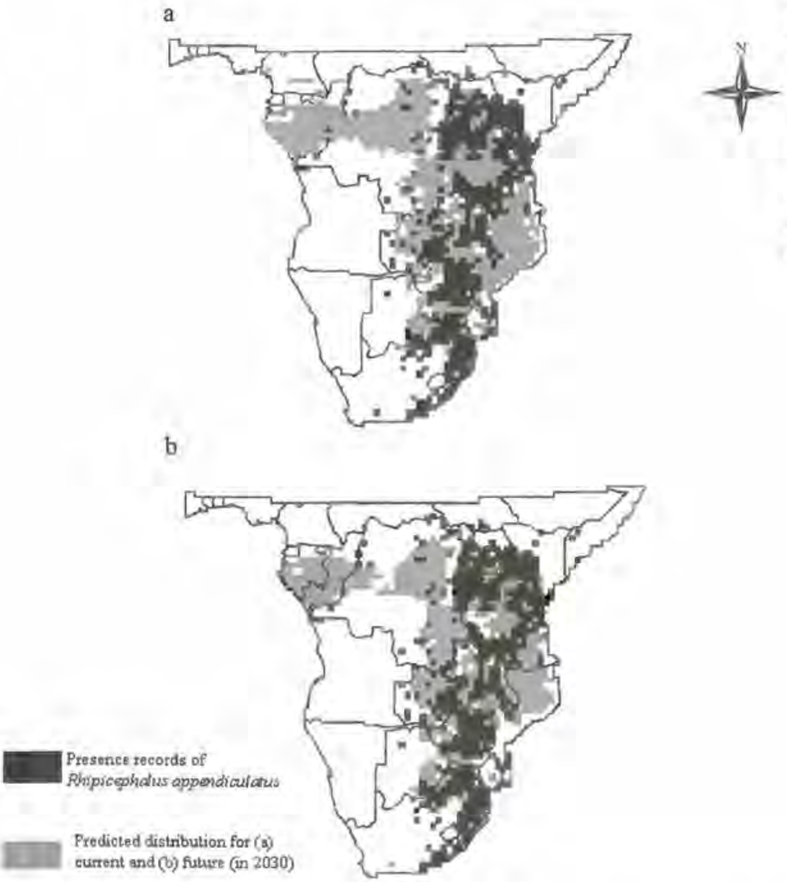


Fig. 1.1. The current and future predicted probability of the occurrence of the *R. appendiculatus* in sub-Saharan Africa obtained by using the predictive species model (Erasmus et al., 2002) and the DARLAM climate surfaces model: (a) current, (b) future–2030. (Adopted from Olwoch et al., 2008)

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-Sharan 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

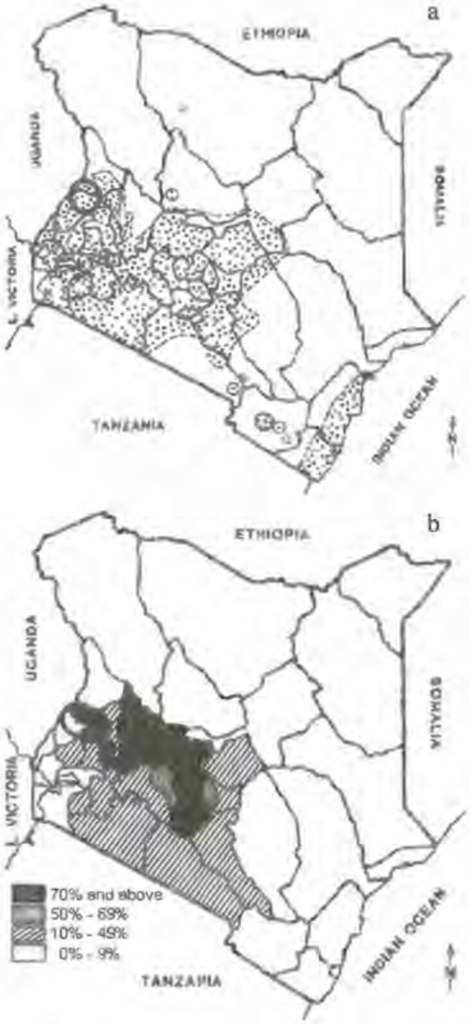


Fig. 1.2. The distribution of *Rhipicephalus appendiculatus*, the vector of *Theileria parva*, in Kenya (a). The distribution of exotic beef and dairy cattle breeds (most preferred cattle, with high productivity, by rural farmers) in Kenya, showing the percentages by District (b). The two figures show 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* Gerstacker, *R. sanguineus* Latreille, *R. simus* Koch, *R. senegalensis* Koch, *R. turanicus* Pomerantsev, 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*, Dhori 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.

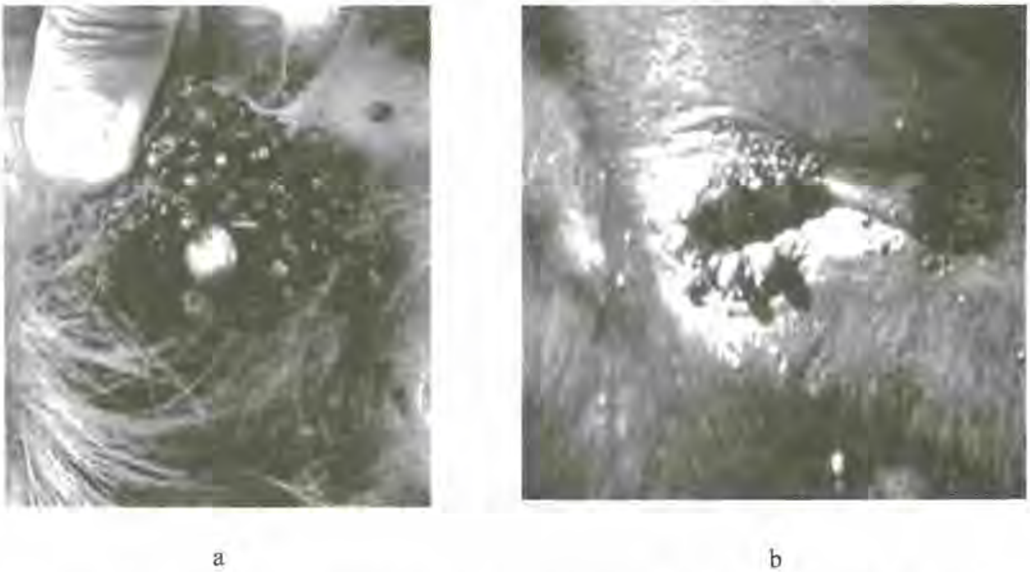

a b

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 toxaemia, 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 pyaemia (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-

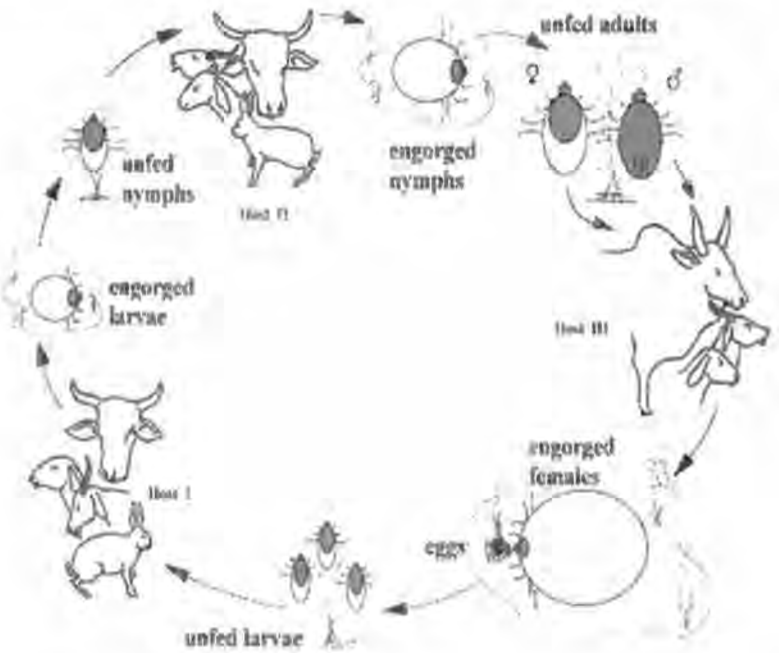


Fig. 1.4. The life cycle of *Rhipicephalus appendiculatus* displaying the teletropic type of behaviour (the three-host cycle)

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).

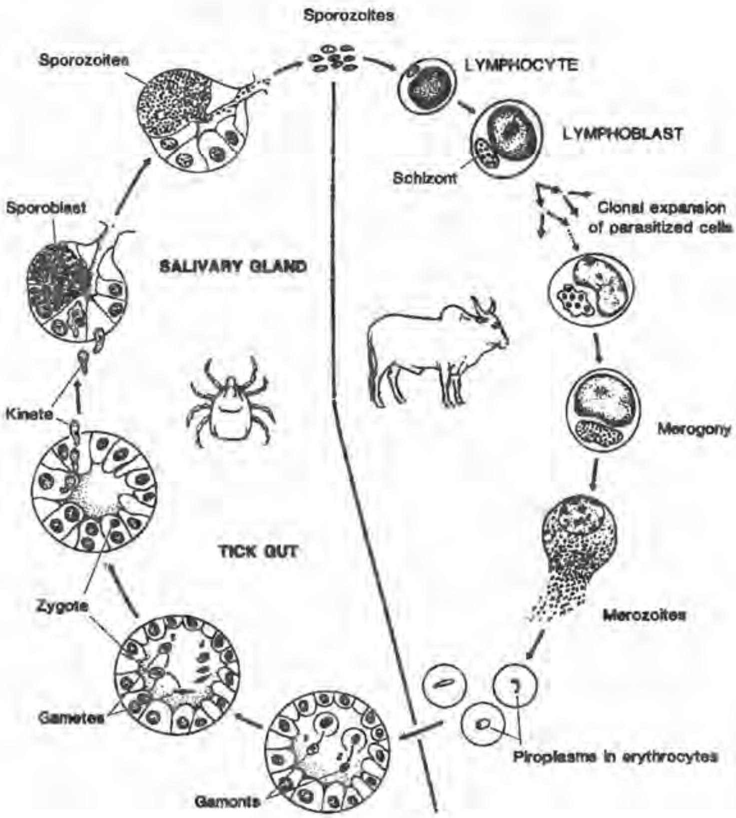
Besides cattle morbidity and mortality as well as severe economic losses as a result of direct and indirect tick parasitism, stock management also requires large financial inputs. For example, costs incurred for control operations have been estimated at US$ 7.02/head/year or US $1.08 per hectare (Mukhebi 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 kudus 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 telotropic type of behaviour (three–host cycle) with a tendency to the monotropic 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 telotropic 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 infected 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 (Fawcet 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 microschizonts). 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[2]) 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).

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[2] 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)

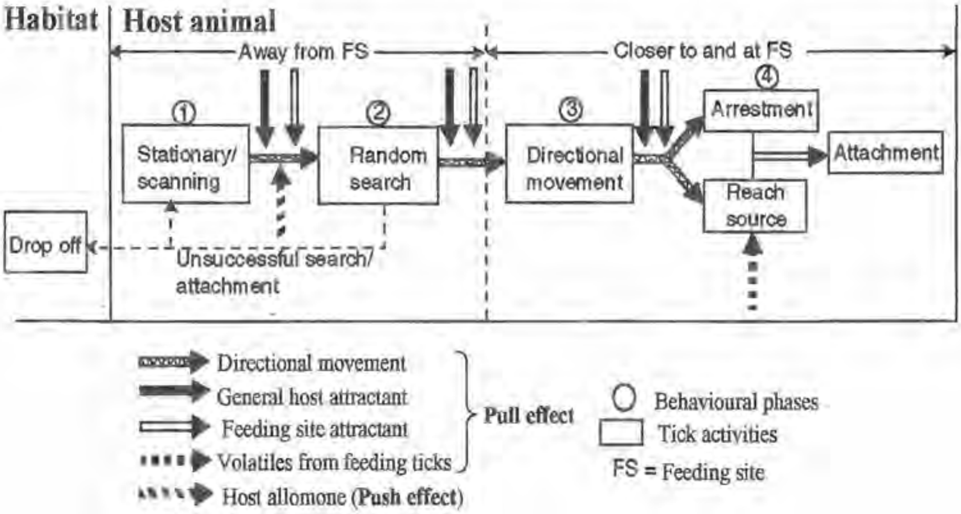

Fig. 1.6. A sequence of orientation behavioural activities of *Rhipicephalus appendiculatus* on the host. The process is characterized by different behavioural patterns (after Sika, 1996).

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).

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[3] 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[3], 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_2 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_2 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_2 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_2's role in host location by ticks is more intricate, as elevated CO_2 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_2 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_2 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 ≥10 m in response to host-derived kairomonal cues (Carroll and Schmidtmann, 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 appetence 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 presence 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) amidines, (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[4] 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 expen-sive process (Graf et al., 2004).

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[4] 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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[5] 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[5], 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 infestation 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_2, 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_2 with o–nitrophenol (ONP) in the field. According to these workers, increased catch of *A. hebraeum* was obtained by the activating effect of CO_2 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 acaricide 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 (AAAP) 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 (*Psiadia punctulata* (DC) Vatke.) mixed with Aloe (*Aloe secundiflora* Engl.) and Africa olive tree [*Olea europea* (L.) *ssp. africana* (Vent.) P. Green] mixed with Ilkerereai (*Cadia 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

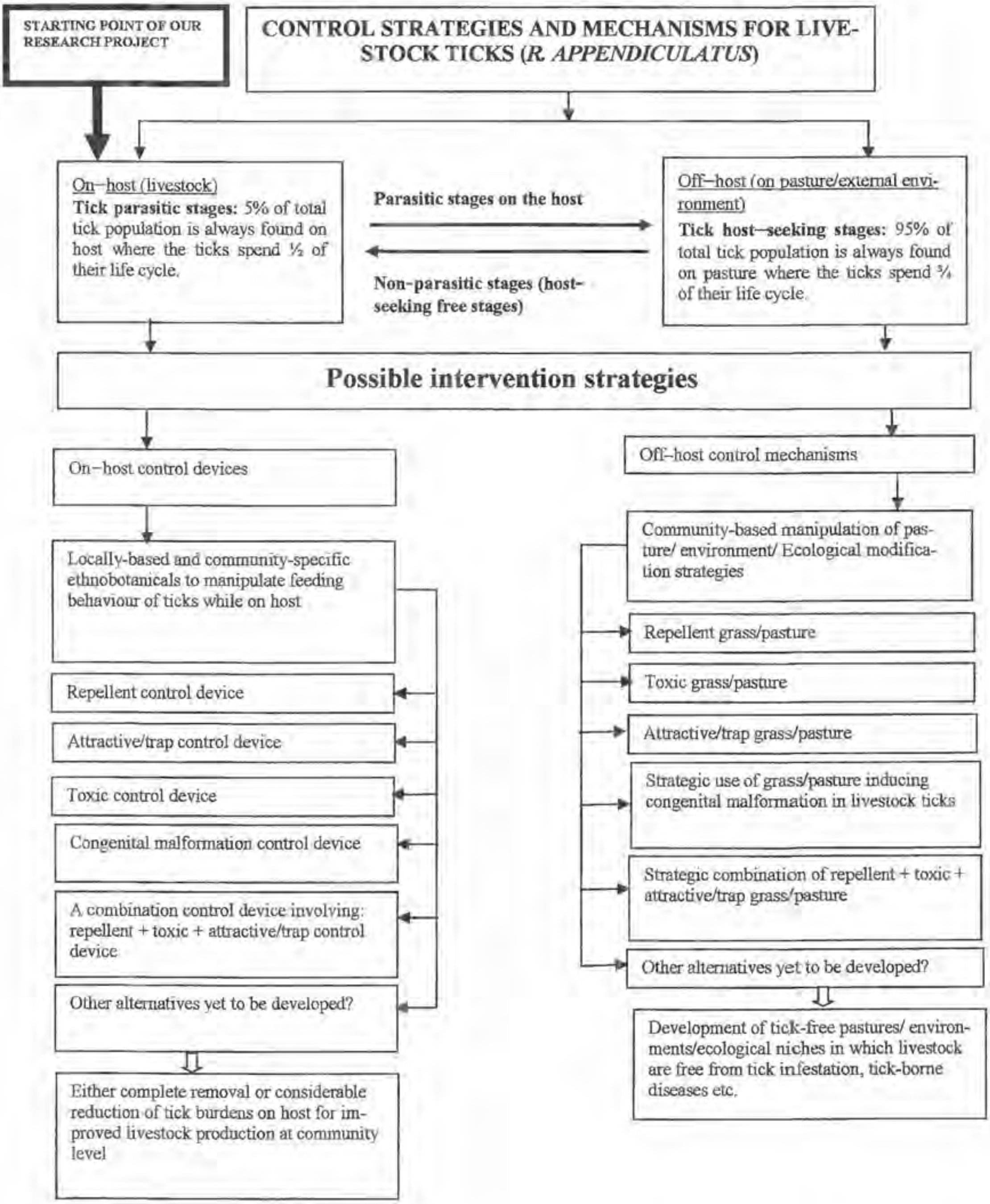

Fig. 1.7. Possible interplay of factors suitable for the development of on–host and off–host community-based integrated tick control and management strategies, which are effective and sustainable under local

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 longepedunulata* and 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.

Hoslundia 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[1]

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^2, 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

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[1] 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

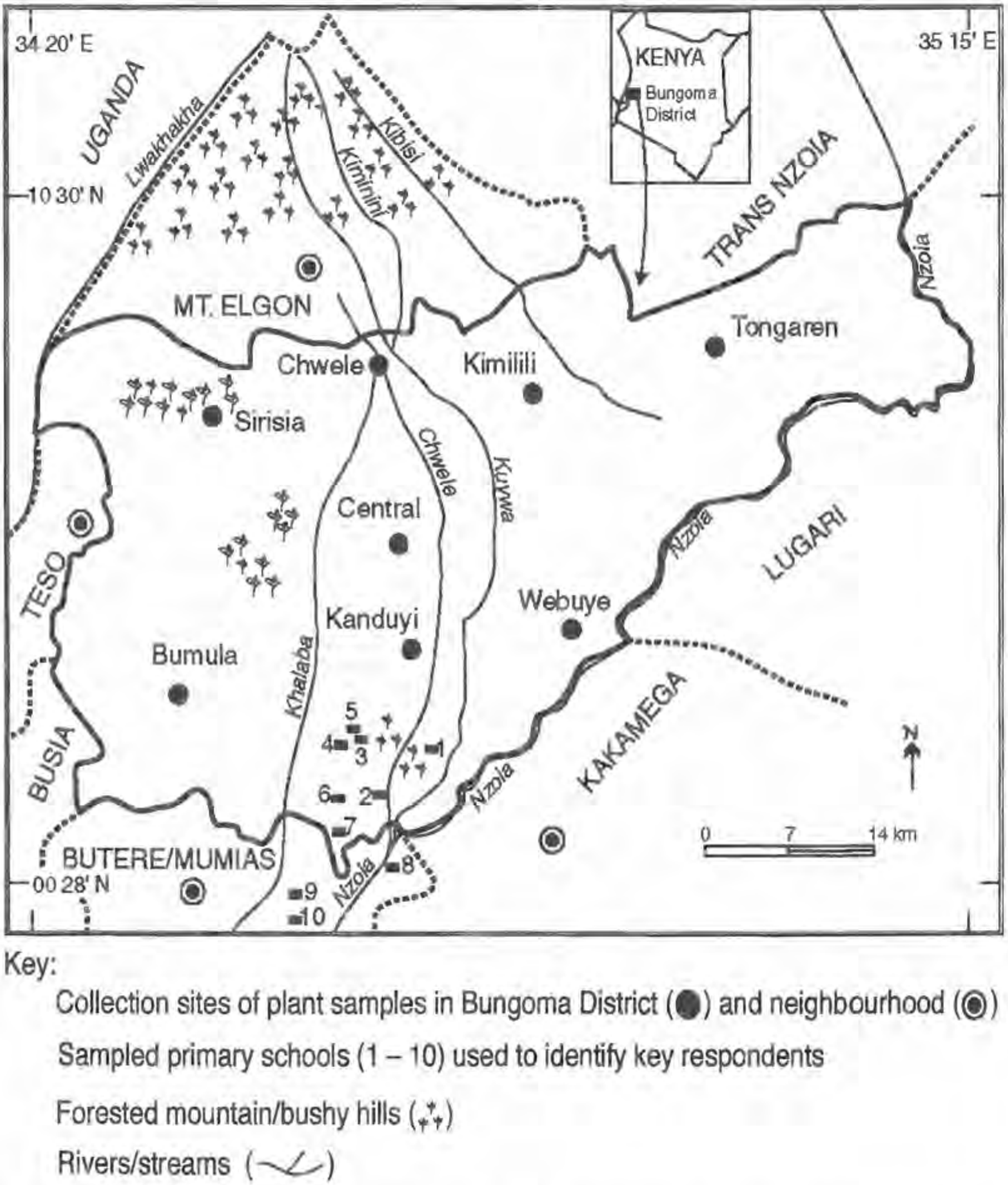

Fig. 2.1. The location of Bungoma District in Kenya. Note the positioning of the District within the Lake Victoria Basin and towards the southern slopes of Mt. Elgon.

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 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 (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 interlacostrine 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 heav-ily influenced geographically and with time by immigrants of both Bantu and Nilotic ori-gin (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 ethnoscientific 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 coracan*. 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 Demissew, 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, McCorckle 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 Cervinskas, 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 partici-patory 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 sensi-tivities 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		
Colour													
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
Size													
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
Life cycle type													
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
% total	**25.43**	**7.11**	**10.15**	**7.30**	**2.63**	**3.63**	**8.21**	**13.78**	**17.33**	**0.50**	**1.82**	**2.11**	**100.00**

*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 conventially–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 *per se* 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 conventially–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.

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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*[1]

Abstract: Essential oils of eight plants (*Tagetes minuta*, *Tithonia diversifolia*, *Juniperus procera*, *Solanecio manii*, *Senna didymobotrya*, *Lantana camara*, *Securidaca longepedunulata* 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%), β-ocimene (3.25%), *cis*-tagetone (1.95%), β-caryophyllene (0.84%), bicyclogermacrene (0.62%) and AR-turmerone (0.50%). In the essential oil of *T. diversifolia*, α-pinene occurred in largest amount (63.64%) followed by β-pinene (15.00%), isocaryophyllene (7.62%), nerolidol (3.70%), tetradecanol (1.75%), limonene (1.52%), sabinene (1.00%), α-copaene (0.95%), α-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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[1] This chapter will be submitted for publication as: 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*.

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 alternative 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
Tagetes minuta 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
Tithonia diversifolia (Hemsl.) Gray	Kamang'ulie/ Kamaua/ Kiming'ulie	Asteraceae	7	Whole plant used to make mainly paste for application	I,P,T,B,F,Af	Adoyo et al., 1997 Anon., 1997; Adedire and Akinneye, 2004; Njoroge and Bussmann, 2006
Solanecio manii (Hook. f.) C. Jeffrey.	Nandebe	Asteraceae	5	Whole plant used in many different forms	A,C	University of Nairobi Herbarium records
Juniperus procera procera Hochst. ex Endlicher.	Kumutarakwa	Cupressaceae	5	Leaf/root/back	I,B	Getahun, 1976; Anon., 2001
					I	Hines and Eckman, 1993
Securidaca longepedunculata Fres.	Kumulondamwombe / Kumunyakasia/ kumuyanjabakeni	Polygalaceae	2	Whole plant	I,C,B,F, Ps	Jayasekara et al., 2002; Rukangira, 2001
Lantana camara L.	Lantana/ Mukhekhe	Verbenaceae	2	Whole plant (used to make concoction for pour on or for steaming)	B, F, C, I,	Adebayo et al., 1999
Hoslundia opposita Vahl.	Bifwofwo	Labiatae	1	Whole plant	T, A	Jembere et al., 1995
Senna didymobotrya (Fresn.) Irwin & Barneby	Kumubumbu/ Kumupinupinu	Caesalpiniaceae	1	Leaf/back/root (used to make suspension)	T,B, I	Fernandes et al., 1985; Mansingh and Williams, 1998 UWMG, 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 found to exhibit insecticidal properties
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 (Clevenger, 1928). Pure oil was collected from every plant into 2 ml vials and stored at –20 °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 °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 μm 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 °C. The oven temperature was programmed from 60 °C isothermal for 7 min, to 120 °C at 5 °C per min, then to 180 ° C at 10 °C per min, and finally to 220 °C at 20 °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 quadruple 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 μm 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

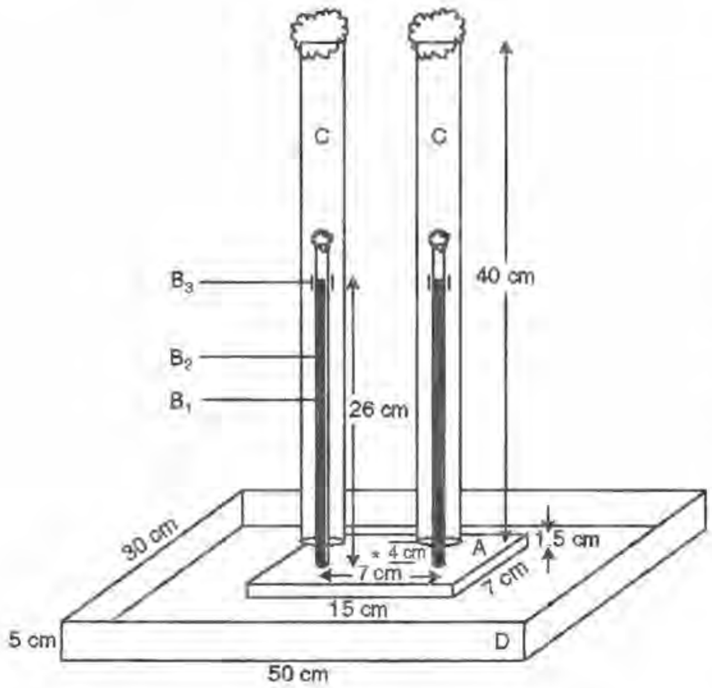


Fig. 3.1. Tick climbing bioassay apparatus (placed in a tray measuring 50 x 30 x 5 cm) with water up to 1.5 cm deep; A, aluminium base; B_1, aluminium rod (26 cm long x 1.7 cm in diameter); B_2, 0.8-cm glass tube plugged with wet cotton wool; B_3, filter paper collar; C, 4.5-cm glass tube plugged with dry cotton wool. The two aluminium rods, B_1 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_1 (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^2 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 ± 1 °C and 75 ± 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 (± 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 ± 3.9	82.8 ± 3.6	75.6 ± 4.5	70.5 ± 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® 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® 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:

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

Where, β_0 = coefficient of the model representing y-intercept,

β_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%).

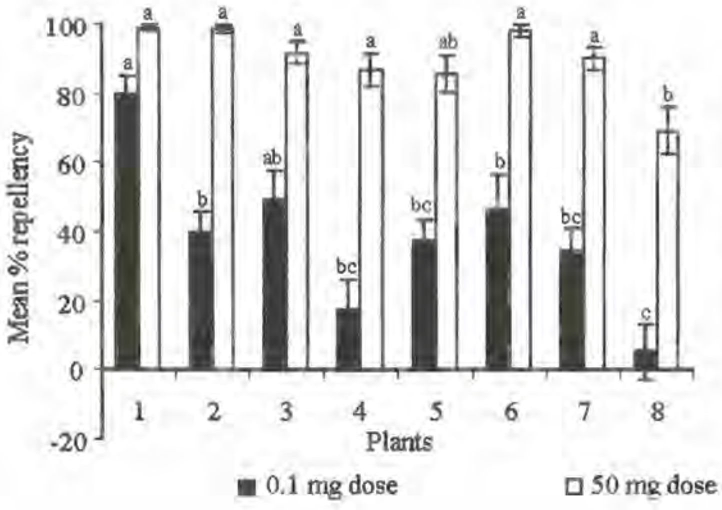


Fig. 3.2. The repellent effect of essential oils of eight plants in doses of 0.1 mg and 50 mg (neat oil) against *Rhipicephalus appendiculatus*. Plant species 1= *Tagetes minuta*, 2 = *Tithonia diversifolia*, 3 = *Hoslundia opposita*, 4 = *Solanecio manii*, 5 = *Lantana camara*, 6 = *Juniperus procera*, 7 = *Senna didymobotrya* and 8 = *Securidaca longepedunulata*. 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_{50} and RD_{75} levels is shown in Table 3.3, whereas the dose-response link function of the regression model was:

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

where dose1 = Log_{10} (dose), and P = repellency probability function as shown in Fig. 3.4a.

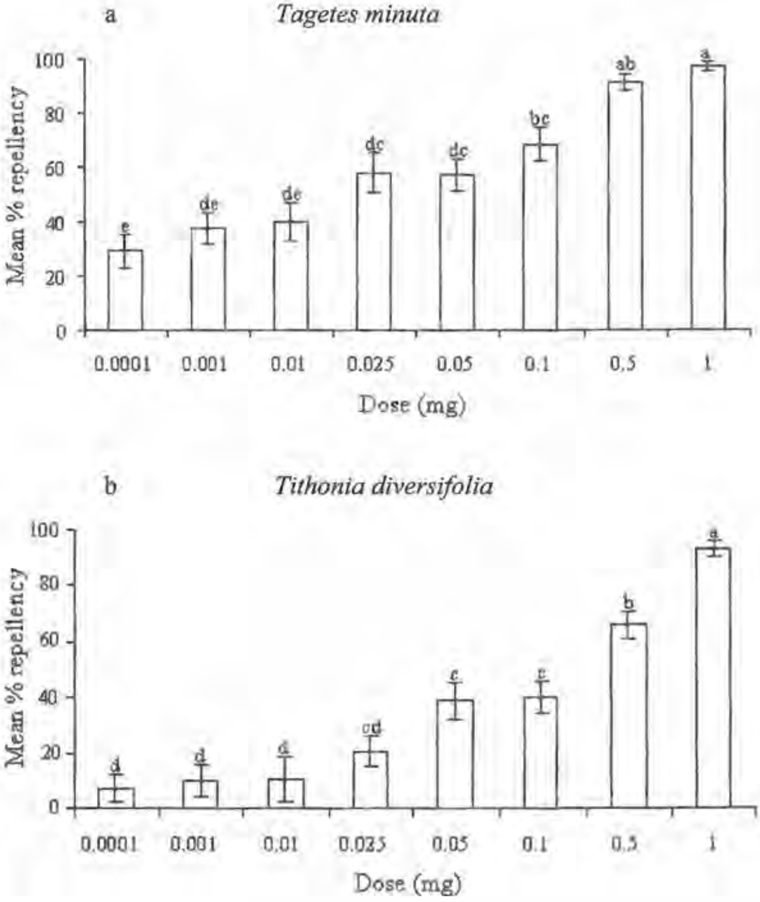


Fig. 3.3. Mean repellency percentage of different doses of *Tagetes minuta* (a) and *Tithonia diversifolia* (b) essential oils against newly emerged adult *Rhipicephalus appendiculatus* in a dual choice assay. Means with the same letters are not significantly different at $P<0.0001$ (Student-Newman-Keuls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

Table 3.3. Probit analysis of dose-response relationship of *Tagetes minuta* and *Tithonia diversifolia* essential oils at RD_{50} and RD_{75}.

Plant species	Repellence probability	Repellent dose (mg)	Upper confidence limit at 95%	Lower confidence limit at 95%
Tagetes minuta	0.50	0.00213	0.00237	0.00191
	0.75	0.09151	0.10124	0.08299
Tithonia diversifolia	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_{50} and RD_{75} levels is shown in Table 3.3, while the dose-response link function of the regression model was:

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

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).

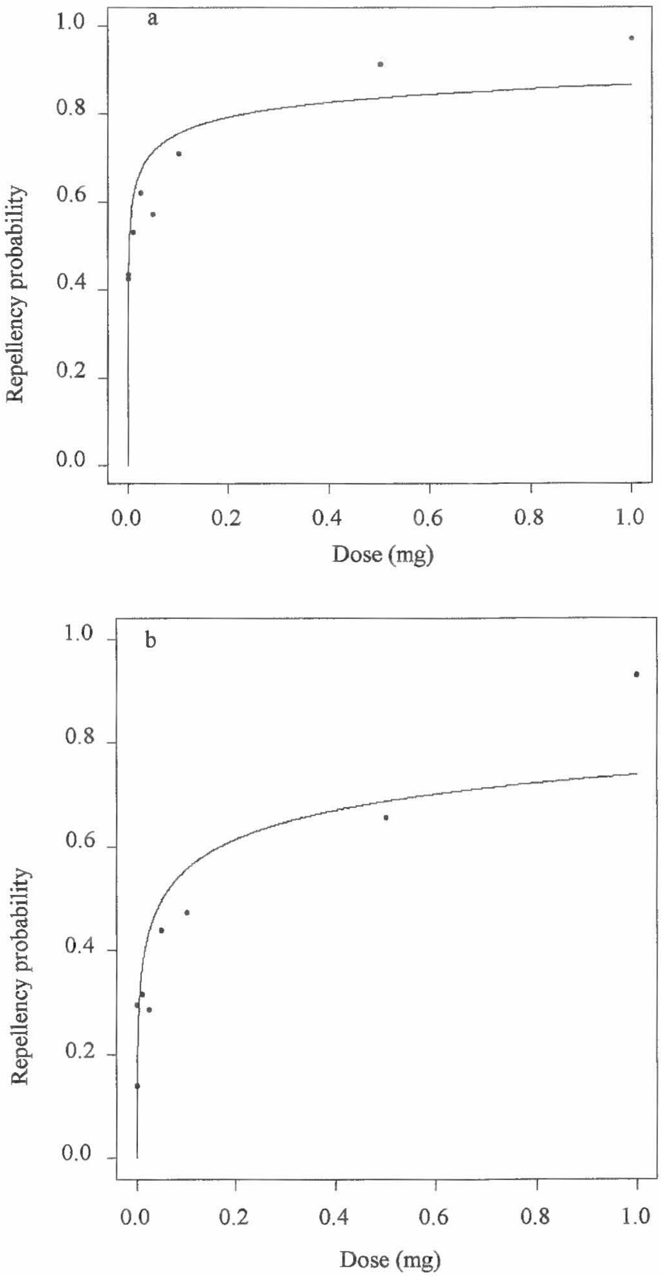
Mass spectroscopy of the essential oil of *Tagetes minuta*

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 dihydrotagetone (16.71%), piperitenone (10.15%), *trans*-tagetone (8.67%), 3,9 epoxy-p-metha-1,8 (10) diene (6.47%), *β*-ocimene (3.25%), *cis* -tagetone (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 = 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, α-pinene occurred in the largest amount (63.64%), followed by β-pinene (15.0%), isocaryophyllene (7.62%), nerolidol (3.70%), 1-tridecanol (1.75%), limonene (1.52%), sabinene (1.00%), α-copaene (0.95%), α-gurjunene (0.56%) and cyclodecene (0.54%) in that order (Table 3.4b).

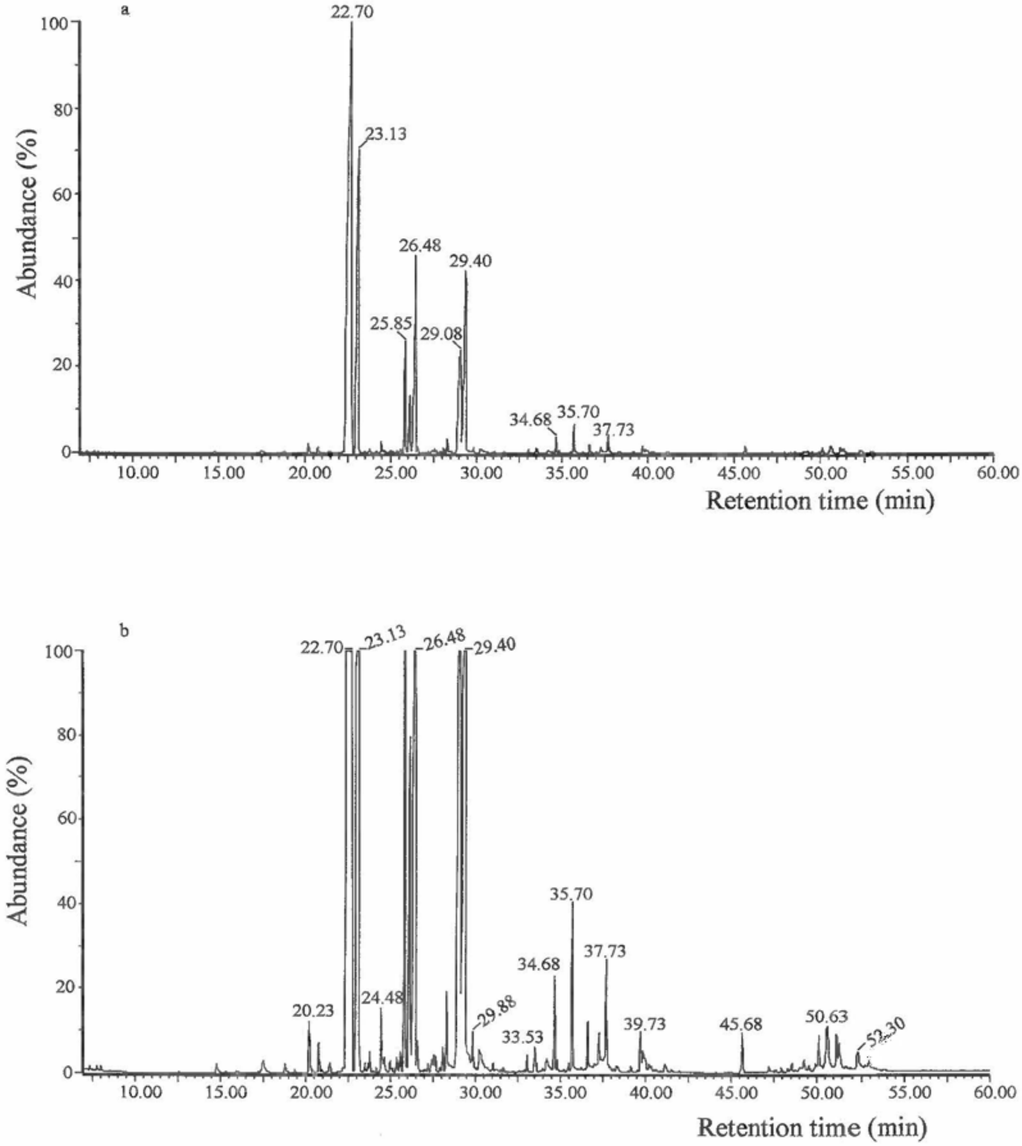


Fig. 3.5. Chromatograms generated by gas chromatography for the essential oils of (a), *Tagetes minuta* and (b), *Tithonia diversifolia*. Each of the peaks in the chromatogram represents the signal created when a compound elutes from the chromatographic column into the detector.

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^+ (g/ mol)	Base peak	Major peaks	Relative abundance (%)	Identification of compounds
14	*cis*-ocemene	$C_{10}H_{16}$	22.701	136.20	93	41, 79, 105, 121	43.78	**GC-MS Co**
15	dihydrotagetone	$C_{10}H_{18}O$	23.126	154.00	85	41, 57, 69, 97	16.71	GC-MS
42	piperitenone	$C_{10}H_{16}O$	29.401	152.00	150	41,91,107,135	10.15	GC-MS
31	*trans*-tagetone	$C_{10}H_{16}O$	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	$C_{10}H_{14}O$	29.076	150.00	135	41, 79, 122, 150	6.47	GC-MS
29	*β*-ocimene	$C_{10}H_{16}$	25.851	136.20	121	41, 79,105, 136	3.25	**GC-MS Co**
30	*cis*-tagetone	$C_{10}H_{16}O$	26.126	152.00	95	41, 67, 109, 152	1.95	**GC-MS Co**
57	*β*-caryophyllene (*Trans*)	$C_{15}H_{24}$	35.701	204.00	41	69,93,133	0.84	**GC-MS Co**
61	bicyclogermacrene	$C_{15}H_{24}$	37.726	204.40	121	41, 93, 136, 161	0.62	GC-MS
93	AR-turmerone	$C_{15}H_{20}O$	50.626	216	83	41,79, 124	0.501	GC-MS

(b)

Peak no.	Compound	Molecular formula	RT (min)	M^+ (g/ mol)	Base peak	Major Peaks	Relative abundance (%)	Identification of compounds
2	α-pinene	$C_{10}H_{16}$	18.975	136.24	93	39, 77, 79, 121, 136	63.64	**GC-MS Co**
5	*β*-pinene	$C_{10}H_{16}$	20.425	136.24	93	41, 121, 136	15.0	**GC-MS Co**
34	Isocaryophyllene	$C_{15}H_{24}$	35.476	204.36	93	41, 69, 105, 133, 161	7.62	GC-MS
45	Nerolidol	$C_{15}H_{26}O$	38.651	222.37	69	41, 43, 93, 107, 161	3.70	**GC-MS Co**
38	1-Tridecanol	$C_{14}H_{13}O$	36.876	197.00	55	43, 69, 83, 97	1.75	**GC-MS Co**
10	Limonene	$C_{10}H_{16}$	22.200	136.24	68	39, 41, 53, 67, 93	1.52	**GC-MS Co**
4	Sabinene	$C_{10}H_{16}$	20.150	136.24	93	39, 41, 77, 79, 136	1.00	GC-MS
28	α-copaene	$C_{15}H_{24}$	34.101	204.36	105	41, 77, 81, 93, 119, 161	0.95	GC-MS
29	α-gurjunene	$C_{15}H_{24}$	34.376	204.36	189	41,55,91,105,119,161,161,204	0.56	GC-MS
47	Cyclodecene	$C_{12}H_{22}$	41.251	166.00	67	41, 54, 81, 95	0.54	GC-MS

Key
M^+ = 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

Discussion and conclusion

The selection of plants for screening was based on the non-experimental validation of ethnic knowledge on tick control involving comparative analysis of ethnobotanical, phytochemical and pharmacological information in the literature for each plant (Lans, 2001) and following the four levels of validity described by Heinrich et al. (1992). This methodological approach ensured that local tick problems were correctly matched with candidate repellent plants (Lans, 2001), at the same time helping to separate plants having effects on livestock ticks from those with religious and cultural values to the community, thereby ensuring that future field trials are undertaken only on known anti-tick plants. Such a selection process and approach is preferred, as it gives a more successful score than large-scale screening programmes (Vanden Berghe and Vlictinck, 1999).

The essential oils of the eight plants selected from a group of 157 plants surveyed in the Bukusu community showed varying degrees of repellent effects against the brown ear tick *R. appendiculatus* at 0.1 mg and 50 mg doses. Other than the essential oils of *S. manii*, *L. camara* and *S. longepedunculata*, those of the plants tested were found to be more repellent than the commonly used commercial arthropod repellent N,N-diethyl-3-methylbenzamide. However, at lower doses (0.1 mg), the repellent effects of the essential oils on *R. appendiculatus* were lower than those of DEET except for the essential oil of *T. minuta*. Similar results were obtained with essential oils of *C. monophylla* (Ndung'u et al., 1995), *C. hirta* (Ndung'u et al., 1999) and *G. gynandra* (Lwande et al., 1999) on *R. appendiculatus*.

Of the eight essential oils, those of *T. minuta* and *T. diversifolia* showed relatively stronger repellent effects against the brown ear tick *R. appendiculatus* than the other six essential oils. These results validate the high ranking of the two plants in the Bukusu and Kikuyu communities in Kenya for their traditional use to control livestock ticks (Njoroge and Bussmann, 2006).

Chemical characterization of the essential oils of *T. minuta* and *T. diversifolia* revealed a considerable number of chemical compounds present in the mixture, although the GC chromatogram profiles of the essential oil of *T. diversifolia* showed that it contained more compounds than the essential oil of *T. minuta*. The essential oils of both *T. minuta* and *T. diversifolia* contain considerable proportions of monoterpenes. A number of monoterpenes have been previously reported to be repellent against insects such as *Phoebis sennae amphitrite* Feisthamel, *Pieris brassicae* L., *Tatochila autodice blanchardi* Butler, *T. mercedis mercedis* Eschscholtz, *Battus polydamas archidamas* Boisduval, *Cosmosatyrus chilensis chiliensis* Guérin, *Vanessa carye* Hübner, *Helephila venusta* Hayward, *Culex pipiens pallens* Rank and *Castnia psittachus* Molina (Urzua, 2002;Won-Sik et al., 2002) as well as host-seeking nymphs of *Ixodes ricinus* (Jaenson et al., 2006; Pålsson et al., 2008; Garboui, 2008). We therefore suppose that differences in the monoterpene proportions may affect the repellent properties of the two essential oils. This would explain the fact that the essential oil of *T. minuta* that had a greater repellent effect against *R. appendiculatus* than its counterpart, as it contained a higher proportion of monoterpene compounds. However, both the essential oils caused significant repellent effects against *R. appendiculatus* albeit a considerable difference in the proportion of monoterpene compounds.

The results of chemical characterization of the essential oil of *T. minuta* compare favourably with the results obtained in Egypt, where the main components of the essential oil of *T. minuta* were monoterpenes of which *trans*- and *cis*-tagetone together accounted for 52.3-64.2% (Mohamed et al., 2002). In the recent past, monoterpenes have been proposed as an attractive alternative to chlorofluorocarbons in many industrial applications (Li et al., 1998). Physicochemical properties of a number of monoterpenes (α-pinene, limonene, γ-terpinene, terpinolene, arbanol, α-terpineol, linalool, and plinol) (some also found in this study), have been studied to evaluate the possibility of these compounds to cause environmental pollution in the industrial applications.

The essential oils of *T. minuta* and *T. diversifolia* differed in their dose-response relationships reflected in their repellent effects at 50 (RD_{50}) and 75 (RD_{75}) percent. The essential oil of *T. minuta* showed a stronger repellent effect against *R. appendiculatus* than that of *T. diversifolia*. This may be due to differences in the nature, proportion and number of respective constituent chemical compounds in the two blends as shown by the GC-MS profiles. Additive or synergistic effects resulting from a blend of specific components of the essential oil of *T. minuta* may confer it greater repellency than the active constituents of *T. diversifolia* (Ndungu et al., 1995; Lwande et al., 1999; Odalo et al., 2005). Synergistic effects resulting from blends of different components are widespread in phytochemicals (Bekele and Hassanali, 2001; Kametani et al., 2007). Comprehensive dose-response assays of constituents of the two essential oils individually and in different blends may help to identify active compounds that contribute to the respective repellent actions of the oils against *R. appendiculatus*.

The bioassay results in our study of the essential oil of *T. minuta* are comparable to those obtained by Nchu (2005) in South Africa, while working with *Hyalomma marginatum rufipes* Koch, 1844 and *R. pulchellus* Gerstacker, 1873. This supports the suggestion that the essential oil of *T. minuta* may have a broad spectrum of bioactivity for use as a general-purpose livestock tick repellent. In addition, the leaves and flowers of *T. minuta* are known to be insect repellent and are often seen hanging from native huts to deter swarms of flies and mosquitoes (Soule, 1993). Essential oil of *T. minuta* has also been reported to be a broad spectrum pesticide (Campin, 2005) and it would be interesting to see if this effect also extends to ticks. Likewise, the repellent activity of the essential oil of *T. diversifolia*, its insect feeding deterrent activities and its reputed anti-leishmanial activity (Toledo et al., 2003) and insecticidal properties (Taiwo and Makinde, 2005), make it, as *T. minuta*, an attractive candidate for field evaluation as a tick control agent. Hence, detailed knowledge on the efficacy of the two oils over time, their effective dosages, release rates and toxicity on target tick species in field settings is needed. This is currently being undertaken.

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4

Attractive and repellent host odours guide ticks to their respective feeding sites[1]

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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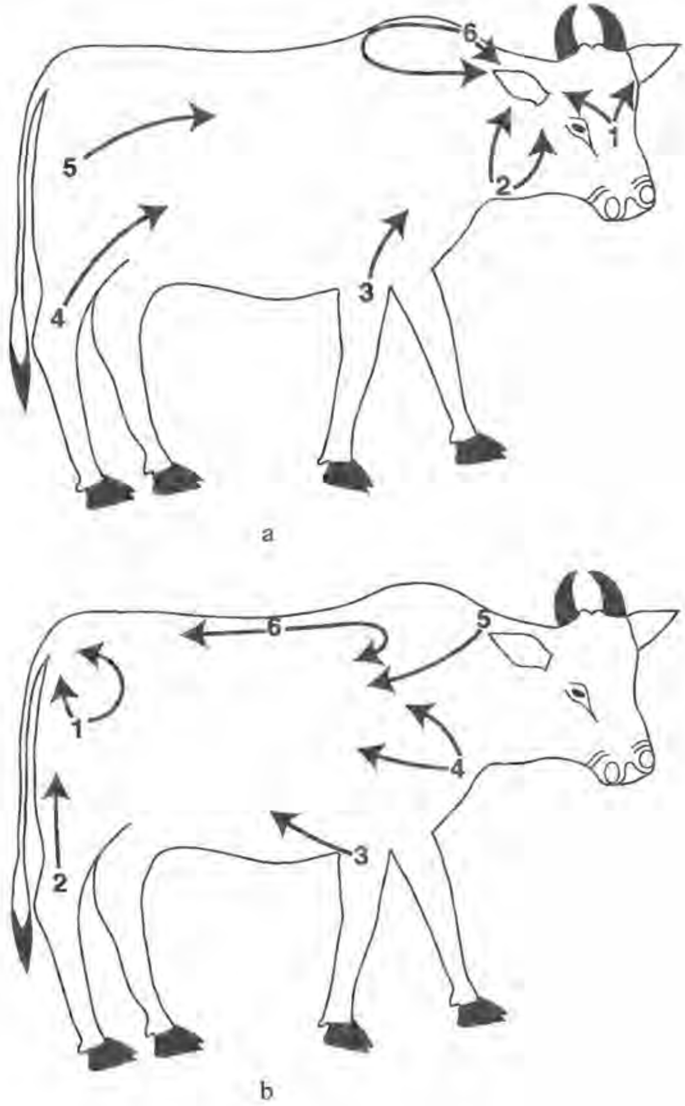

Fig. 4.1. General orientations of *R. appendiculatus* (a) and *R. evertsi* (b) that initiated movements from different release points (1–6).

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 areas 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_{18}–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 dichlromethane (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 $P \geq 0.05$. 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)
(a) *R. appendiculatus*					
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
(b) *R. evertsi*					
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≥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

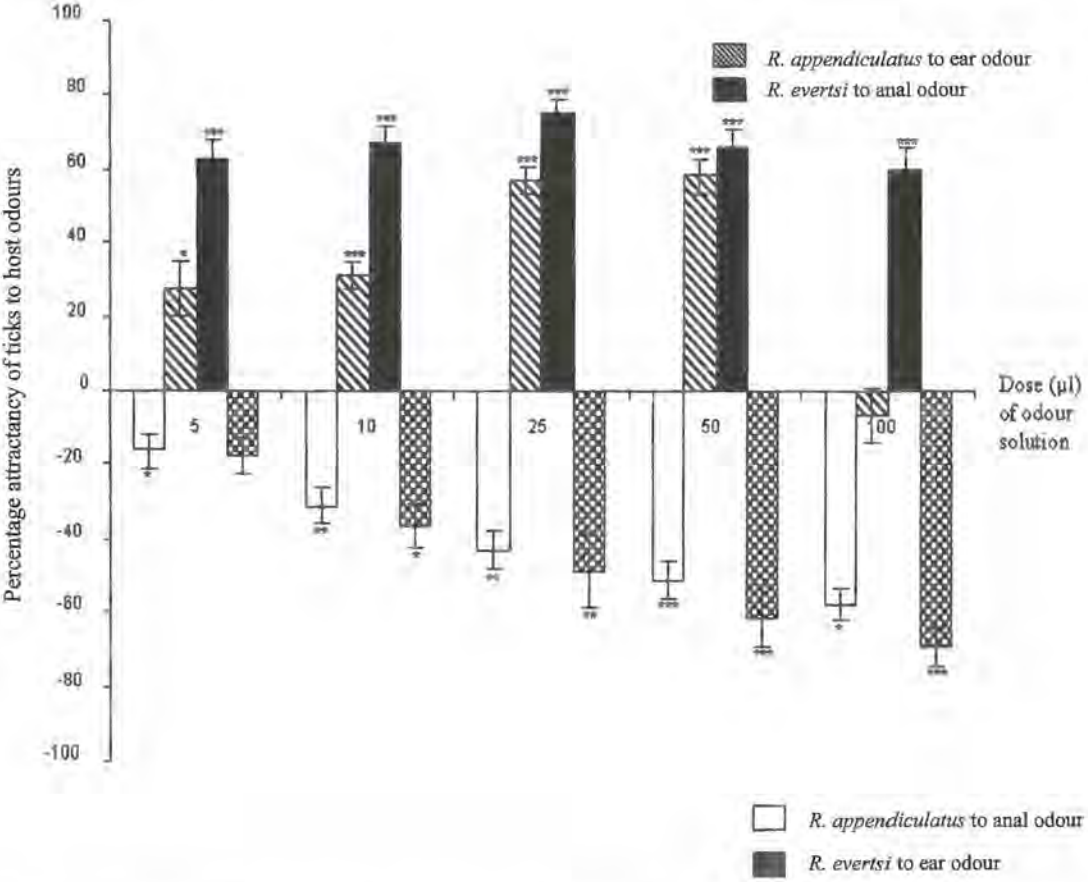

Fig. 4.2 Mean percentage attractancy or repellency of ear and anal volatiles to *R. appendiculatus* and *R. evertsi* in climbing bioassays (*, ** and *** indicate statistical significance at $P \leq 0.05$, 0.01 and 0.001, respectively).

significantly short time (0.06 ± 0.02 h from escutcheon, compared to 0.72 ± 0.25 h and 0.84 ± 0.40 h from the upper rear and fore legs, respectively) at relatively high speed (2.12 ± 0.42 cm/min) comparable to conspecifics placed on the forehead close to the ears (2.00 ± 0.49 cm/min). Likewise, the reaction time and initial speed of *R. evertsi* deposited at the shoulder closer to the ear (0.08 ± 0.07 h and 3.04 ± 0.24 cm/min, respectively) were not significantly different from those deposited at the escutcheon near the anal region (0.03 ± 0.01 h and 2.77 ± 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 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.

Acknowledgements

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5

Dual and no-choice assays on the effect of the essential oil of *Tagetes minuta* on *Rhipicephalus appendiculatus*[1]

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; Garboui 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 °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 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)

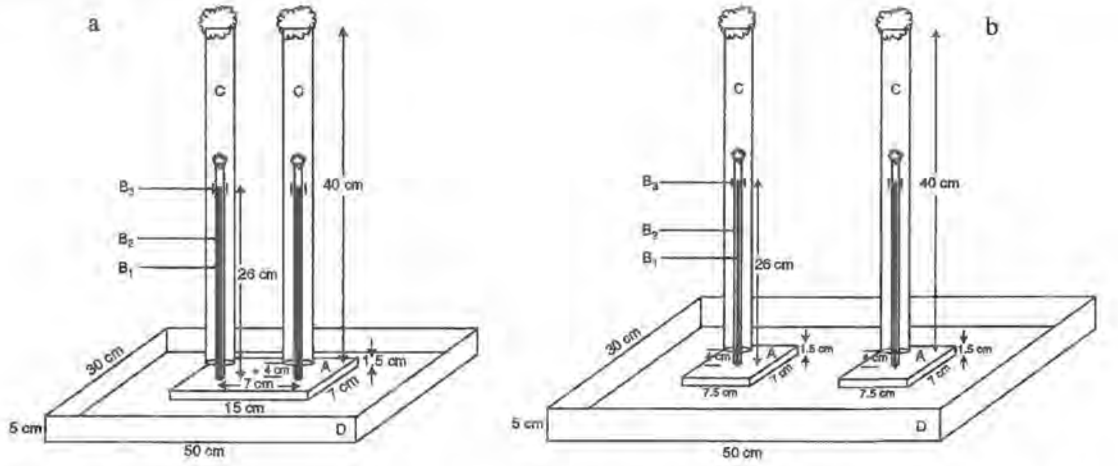

Fig. 5.1. Two bioassay devices to study the impact of putative repellents on tick climbing response (modified from Browning, 1976). Dual-choice climbing assay apparatus (a). For details, see Fig.3.1. For no-choice assay (b), the two aluminium rods (B_1) on the aluminium base, A, were separated by water. 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 col-umns. 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 ± 1 °C and 75 ± 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 (Nc) and on the treated glass tube (Nt) 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) = [(Nc-Nt)/(Nc+Nt)] x 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.

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-h-hydrated adult ticks of mixed sexes were placed at a distance of 3.5 cm from the base of aluminium rod, B_1 (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 essay 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 (± 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 ± 15.07b1	66.7 ± 14.05a1	56.7 ± 15.24bc1	61.7 ± 13.94a1	0.795
0.1	51.7 ± 13.71b1	72.0 ± 12.27a1	72.0 ± 12.27b1	78.0 ± 12.09a1	0.483
1	100.0 ± 00a1	100.0 ± 00a1	100.0 ± 00ab1	88.0 ± 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 (± SE) percentage of repellency of the essential oil of *Tagetes minuta* obtained using a dual -choice assay

Doses of essential oil of *Tagetes minuta* (mg)	Mean percentage of repellency
0.025	57.9 ± 7.11b
0.100	68.4 ± 6.26b
1.000	97.0 ± 1.69a
P-values	<0.05

Within a given column, means (± SE) with the same letter(s) are not significantly different at P = 0.05 (Student-New-man-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-

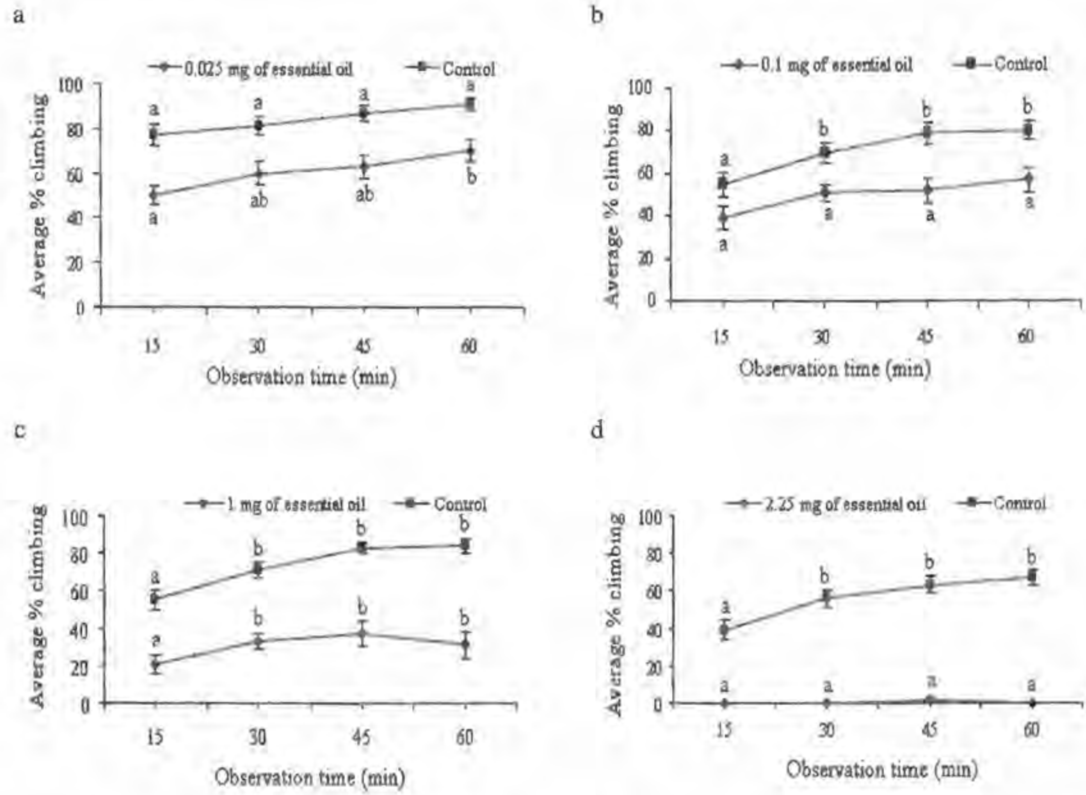

Fig.5.2. Mean climbing response (%) over time of different doses of essential oil of *Tagetes minuta* with adult *Rhipicephalus appendiculatus* using a no-choice assay. The figures from **(a)** to **(d)** represent doses of essential oil of *Tagetes minuta* exposed to newly emerged *Rhipicephalus appendiculatus* in a no-choice climbing assay (n = 5). For either control or essential oil treatment, the means (± 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 (± 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

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

Within a given column, means (± SE) with the same letter(s) are not significantly different at P = 0.05 (Student-New-man-Keuls test). In a given row, means (± 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 essay 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)] x 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)] x 100), and therefore, this does not put into consideration the time over which the observa-tions 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 interactive. 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*[1]

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 essen-tial 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 main-tain them. Another complication associated with the use of acaricides is that they are environmental pollutants and have been found to contaminate milk and meat (Mitchell, 1996).

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

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

The life cycle of an ixodid tick often has a total duration of 6 years and host attachment may constitute less than 2% (1½ months) of this time. Ticks spend most of their life cycle away from their hosts, hiding either in the soil and vegetation or in the nests of their hosts. However, ticks select habitats with good opportunities to encounter a host for feeding. Host–seeking and –recognition are two most important and challenging activities in the life cycle of ticks. Many ticks, in particular certain *Hyalomma*, *Amblyomma*, *Ornithodoros* and *Dermacentor* species (Sonenshine, 1991; Anon, 2008), seek their hosts by hunting, whereas others use an ambushing strategy, e.g. the larvae of *Boophilus* spp. and most ixodid ticks (Sonenshine, 1993; Eckert et al., 2005). Other tick species respond to volatile host emanations, vibrations, visual cues, radiant heat and touch with questing behaviour, e.g. *R. appendiculatus* (Speybroeck et al., 2003). However, little is known on how different host specificities are encoded in the odours. Questing ticks climb up the stems of grass or perch on the edges of leaves on the ground in an erect posture, while the first pair of legs is waved in the direction of the stimuli of the host passing by. Subsequently, the ticks climb and grab onto the potential host body using their front leg claws. Once on the host, gustatory and olfactory cues seem to aid the ticks in deciding whether it will remain on the host. By 'push' and 'push–pull' action modes of the host's volatiles, ticks may be guided to particular feeding sites (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–dichlorphenol, nitrophenol, pentanoic acid, 2–methylpropanoic acid, butanoic acid, ammonia, and 3–pentanone, may be involved in host identification by the ticks (Anon. 2008). Masking these natural volatiles and diverting ticks away from their prospective hosts may be an attractive strategy to incorporate into the existing ITM for livestock tick control and management.

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

In this study, we investigated the on–host behaviour of unfed adults of the brown ear tick *R. appendiculatus* and assessed the effect of the application of essential oils of *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

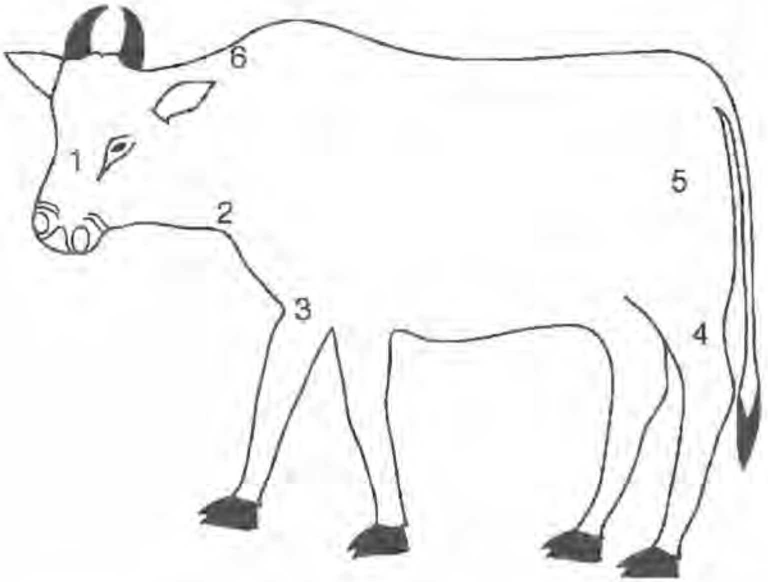

Fig. 6.1. Sites on the host animal (bovid) used for the study of on-host navigation behaviour of *R. appendiculatus* towards the predilection feeding site (the ear) in the presence of essential oils. Marked site 1 = Forehead, 2 = Dewlap, 3 = Foreleg, 4 = Rear leg, 5 = Escutcheon and 6 = Shoulder.

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 (Sereshti and Samadi, 2007). Essential oils were diluted to 10% in odourless vaseline petroleum jelly (BP–USP 100% Grade) (Unilever, Kenya) skin protectant. This carrier material contains no colours, fragrances or irritants. One ml of the stock 10% formulation of essential oils was placed in a 5 cm^3–Eppendorf tube which was attached by a string to a plastic ear tag on the inner side of the ear pinna. The Eppendorf tube was left open so that there was continuous release of either essential oil volatiles or petroleum jelly. The implication was that at all times a dose of 100 ml of pure essential oil in 900 ml of vaseline petroleum jelly was exposed to the ticks on the inner side of the ear of the animal. The odour from the applied essential oils was presumed to gradually contaminate the ear and its surrounding area. Both ears were simultaneously treated and ticks were released from previously marked sites (Wanzala et al., 2004) (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 filed (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[1]	No. of ticks that dropped off	Percentage of non-responding ticks[2]
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

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

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

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).

Table 6.2. On–host walking behaviour of *Rhipicephalus appendiculatus* to the predilection feeding site; ticks were released at six different locations on zebu cattle (n = 20).

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

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

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

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

[3] Time taken by ticks from placement on a given release point to the arrival at the predilection feeding site on the host animal.

- Indicates that no tick from the corresponding site of release on the host body reached the predilection feeding site, the ear pinna.

Discussion and conclusions

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

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

On-host observations of the ticks in our field site showed the typical sequence of behavioural patterns reported previously (Sika, 1996; Wanzala et al., 2004). In fact, the tick response pattern in the control experiment almost followed the pattern of on-host responses of ticks observed in Chapter 4. Although the responses of ticks at all release points were the same as that obtained in Chapter 4, the number of ticks successfully reaching the predilection feeding site was relatively low, implying that the essential oils applied had a significant repellent effect. This effect was further manifested in the high number of ticks losing their way and dropping off in the essential oil–treatments, while another sizeable percentage of ticks did not respond at all. Few ticks in the control dropped off. Although most ticks responded at all release points, the orientation and appropriate navigation toward locating and attaching at the predilection feeding site was undermined by repellent effects of the essential oils. This also meant that the previously suggested mediation of specific attractive and repellent host–derived stimuli in the process of orientation and navigation toward predilection feeding sites (Wanzala et al., 2004) was masked by the volatiles of the essential oils, as initially hypothesized.

With the disruptive effect of the two essential oils, the stereotyped sequence of orientation behaviours of *R. appendiculatus* on the host animal observed in the control experiment and described in Chapter 4, was not evident. This implied that the percentage of ticks reaching the predilection feeding site and exhibiting the unique complex set of behaviours, did so against the repelling forces of essential oils of *T. minuta* and *T. diversifolia*. Most notably missing in these stereotyped sequences of orientation behaviours were the second and third phases, involving random and directional movements, respectively. In the two essential oil treatments, ticks were observed randomly navigating on the host body and seeming to have accidentally arrived at the predilection feeding site, as they behaved in a manner that suggested that they were not affected by a feeding–site–specific cue.

The critical key variables of this study, the reaction time, initial speed and time between release and arrival at the predilection feeding site, were evaluated. However, other variables, such as the percentage of ticks at different sites that initiated movement (respondents), the percentage of ticks reaching feeding sites and the percentage of ticks that attached in the neighbourhood of release points, the percentage of ticks that dropped off after losing the way, and 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* Adr. Juss. and African spiderflower, *Gynandropsis gynandra* (L.) Briq. (Mwangi et al., 1995a, b; Ndung'u et al., 1995; Malonza et al., 1992; Waka et al., 2004; Garboui et al., 2006), they offer possibilities of exploitation of this potential in effective and environmentally acceptable methods of tick control mechanisms.

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

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 ± 1.9% and 26.0 ± 2.8%) and the highest mean percentage reduction of attaching ticks (76.5 ± 3.9% and 67.0 ± 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 ± 2.1%). The ear tube resulted in the highest mean percentage of ticks on the host (47.5 ± 5.1% and 55.8 ± 5.1%) and a lowest mean percentage reduction of attaching ticks (44.8 ± 5.1% and 36.5 ± 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 forma-midine (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 °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.

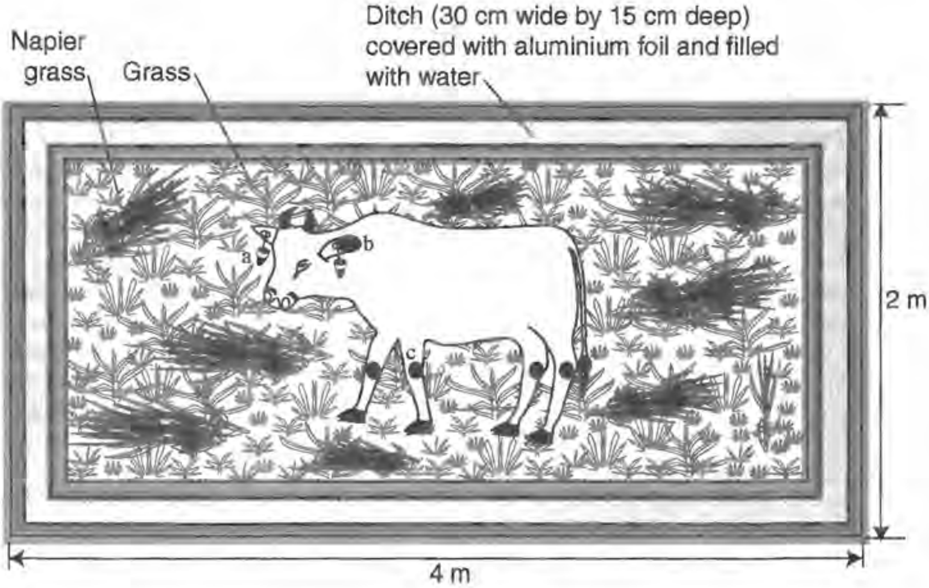

Fig. 7.1. Experimental plot in which 200 adult *Rhipicephalus appendiculatus* were released, indicating various experimental treatments: (a) tubes with petroleum jelly (control) or essential oils of *T. minuta* or *T. diversifolia*; (b) ears treated by smearing petroleum jelly (control) or essential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

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

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® 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 (± 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 (± SE)	Mean % of released ticks counted on the host after 3 h of host exposure(± SE)	% reduction in ticks on host after 3 h of host exposure
Cet	Control	Eartube	184.7 ± 4.67d	92.3 ± 2.33d	-
Ces	Control	Ear smear	172.3 ± 3.18d	86.2 ± 1.59d	-
Ctl	Control	Legs + tail smear	186.0 ± 4.16d	93.0 ± 2.08d	-
Tmet	Tm	Ear tube	95.0 ± 10.21bc	47.5 ± 5.11bc	44.8 ± 5.10bc
Tmes	Tm	Ear smear	50.3 ± 2.85a	25.2 ± 1.42a	61.0 ± 0.87ab
Tmtl	Tm	Legs + tail smear	33.0 ± 3.79a	16.5 ± 1.89a	76.5 ± 3.91a
Tdet	Td	Ear tube	111.7 ± 10.14c	55.8 ± 5.07c	36.5 ± 7.40c
Tdes	Td	Ear smear	83.3 ± 9.39b	41.7 ± 4.69b	44.5 ± 5.80bc
Tdtl	Td	Legs + tail smear	52.0 ± 5.69a	26.0 ± 2.84a	67.0 ± 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 ± 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 ± 4.9%) was higher than that by the essential oil of *T. diversifolia* (49.3 ± 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 ± 4.8%) was higher than that of the essential oil of *T. minuta* (29.7 ± 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, Hl; Tail, T; and Head-eyes/nose/ears/horn-base, Hd) (n = 200).

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

Acknowledgements

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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[1]

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

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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.

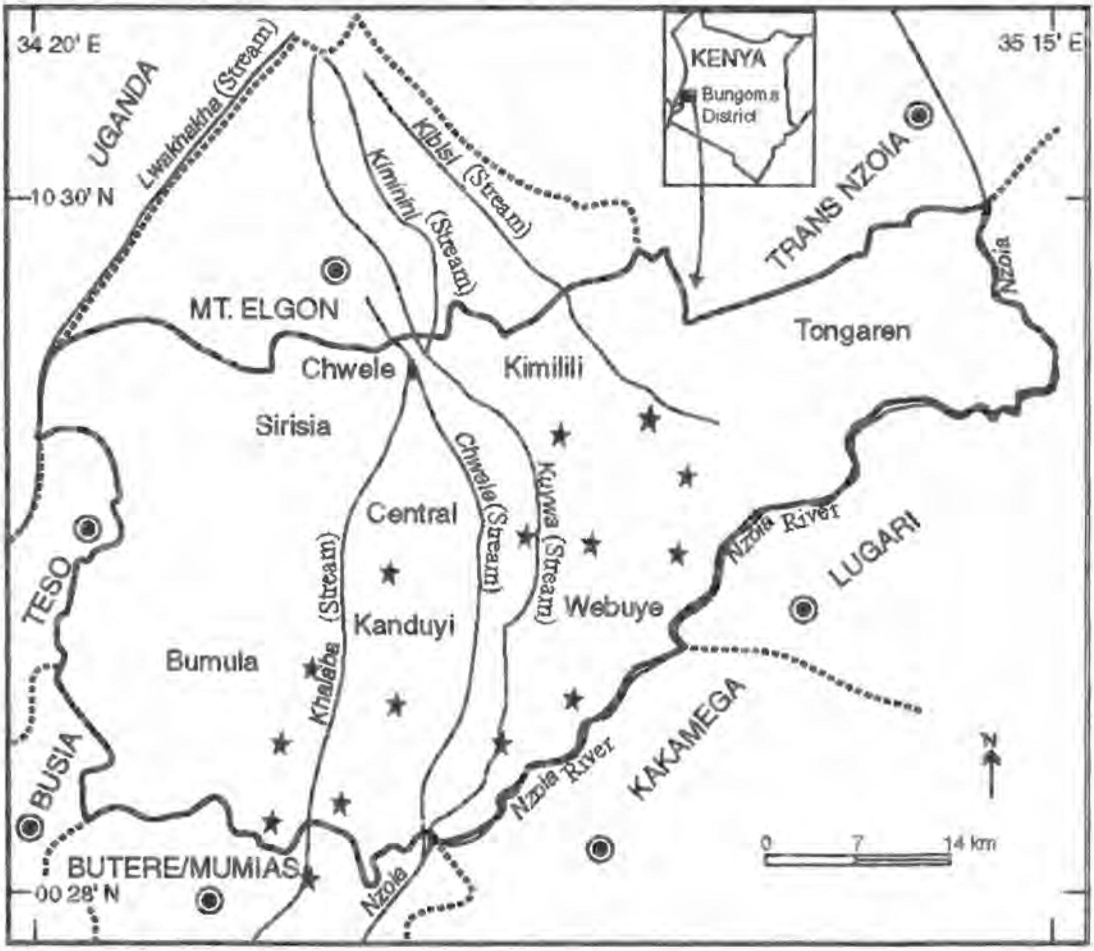


Fig. 8.1. The location of Bungoma District in Kenya. The field experiments were located on 15 different livestock farms (indicated with stars).

Methods and materials

Geography of experimental field site

The study was carried out in Bungoma District, western Kenya, situated between latitude 00°28' and 10°30' N and longitude 34°20' and 35°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 Bungoma 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 °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), Matthysse and Colbo (1987), Okello–Onen et al. (1999) and Walker et al. (2003). Some tick specimens could not be identified.

Data management and statistical analysis

Data collected were 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 (Log_{10}(tick counts + 1) (Zar, 1996), was applied to the data and the transformed data subjected to a paired sample t–test to evaluate a generally–observed trend in the field that the number of male ticks was higher than that of female ticks on the ani-

Table 8.1. The mean (± SE) tick counts on day zero on hosts naturally infested with ticks and selected for different treatments before start of experiment (I = those hosts selected for treatment with petroleum jelly (as control), II = those hosts selected for treatment with 10% essential oil 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 (± SE) tick counts [1]		
	I	II	III
R. appendiculatus (♂)	32.3 ± 18.19a	38.7 ± 18.47a	24.7 ± 14.59a
R. appendiculatus (♀)	31.3 ± 18.60a	23.2 ± 12.92a	24.1 ± 14.73a
Other tick species	32.2 ± 21.07a	38.7 ± 21.39a	21.9 ± 11.09a

[1] 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.

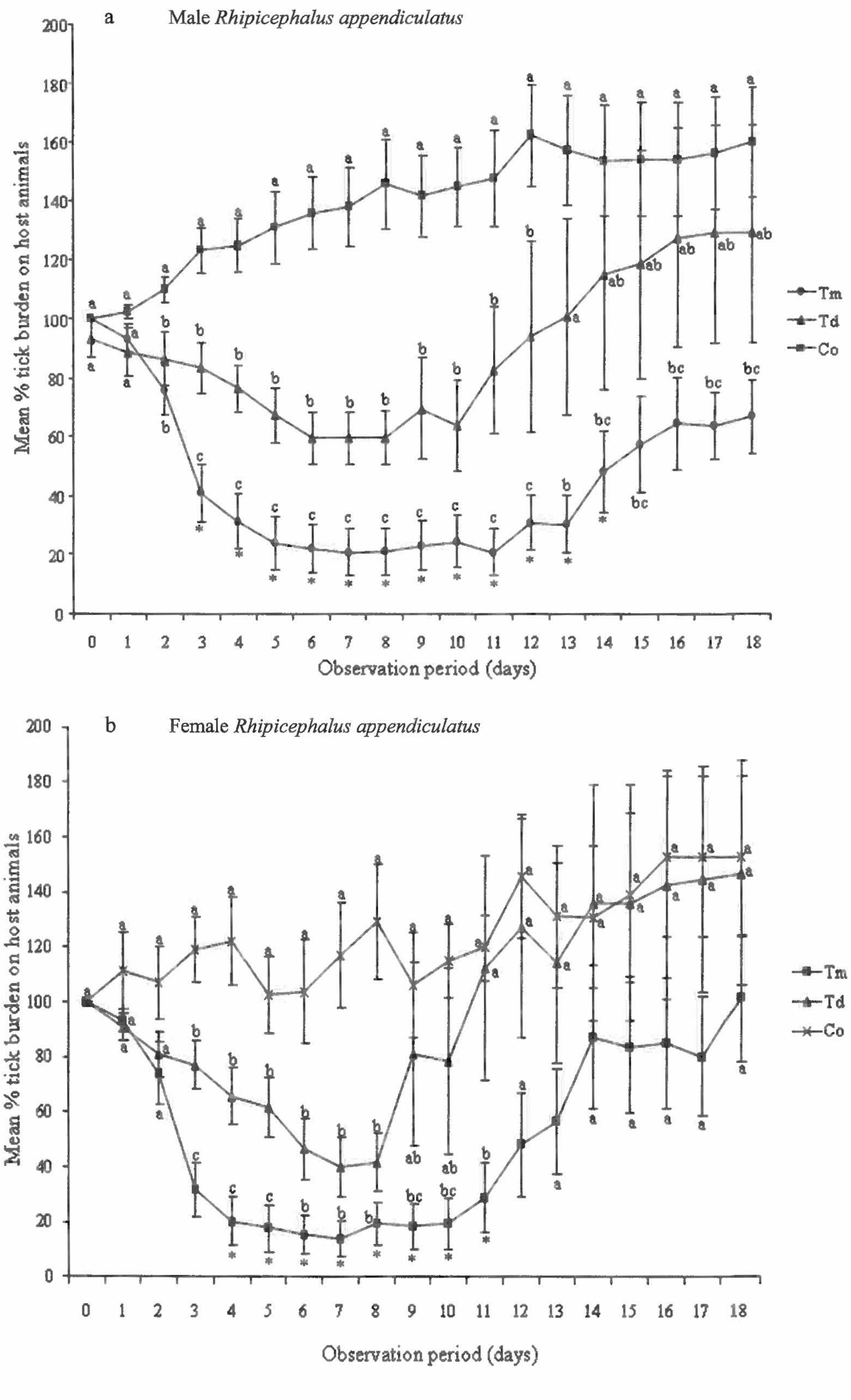
Results

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-



a Male *Rhipicephalus appendiculatus*

b Female *Rhipicephalus appendiculatus*

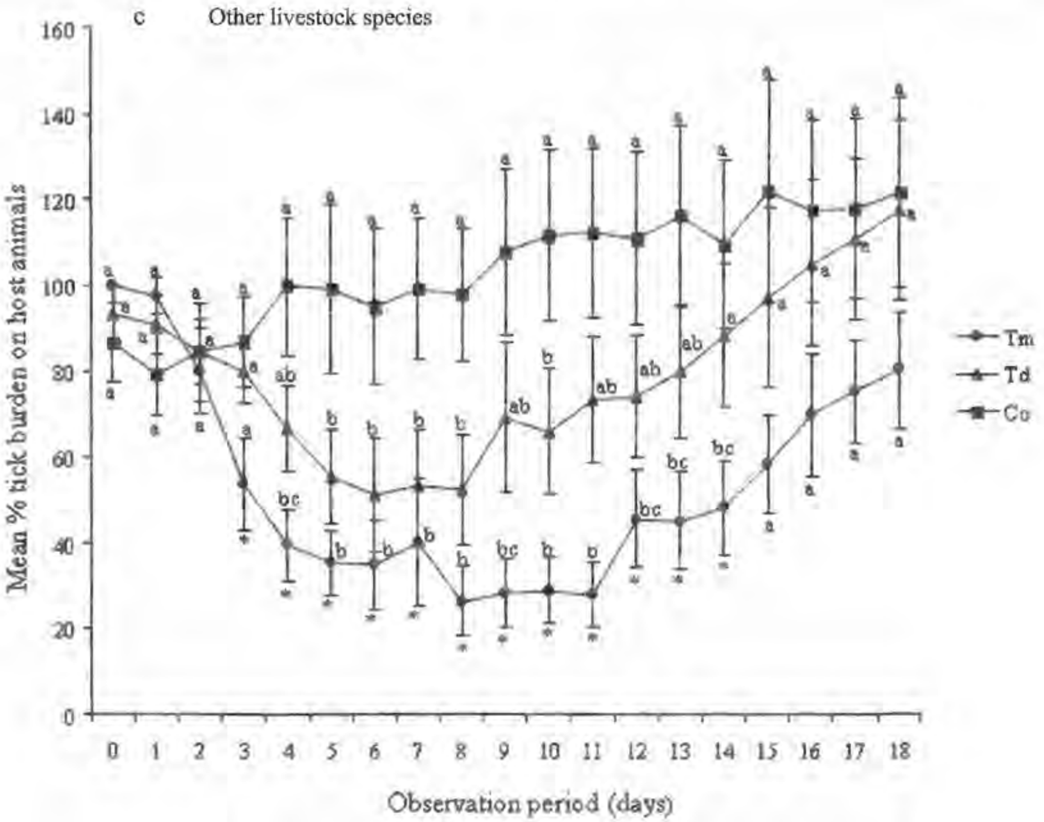


Fig. 8.2. mean (± SE) percentage of tick infestation on the hosts treated with essential oil of *Tagetes minuta* (Tm), *Tithonia diversifolia* (Td) or petroleum jelly (Co) (control) and monitored daily for 18 days. The effects of these treatments were evaluated on (a) male *Rhipicephalus appendiculatus*, (b) female *Rhipicephalus appendiculatus* and (c) other livestock tick species. On each day, values capped with the same letters are not significantly different at α = 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–treat-ment (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 *Tagetes minuta*	Hosts treated with essential oil of *Tithonia diversifolia*
1	16	9
2	5	5
3	11	7
4	13	5
5	10	8
6	4	6
7	10	12
8	18	10
9	15	8
10	16	4
11	13	10
12	18	9
13	14	6
14	14	11
15	11	8
Range	4-18	4-12
Mean ± SE	12.53 ± 1.07	7.87 ± 0.61

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.

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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., 2004). This on–host evolutionary specialisation of the two tick species is important in the formulation of a successful on–host control measure, specific to each tick species. Studies on the effects of the essential oils of *T. minuta* and *T. diversifolia* on *R. appendiculatus* described in Chapter 6 and the conclusion reached in selecting the appropriate dispensing method and application site of the essential oils on host animals in Chapter 7 and later field experiments with livestock farmers in Chapter 8, all used knowledge on behavioural biology obtained in Chapter 4. Data on biological and ecological characteristics of various tick species are required to develop simulation models that are incorporated into intervention strategies for tick control and management (Nokoe, 1992; Nokoe et al., 1992; Mwambi et al., 2000).

Appropriate methods and sites of application of putative repellents on the host

The successful interception of tick orientation toward predilection feeding sites on the host by repellents is dependent on the method of application as well as on the site of application. In Chapter 6, it is shown that the essential oils of *T. minuta* and *T. diversifolia* caused a disruption of orientation, dispersal and attachment behaviour of *R. appendiculatus*. More ticks dropped off in the treatments with the two essential oils than with the control, and host ears treated with *T. minuta* caused the highest percentage of ticks to drop off. The study in Chapter 7 focused on evaluating different methods of application of the repellents as well as on the sites of application on the host animal. In this study, the three treatment sites on the host animal all gave some degree of protection against host–seeking *R. appendiculatus,* but there was a considerable difference in effects of the essential oils of *T. minuta* and *T. diversifolia. Rhipicephalus appendiculatus* were more affected by the essential oils of *T. minuta* and *T. diversifolia* when smeared around the legs + tail of the animal than when applied on the ear by either smearing or by suspending a tube containing the oil on the inner side of the ear. The ear tube had the least effect on host–seeking *R. appendiculatus,* and its difference with the legs + tail treatment was significant for both essential oils. The ear tube could possibly offer a slow release of the odours of the essential oils, which would make it likely to affect ticks over a longer period of time than the other two methods (Gupta and Rutledge, 1989; 1994; Won-Ja et al., 2007); however, this is yet to be evaluated. Treatment of the inner side of the ear of the animal with a smear of essential oil in petroleum jelly seems to be the most appropriate and therefore recommended technique for the time being. However more studies are needed to evaluate the effect of different concentrations of essential oils under varying environmental conditions (Hamburger, 2003). It assumed that higher concentrations might afford better protection and have a longer residual time. In future, other sites on the animal for applying the tick repellents may be tested as well. For a broad–spectrum repellent, a strategic position on the host animal for applying the repellent is very important, as from this position, many different tick species locating the host animal for a blood meal will be affected.

The integration of locally–derived repellents in tick control and management programmes by livestock farmers

Locally–derived tick repellents are part of the traditional animal health care system (TAHS) already in use in the community. However, TAHS may not be in use uniformly across the community due to the development of conventional animal health care systems that may be preferred (Martin et al., 2001; Mathias and McCorkle, 2004; Mathias, 2004). Recent studies demonstrate that in many communities worldwide, TAHSs are acceptable, operate effectively in remote rural areas of poor livestock holders and are more sustainable than conventional health care systems (Sones and Catley, 2003; Mathias and

McCorkle, 2004; Wanzala et al., 2005). For example, on–farm experiments in the Andes mountains in Peru demonstrated that a homemade tobacco–based acaricide reduced mite infestation in sheep by a rate comparable to the best commercial acaricide on the local market. By contrast, the commercial product would cost a family approximately US$ 9 in an area where daily wages were well under US$ 1 (McCorkle and Bazalar, 1996). This is encouraging because traditional methods are culturally more acceptable and the plants often grow readily around the local villages (Mathias–Mundy and McCorkle, 1989; Wynn, 1999; Wanzala et al., 2005). Furthermore, villagers do not need an expert's advice on using such plants as some people in the community are already using them (Wynn, 1999; Catley and Mariner, 2002). In case of emergencies, healers and their treatments are often more easily available and transport expenses may be avoided (Mathias and McCorkle, 2004).

The development of products such as the formulated essential oils of *T. minuta* and *T. diversifolia* for on–host tick control can be integrated into livestock–tick control programmes by livestock farmers with relative ease. This is manifested in Chapter 8 of this thesis by the willingness of local livestock farmers to allow their farms and animals to be used in field experiments, which are a significant aspect of the evaluation of any repellent test, as they assess how the candidate repellent performs under field conditions (Mathewson et al., 1981). The integration of the experimental results obtained in this study with traditional practice and attitudes concerning tick control, may lead to a sustainable utilization of these plants' resources and further enhance the conservation of the ethnoknowledge present in the community (Ayensu, 1978; Martin, 1996).

Advantages and disadvantages of using repellents to control and manage livestock ticks

Most of the cattle in Africa live in a state of enzootic stability with respect to tick–borne diseases (TBDs), where the majority of the indigenous cattle population is infected and immune and little or no clinical disease occurs (Norval et al., 1992). This enzootic immunity to TBDs in a cattle population limits the worst of the impact of TBDs. From the results in Chapter 8 it is evident that one of the advantages of using repellents is to help maintain an enzootic immunity in cattle populations, as ticks on the host are not equally affected and they do not drop off the host all at once.

Compared to acaricides, repellents are less costly and provide a practical means of protection against many nuisance and disease vectors in conjunction with or when other control measures are not feasible (Schwantes et al., 2008). They offer preventative and prophylactic control measures at an individual level and easily applied (Rossi et al., 2007). Considering the challenges involved in vaccinating large populations as a prophylactic measure, repellents are likely to have a role in protection from vector–borne diseases for a long time to come, regardless of other technological developments (Dyer, 2004). The combination of vector control methods and protection of hosts at an individual level with repellents may be surprisingly effective (Strickman et al., 2001).

Natural repellents, which are biodegradable, may be safer for human use and more environmentally acceptable than synthetic, non-biodegradable products such as DEET. Newer natural repellent products such as picaridin and essential oil of lemon eucalyptus are becoming increasingly popular because of their low toxicity, comparable efficacy and customer approval (Katz et al., 2008).

Unfortunately, the short residual life of many repellents does not permit their long–term use for protection at the individual level. The repetitive application of repellents is a disadvantage and may be costly to some users (Waka et al., 2004). In addition, the costs involved in developing and delivering repellents, depending on the method, may make 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 worthwhile 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 stablilise 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
Euphorbia heterochroma (Pax.)	Kumutua (1)	Euphorbiaceae	Succulent shrub	✓✓✓	Sap/stem (used to make suspension)	T	Kaaya et al., 1995
						B	Watt and Breyer-Brandwijk, 1962 Philippe and Sura Faucon, 2003 Schmidt, 2003b
						A	ICIPE Annual Report, 1998/99 Regassa, 2000
Euphorbia tirucalli (L.)	Kumusongofwa	Euphorbiaceae	Perennial thornless cactus-like tree	✓✓✓✓✓	Whole plant (used to make suspension)	I	Hines and Eckman, 1993 Schmidt, 2003b Clevinger, 2003
						T	ICIPE Annual Report, 1998/99 Kaaya et al., 1995 Regassa, 2000
						B	Watt and Breyer-Brandwijk, 1962 Getahun, 1976 Duke, 1983a Hines and Eckman, 1993 Bowen and Hollinger, 2002
						C	Hines and Eckman, 1993
Euphorbia candelabrum (Kotschy.) (Trémaut) var. *candelabrum*	Kumutua (2)	Euphorbiaceae	Tree	✓✓✓	Leaf/stem/sap (used to make suspension)	T	Kaaya et al., 1995 Regassa, 2000
						A	ICIPE Annual Report, 1998/99
						C	Hines and Eckman, 1993
						B	Watt and Breyer-Brandwijk, 1962 Hines and Eckman, 1993 Schmidt, 2003b
Margaritaria discoideus (Webster./Baillon)	Erionoi (Teso) Atego (Luo) Kamenyabazi (Luganda) Muremampango (Runyankore)	Euphorbiaceae	Tree	✓	Whole plant (used to make suspension), wood smoke repels mosquitoes and snakes	I	ICIPE Annual Report, 1998/99 Schmidt, 2003b
						T	Regassa, 2000
						A	Kaaya et al., 1995
						B	UWMG, 2003 Schmidt, 2003b
						C	Hines and Eckman, 1993 Schmidt, 2003b

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

Neoboutonia melleri (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
Croton sylvaticus (Hoschst. ex Krauss)	Kumuchwichwi	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
Croton macrostachyus (Hochst. ex Delile.)	Kumuchwichwi 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
Croton megalocarpus (Hutch.)	Kumukunusia kumukeni or Kumutotoa kumukeni	Euphorbiaceae	Tree	✓✓	Leaf/bark/root (suspension)	B	Harjula, 1980 Politz and Lekeleley, 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

Ricinus communis (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 UWMG, 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
Hymenocardia acida (Enkeleñ Tul.)	Nangoso	Euphorbiaceae	Mostly found in savanna as woody plant/ small shrubs/tree	✓	Bark/leaf (used to make decoction)	B	Irvine, 1961 Marin, 1999 Schmidt, 2003b
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
Drypetes gerrardii (Hutch.)	Kumwilima	Euphorbiaceae	Tree	✓✓	-	B	Schmidt, 2003b
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
Flueggea virosa (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 (Fuits)	Zemede and Tadesse, 2001
						B	Samuelsson et al., 1992 Schmidt, 2003b
Sapium ellipticum (Krauss/ Pax.)	Kumuchaso	Euphorbiaceae	Tree	✓✓✓	Root/bark	B	Schmidt, 2003b
						T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
Phyllanthus ovalifolius (Forssk)	Kumusekese	Euphorbiaceae	Many stemmed shrub	✓✓	-	T	Kaaya et al., 1995 ICIPE Annual Report, 1998/99 Regassa, 2000
						B	Schmidt, 2003b

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

Turraea holstii (Gürke)	Nawili	Meliaceae	Tree	✓✓	Whole plant	B	Mulholland et al., 1998 Rajab et al., 1998
						T	Lindsay and Kaufman, 1988 Cabral et al., 1996 Mansingh and Williams, 1998 Ndumu et al., 1999 Abdel-Shafy and Zayed, 2002 Borges et al., 2003
Turraea robusta (Gurke)	Nabili	Meliaceae	Small tree	✓✓	Whole plant	T, B	Rajab et al., 1988; 1998 Bentley et al., 1992
Azadirachta indica (Adr. Juss.)	Kumwarubaine (2)	Meliaceae	Tree	✓✓✓✓✓ ✓✓✓✓✓ ✓✓✓✓✓ ✓✓✓✓✓	Stem/bark/fruit/leaf/root (suspension/ dusting/ rubbing	I	IDTG and IIRR, 1996 ICIPE Annual Report, 1998/99 Mansingh and Williams, 1998 Mulla and Su, 1999 Ndumu et al., 1999 Anonymous, 2001d Abdel-Shafy and Zayed 2002 Singh, 2002
						A	Lindsay and Kaufman, 1988 Williams, 1993 IDTG and IIRR, 1996 ICIPE Annual Report, 1998/99 Ndumu et al., 1999 Wilson and Mansingh, 2002 Blair and Mansingh, 2002 Abdel-Shafy and Zayed, 2002 Mansingh and Williams, 2002
						T	Lindsay and Kaufman, 1988 Cabral et al., 1996 Mansingh and Williams, 1998 Borges et al., 2003
						P	Isman et al., 1996 Brechelt, 2002 Singh, 2002
						B	UWMG, 2003 Thapa and Pathak, 2002
						Af	ICIPE Annual Report, 1998/99 Vellaikumar, 1997

Melia azedarach (L.)	Kumwarubaine (1)	Meliaceae	Tree	✓✓✓✓✓ ✓✓✓✓✓ ✓✓✓✓✓ ✓✓✓✓✓	Stem/bark/fruit Leaf/root (used to make suspension/dusting/rubbing	T	Williams, 1993 Ndumu et al., 1999 Anonymous, 2001d Abdel-Shafy and Zayed, 2002
						A	Lindsay and Kaufman, 1988 Cabral et al., 1996 Mansingh and Williams, 1998 Borges et al., 2003
						P	Stoll, 1988 Neupane, 1992 Berger, 1994 Kroschel, 1996 Batcher, 2000
						Af	Chen et al., 1996 Nardo et al., 1997 Vellaikumar, 1997
						B	Andrei et al., 1990 Zakir et al., 1991 Villmil et al., 1995 HerbWeb, 2000
						I	Al-Sharook et al., 1991 Arias et al., 1992 Isman et al., 1996 Cabral et al., 1996 Nardo et al., 1997 Valladares et al., 1997 Adoyo et al., 1997 Schmidt et al., 1998 Singh, 2002
Markhamia lutea (Benth. /K. Schum.)	Kumusiola	Bignoniaceae	Tree	✓✓	Leaf/stem	I	Anonymous, 2003c
						B	Kayonga and Habiyaremye, 1987 New Agriculturist, 2003
						C	UWMG, 2003
Stereospermum kunthianum (Cham.)	Kumutomolo (1)	Bignoniaceae	Small tree	✓	Root/bark (used to make hanging bouquet/suspension)	B, F, C, I	Petit, 2003
						B	Malerich and Trauner, 2003
Zizyphus abyssinica (A. Rich.)	Kumukomboti (1)/ Kumwikalangwe	Rhamnaceae	Tree	✓✓✓	Root/bark	F	Hentgen, 1985
Zizyphus mucronata (Willd.) ssp. *mucronata*	Kumukomboti (2)/ Kumwikalangwe/ Tirokwo (Pokot)	Rhamnaceae	Tree	✓✓✓	Root/bark	F	Hentgen, 1985
Lophira alata (Banks ex Gaertn.f.) (Red ironwood).	Oteng (Luo)	Ochnaceae	Tree	✓	Whole plant	B	Anonymous, 1977

Ximenia americana (L.) var. *amaricana*	Kumutuli	Olacaceae	Woody shrub or small tree	✓ ✓ ✓	Leaf/bark/root/fruit	P, B, C, F	Hines and Eckman, 1993
						B	Anonymous, 2003b
Sesbania sesban (L./ Merrill.) Var. *nubica* (Chiov.)	Chisubasubi (1)	Papilionaceae	Tree	✓ ✓ ✓	Leaf/stem (used to make suspension)	T	Matzigkeit, 1990
Kotschya africana (Endl.) Var. *bequaertii* (De Wild./ Verdc.)	Chisubasubi (2)	Papilionaceae	Tree	✓	Parts of the plant	T	Matzigkeit, 1990
Sesbania macrantha (Phil. and Hutch.)	Chisubasubi (3)	Papilionaceae	Tree	✓ ✓ ✓	Leaf/stem (used to make suspension)	T	Matzigkeit, 1990
Indigofera emarginella (Fresen.)	Lusisakwe	Papilionaceae	Shrub	✓ ✓ ✓	Root/stem	T	Matzigkeit, 1990
Erythrina abyssinica (Lam ex DC.) Subsp.*abyssinica*	Kumurukuru 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
Tephrosia vogelii (Hook F.)	Kumufila or Esumu	Papilionaceae	Shrub	✓	Leaf/stem/fruit/bud/ bark/seed/root (whole plant)	A, T	Lemmens and Fryer, 1917 Cremlyn, 1978 Matzigkeit, 1990 Toyang et al., 1995 Kambewa et al., 1997
						I	Kambewa et al., 1997
						Af	Niang, 1987 Bertram, 1988
						B	Niang, 1987 Getahun, 1976
						P	Matzigkeit, 1990 Cremlyn, 1978
Rhynchosia resinosa (A.Rich.) Baker	Kumusiuli	Papilionaceae	Tree	✓	Leaf/stem/root/bark	T, B	Matzigkeit, 1990 William, 1999
Macuna poggei Taub.var. *pesa* (De Wild) Verdc.	Kumuchuli or Kumusiuli	Papilionaceae	Perennial climbing herb or shrub	✓	–	T	Matzigkeit, 1990
Macuna poggei Taubert var. *poggei* (De Wild) Verdc.	Kumuchuli or Kumusiuli	Papilionaceae	Perennial climbing herb or shrub	✓	–	T	Matzigkeit, 1990

Acacia macrothyrsa Harms.	Kumukholondo	Mimosaceae	Shrub/tree	✓ ✓ ✓	Root/leaf/bark (used to make hanging bouquet/ suspension	B, C, T, F	Marin, 1999 Le Houérou, 2003
Albizia coriaria Welw. ex Oliver.	Kumupeli Ober (Luo)	Mimosaceae	Perennial tree	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	Stem/root/bark/leaf	T, B	Kokwaro, 1993 Bekalo et al., 1996 Schmidt, 2003d
Albizia grandibracteata Taub.	Kumufunjamwe/ Kumulongo/ Kumunianyuni	Mimosaceae	Perennial tree	✓	-	T, N	-
Entada abyssinica A.Rich.	Kumusembe	Mimosaceae	Perennial tree	✓ ✓	Root/bud/leaf/bark (used to make suspension)	T, C, F,B	Hines and Eckman, 1993 Toyang et al., 1995 Freiburghaus et al., 1998 Duke, 2002
Acacia sieberiana (DC.) (Paperback acacia)	Kumulemba	Mimosaceae	Perennial Tree	✓ ✓ ✓ ✓	-	F, C, B, T	Anonymous, 2001a
Acacia nilotica (L.) Will ex Del.	Kumulemba	Mimosaceae	Tree	✓ ✓ ✓ ✓	Bark/roots	B, I, F	Duke, 1983b Anonymous, 2001d
						B, P	Heine and Heine, 1988 Heine and König, 1988 Timberlake, 1987
Piliostigma/Bauhinia thonningii (Schumach.) Milne-Redh.	Kumulamalama	Caesalpiniaceae	Perennial Tree/shrub	✓ ✓	Parts of the plant–barks/ leaf/root	T, B, C, F	Ogundaini, 1999 Oni, 2001 Anonymous, 2001b Le Houerou, 2003
						F	Zemede and Mesfin, 2001
Senna siamea (Lam.) Irwin & Barneby.	Mbekoraisi or Kumuraisi	Caesalpiniaceae	Tree	✓ ✓ ✓ ✓	Root/stem/leaf (used to make suspension)	T, I, R	Adoyo et al., 1997 Mansingh and Williams, 1998 Anonymous, 2001d
Senna singueana (Del.) Lock.	Kumusilamosi	Caesalpiniaceae	Tree	✓ ✓ ✓	Fruit/root/leaf/bark (used to make suspension)	B, T, I	Adoyo et al., 1997 Mansingh and Williams, 1998
Tamarindus indica L.	Kumukhua	Caesalpiniaceae	Medium to large tree reaching 30 m in height.	✓ ✓ ✓ ✓	Root/bark/leaf (used to make suspension)	I, B	Imbabi et al., 1992 El-Siddig et al., 1999 Rao et al., 1999 Gurumurthy and Sreenivasa, 2000
						B	Zemede and Mesfin, 2001
Senna didymobotrya (Fresn.) Irwin & Barneby	Kumupinupinu	Caesalpiniaceae	Tree	✓	Root/bark/leaf (used to make suspension)	T, B	Fernandes et al., 1985 Mansingh and Williams, 1998 UWMG, 2003

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

Ficus amadiensis De Wild.	Kumutoto kumu-kobianyuni	Moraceae	Tree	✓ ✓	Root/fruit/bark/latex	T, C	Puyvelde (van) et al., 1985 Regassaa, 2000
Ficus thoningii BL.	Kumutoto ku-musecha	Moraceae	Tree	✓	Root/fruit/bark/latex	T, F , B, C	Puyvelde (van) et al., 1985 Hines and Eckman, 1993 Regassaa, 2000 Stockbauer, 2003
Ficus glumosa Del.	Kumusilisisi	Moraceae	Tree	✓	Root/fruit/bark/latex	-	-
Ficus sur Forssk. Broom cluster fig	Kumukhuyu nandere	Moraceae	Tree	✓ ✓	Root/fruit/bark/latex (used to make sus-pension)	T, F, C	Puyvelde (van) et al., 1985 Regassaa, 2000
Garcinia buchananii Bak.	Kumukhomeli	Guttiferae	Tree	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	Twigs/bark/root/ fruit (used to make sus-pension)	Ps, B	Smith, 1969 Duke-Elder and MacFaul 1972 Schmidt, 2003
Psorospermum febrifugum Spach.var. *febrifugum*	Nangoso	Guttiferae	Tree	✓	Fruit/bark/stem/leaf	B	Aubréville, 1936 Dalziel 1937 Irvine 1961 Cassady et al., 1990 Schmidt, 2003c
Harungana madagascariensis Lam. ex Poir.	Namalasile	Guttiferae	Tree	✓	Leaf/bark/stem	B	Woodland, 1997 Irvine, 1961 Schmidt, 2003c The World Bank Group, 2003 Erah et al., 2003
Maesa lanceolata Forssk. var golungensis	Namaru	Myrsinaceae	Tree	✓ ✓	-	T, B	Getahun, 1976
Nicotiana tabaccum L.	Eraba	Solanaceae	Herb	✓ ✓ ✓ ✓ ✓	Leaf/stem–dust (used to make sus-pension smoke and hanging bouquet also)	P, A, I, B, C	Matzigkeit, 1990 Mwangi, 1996 IDTG and IIRR, 1996; Cremlyn, 1978 Dipeolu and Ndungu, 1991 Juliette de Ba Levy, 1991 Stoll, 1988 Berger, 1994 Adoyo et al., 1997 Mansingh and Williams, 1998
Capsicum annum L.	Pilipili 1	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 Regassa, 2000

Capsicum frutescens L.	Pilipili 2	Solanaceae	Shrub	✓✓✓✓✓ ✓✓✓✓✓ ✓✓✓✓✓ ✓✓✓✓✓ ✓✓✓✓✓	Fruit/leaf/stem suspension, dust, smoking and hanging bouquet	P, I, T, B , F	Stoll, 1988 Berger, 1994 Adoyo et al., 1997 Mansingh and Williams, 1998 Regassa, 2000
Solanum incanum L. Bitter apple	Chindurandura	Solanaceae	Shrub	✓✓✓✓✓	Root, fruit–juice (suspension)	B, C, F	Hines and Eckman, 1993 Lukwa et al., 2001
Physalis peruviana L.	Embunwe Emiilwa (Wanga)	Solanaceae	Herb	✓✓	Stem/root/fruit/leaf. Heated leaves are applied as poultices on inflammations and leaf infusion as an enema to relieve abdominal ailments in children.	F, B, C	Morton, 1987 Barbadine, 2003
Securidaca longepedunculata Fres.	Kumunyakasia Kumuyanjabakeni	Polygalaceae	Tree	✓✓	Whole plant	I, C, B, F,Ps	Jayasekara et al., 2002 Rukangira, 2001
Lippia grandifolia var. *longipedunculata* Moldenke	Lukumakuma	Verbenaceae	Herb	✓✓✓✓✓ ✓	Root/leaf/stem	T^{I}	Kunle et al., 2003 Mwangi et al., 1989, 1991a,b, 1994, 1995, 1998 Koumaglo et al., 1996a,b Garneau et al., 1996
Lantana camara L. (Shrub verbena or sage)	Mukhekhe (Bukusu) Lantana (Wanga)	Verbenaceae	Herb/shrub	✓✓	Whole plant (Pour on or steaming)	B, F, C, I,	Adebayo et al., 1999
Vitex doniana Sweet (Meru-Oak)	Kumufutu or Kumufututu	Verbenaceae	Tree	✓	Bark/root/leaf/fruit (used to make smoke and hanging bouquet)	I, B, F, C	FAO, 1983 RSCU, 1992 Hines and Eckman, 1993 Olusola et al., 1997 Rukangira, 2001
Clerodendrum myricoides '*Ugandense*' (Hochst.) R. Br. & Vatke.	Nangoso (3)	Verbenaceae	Tree	✓✓✓	leaves, juice, roots	lice, fleas, mites, insecticides, ticks & leech	Baerts and Lehmann, 1991 Kasonia et al., 1991

						B, P	Staner and Boutique, R. 1937 Watt and Breyer-Brandwyk, 1962 Glover et al., 1966 Lemordant, 1971 Van Puyvelde et al., 1977 Lindsay and Hepper, 1978 Harjula, 1980 Gelfand et al., 1985 Kayonga and Habiyaremye, 1987 Heine and Heine, 1988 Baerts and Lehmann, 1989 Gachathi, 1989 Polygenis–Bigendako, 1990 Kokwaro, 1993 Tedesse, 1994
Vitex fischeri Gürke	Kumufutumbwe	Verbenaceae	Tree	✓	Whole plant (used to make smoke and hanging bouquet)	I	N
Ozoroa insignis Del. ssp. *reticulata* Gillett	Kumwandanda	Anacardiaceae	Tree	✓✓	A milky resin, bark/ root/leaf	I, B, F,	He et al., 2000 Araya, 2001 He et al., 2002 Nyazema, 2002
Rhus natalensis Krauss.	Kumusangula kumukhasi	Anacardiaceae	Tree	✓	Aerial parts/root/seed	I, F, C, B,	Janet, 1986 Berger, 1994
Rhus vulgaris Meikle	Kumusangula kumusecha	Anacardiaceae	Tree	✓	Aerial parts/root/seed	I, F, C, B,	Berger, 1994
Lannea schimperi (Hochst.ex A.Rich.) Engl.	Kumubumbu	Anacardiaceae	Tree	✓	Bark/root/leaf	F, C, B,	Marin, 1999
Rhoicissus tridentata (L.f.) Wild & R.B. Drumm.	Kumukoyakoye	Vitaceae	Lianas	✓	Roots or tuberous root-stock (lignotuber)	F, C, B,	Kayonga and Habiyaremye, 1987 Cunningham, 1990, 1995 Maundu et al., 2001 Michael, 2003
Rhoicissus revoilii Planch. (Bushveld Grape)	Kumukoyakoye	Vitaceae	Climber	✓	-	B	ITDG and IIRR, 1996
Cyphostemma ororondo (Gilg & Bened) Desc.	Kumukoyakoye	Vitaceae	Lianas	✓	-	-	N
Cyphostemma adenocaule (A. Rich) Wild & Drumm	Kumukoyakoye	Vitaceae	Lianas	✓	-	B	Adamantidis, 1956 Polygenis-Bigendako, 1990 Kokwaro, 1993

Paullinia pinnata L.	Kumukoyakoye kumulaasi	Sapindaceae	Lianas	✓	The root and root bark are applied for rubefacient purposes.	B, F	Killip and Smith, 1935 Fanshawe, 1948, 1953 Watt and Breyer-Brandwijk 1962 Abourashad, et al., 1999. van Andel, 2000 Onge, 2002 Duke and Bogenschutz, 2003
Dovyalis macrocalyx (Oliv.) Warb.	Kumusongola-munwa	Flacourtiaceae	Shrub	✓	Whole plant	-	-
Aloe latifolia (Haw.) Haw.	Kimitiokotioko	Aloeceae	Herb/ shrub	✓ ✓ ✓	Leaf/juice	B, T	Watt and Breyer-Brandwjik, 1962 Getahun, 1976 ITDG and IIRR, 1996 Bekalo et al., 1996
Aloe elgonica Bullock	Kimitiokotioko	Aloeceae	Herb/ shrub	✓	Leaf/juice	B, T	Watt and Breyer-Brandwjik, 1962 Getahun, 1976 ITDG and IIRR, 1996 Bekalo et al., 1996
Acanthus pubescens (Thomson ex Oliv.) Engl.	Kamarakaru	Acanthaceae	Shrub	✓		B	Baerts and Lehmann, 1991
Acanthus arboreus Forssk.	Kamarakaru	Acanthaceae	Shrub	✓	Leaf/root	B	UWMG, 2003
Agave sisalana Perrine ex Engelm.	Kamakonge or Kumukonge	Agavaceae	Forb (subacaulescent perennials) (succulent rosette, up to 6 feet tall (1.8 m), 6 feet spread (1.8 m))	✓ ✓	Juice/fleshy leaves *Smoking (using dried leaves) *Leaves and stems fermented in urine and applied *Leaves dried and ground to flour and applied as dust *Aqueous extract made from chopped leaves and stem and applied fresh	B, I	Wilcox and McGeorge, 1912 Schwartz et al. 1957 Faninger and Markovic-Brisk, 1960 Morton, 1977 Lin, 1984 Nobel and Berry, 1985 Ding et al., 1989 Zullo et al., 1989 Pires, (1991) Chand, 1991 Xu et al., 1993 Ding et al., 1993 FAO, 1993 Adoyo et al., 1997 Chang et al., 1997 Assefa and Williamson, 2001 Schmidt, 2003a

Prunus africana (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
Strychnos innocua Delile	Kumulende	Loganiaceae	Tree	✓✓✓✓✓✓	Powder of tried leaves/ seeds (storage pests-weevils)	P, F (fodder)	Assefa and Williamson, 2001
						B	Hines and Eckman, 1993
						Ps	Volfová and Patočka, 2003 Stedman, Wilcox & Follett Co., 1942
Strychnos spinosa Lam.	Kumukhubwe	Loganiaceae	Tree	✓	Leaf/bark/fruit	C, F	Wehmeyer, 1966 Fox and Norwood-Young, 1982 Taylor, 1986 Phillipson, 1995 Saka and Musonthi, 1994 Mizrahi et al., 2002 Kristensen, 2003
Clerodendrum myricoides 'Ugandense' (Hochst.) R. Br. ex Vatke	Nangoso	Lamiaceae	Shrub	✓✓	Whole plant	B	http://members.lycos.co.uk/ ethiopianplants/shinasha.html Getahun, 1976
Tinnea aethiopica Hook. f.	Lubilikhe	Lamiaceae	Shrub	✓✓✓	Leaf	T	Jembere et al., 1995
Plectranthus barbatus Andr.	Ling'ulie or Kamang'ulie	Lamiaceae	Shrub	✓	-	T	Jembere et al., 1995
Plectranthus lactiflorus (Vatke) Agnew	-	Lamiaceae	Shrub	✓	-	T	Jembere et al., 1995
Leonotis nepetifolia (L.) R. Br.	Susuni	Lamiaceae	Shrub	✓	-	T	Jembere et al., 1995
Hoslundia opposita Vahl.	Bifwofwo	Lamiaceae	Herb	✓✓✓	-	T	Jembere et al., 1995
Ocimum kilimandscharicum Baker ex Gürke.	Bifwofwo	Lamiaceae	Herb	✓✓	-	T C	Jembere et al., 1995
Leucas calostachys Oliv. Var. *fascicularis* (Baker) Sebald.	Nasusi	Lamiaceae	Herb	✓	-	T	Jembere et al., 1995
Olea capensis L.	Kumutamaywa	Oleaceae	Tree	✓	Leaf/bark/fruit	F, C	Hines and Eckman, 1993 Mutangah, 1996
						B	Stockbauer, 2003 Hines and Eckman, 1993

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

Gardenia ternifolia Schum. & Thonn.	Siuna	Rubiaceae	Shrub	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	Root/fruit	F	Zemede and Tadesse, 2001
						C, I	Fernandes et al., 1985
Pavetta oliveriana Hiern.	Kumukokhakokhe	Rubiaceae	Tree/shrub	✓	Root/leaf	-	N
Pavetta crassipes (K. Schum)	Kumusindusianjofu/ Kumupepenambusi kumukhasi	Rubiaceae	Tree	✓ ✓	Root/leaf	F, B	Sanon et al., 2003.
Steganotaenia araliaceae Hochst.	Kumutomolo (1) or Kumupepenambusi kumusecha	Umbelliferae	Tree	✓	Leaf/stem/bark	B	Rukangira, 2003
Annona senegalensis Pers. ssp. *Senegalensis*	Kumufwora	Annonaceae	Tree	✓	Leaf/bark/root	I, F	Stockbauer, 2003
						B	Toyang et al., 1995
Annona chrysophylla Boj.	Kumufwora	Annonaceae	Tree	✓	Leaf/bark/root	I, B, F	Stockbauer, 2003
Syzygium guineense (Willd.) Dc. ssp. *guineense*	Kumusitole kumusecha	Mrytaceae	Tree	✓	Bark/root/fruit	C, F, B	Hines and Eckman, 1993
Syzygium cordatum Krauss.	Kumusemwa	Mrytaceae	Medium-large tree.	✓	Stem/root/bark/fruit	F	Trade Winds Fruit, 2003
Syzygium cumini (L.)							
Dolichos kilimandscharicus Taubert Subsp. *parviflorus* Verdc.	Nandwasi	Fabaceae	Herb/shrub	✓ ✓ ✓ ✓	Root/stem/leaves (used to make concoction)	B, P (cattle)	University of Nairobi Heerbarium Library/records on voucher specimen.
					Powdered root	P	Golob et al., 1999
Mondia whytei (Hook f.) Skeels.	Kumukombela	Periplocaceae	Herb	✓	Root/stem?	B	Okwemba, 2002
Teclea nobilis Del.	Kumutare	Rutaceae	Tree	✓	Root/stem/bark/leaf	C, B	Hines and Eckman, 1993 Marin, 1999
Cuppressus lusitanica Mill.	Kumusayiprasi	Cupressaceae	Tree	✓ ✓ ✓ ✓ ✓ ✓	Leaf/stem	C, I	Personal communication with herbalists
Juniperus procera Hochst. ex Endlicher.	Kumutarakwa (1)	Cupressaceae	Tropical African timber tree with fragrant wood	✓ ✓ ✓ ✓ ✓	Leaf/root/bark	I, B	Getahun, 1976 Anonymous, 2001d
						I	Hines and Eckman, 1993

Podocarpus falcatus Mind. (Fern Pine)	Kumutarakwa (2)	Podocarpaceae	Shrub	✓	Leaf/root/bark	I	Stockbauer, 2003
Podocarpus latifolius (Thunb.) R.Br. ex Mirb.	Kumutarakwa (3)	Podocarpaceae	Tree	✓ ✓	Stem/root/leaf	C	Stockbauer, 2003
Pittosporum viridiflorum Sims. ssp. *viridiflorum* ssp. *quartinianum* (cuf) Cuf.	Nambaa (1)	Pittosporaceae	Shrub	✓ ✓	Stem bark /root/leaf	F, B	Coetzee et al., 1999 Berger, 2002 Seo, 2002
Harrisonia abyssinica 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
Withania somnifera (W. ashwagandha)(L.) Dunal	Nambaa (2)	Solanaceae	A perennial herb	✓ ✓	Stem/root/leaf	B	Pande, 1999 Singh and Kumar, 1998 McIntyre, 2002
Zea mays L.	Kamayindi	Sapindaceae	Grass	✓ ✓ ✓	Leaf/stem	B	Lukwa et al., 2001
Sorghum bicolor (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
Saccarhum officinarum L.	Kimiba (Kumwiba)	Gramineae	Grass	✓	Leaf/stem/juice	N	-
Ipomoea batatas (L.) LAM	Kamabwoni (Chindabii Chekamabwoni)	Convolvulaceae	Herb	✓ ✓ ✓	Leaf/stem/sap	B	Cevallos-Casals and Cisneros-Zevallos, 2002
Hibiscus calyphyllus Cav.	Kumwarakumba	Malvaceae	Shrub	✓	Leaf/stem/aerial parts/root	F, N	Puckhaber et al., 2002
Jacaranda mimosifolia D. Don	Chakaranda	Bignoniaceae	Tree	✓ ✓	Leaf/root/stem	-	-
Albizia coriaria 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	***Sericulture*** - 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

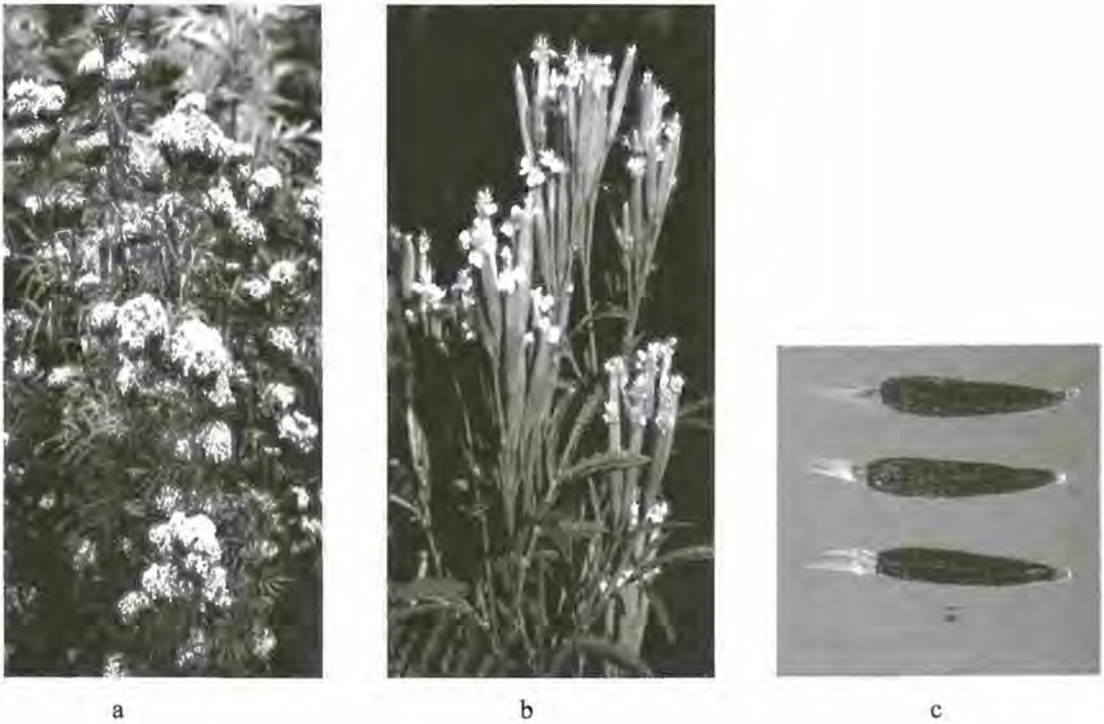

Fig. A2.1. *Tagetes minuta* L. (Asteraceae) plant showing (a) the young and (b) the mature aerial parts. **Flowers** are hermaphrodite (having both male and female organs) and are in erect clusters at the ends of the branches. **Flower heads** are on short stalks. **Leaves** are slightly glossy green, 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 retrosely 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 multipurpose 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). Worldwide 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 unambigously 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 pathenogenic 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 Burckhalter, 1976).

In Argentina, dihydrotagetone, α-phellandrene, limonene, *o*-cymene, as well as the isomers of β-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 dihydrotegetone 32.58% as major constituents (Singh et al., 1992), while in other studies (*Z*)-tagetone, (*Z*)-β-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 α-terpineol (20.8%), (Z)-β-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 dihydrotegetone (Héthelyi et al., 1986c). From these analyses, it could be seen that the composition of the oils varied according to a number of facotors: the harvesting location (Cravelro et al., 1988; Chalchat et al., 1995), growth stage (Héthélyi et al., 1986b), plant parts (Héthélyi 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*-β-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; Kokalis-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*-β-ocimene isolated from *T. minuta* oil showed strong nematicidal activity against eggs and juveniles of *Meloidogyne incognita* with dihydrotagetone showing a higher level of toxicity than *Z*-β-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 α-pinene (32.9%), β-caryophyllene (20.8%), germacrene D (12.6%), β-pinene (10.9%) and 1, 8-cineole (9.1%). Germacrene D (20.3%), β-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_{50} values ranging from 0.13 to 13.0 μM (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).

References

Abdel–Rahman, A., Shetty, A.K. and Abou–Donia, M.B., 2001. Subchronic dermal application of N,N–Diethyl–meta–toluamide (DEET) and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and hippocampus, and Purkinje neuron loss in the cerebellum. Experimental Neurology 172: 153–171.

Abdel–Shafy, S. and Zayed, A.A., 2002. *In vitro* acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). Veterinary Parasitology 106: 89–96.

Abegaz, B.S. and Demissew, S., 1998. Indigenous African useful plants with special emphasis on medicinal plants and issues associated with their management. A paper presented at a regional workshop on: Africa's Natural Resources Conservation and Management Surveys 2–5 March, 1998, Accra, Ghana.

Abo, K.A. and Ashidi, J.S., 1999. Antimicrobial screening of *Bridelia micrantha, Alchormea cordifolia* and *Boerhavia diffusa.* African Journal of Medicine and Medical Sciences 28: 167–169.

Abourashad, A.E, Toyang, N.J., Choinski, J.S., Jr. and Khan, I., 1999. Two new flavone glycosides from *Paullinia pinnata*. Journal of Natural Products 62: 1179–1181.

Abourashed, A.E., Ganzera, M., Khan, A.I., Khan, S., Mossa, S.J. and El–Feraly, F.S., 2003. HPLC fingerprinting and estimation of bioactive components of *Clutia richardiana* L. as a potential hypoglycemic herbal tea. Phytotherapy Research 17: 657–660.

Adamantidis, A., 1956. Monographie pastorale du Rwanda–Urundi. Bulletin Agricole du Congo Belge 47: 632–635.

Adebayo, A.T., Gbolade, T.A. and Olaifa, A.A., 1999. Comparative study of toxicity of some essential oils to larvae of three mosquito species. African Journals Online: Nigerian Journal of Natural Products and Medicine, Vol.3: http:// www.inasp.info /ajol/journals/njnpm/vol3abs.html, as retrieved on 25 October 2003.

Adebowale, K.O. and Adedire, C.O., 2006. Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. African Journal of Biotechnology 5: 901–906.

Adedire, C.O. and Akinneye, J.O., 2004. Biological activity of tree marigold, *Tithonia diversifolia,* on cowpea seed bruchid, *Callosobruchus maculatus (Coleoptera: Bruchidae).* Annals of Applied Biology 144: 185–189.

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

Adoyo, F., Mukalama, B.J. and Enyola, M., 1997. Using *Tithonia* concoctions for termite control in Busia District, Kenya: As part of the Kenya Woodfuel and Agroforestry Programme (KWAP) farmers in the Busia District of western Kenya taking part in an on–farm experiment in pest control. Research and Information Exchange in Ecologically Sound Agriculture (ILEIA). Newsletter 13: 24–25.

Aiello, E.S. and Mays, A. 2003. Ticks: tick control. The Merck Veterinary Manual (MVM), 8th edn. (ed. by Kahn, C.M. and Line, S.) Merck and Co. Inc., Whitehouse Station, NJ, USA.

Akall, G., 2003. Herbs only known to pastoralists. Ethnoveterinary Programme of the Intermediate Technology Development Group–Eastern Africa (ITDG–EA). The Communication Initiative Network: Where communication and media are central to social and economic development. http://www.comminit.com/en/node/241270 /36, as retrieved on 20 November 2004.

Akhtar, M. and Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresource Technology 74: 35–47.

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

Akobundu, I.O., Agyakwa, C.W., 1987. A Handbook of West African Weeds. International Institute for Tropical Agriculture, Ibadan, Nigeria., pp. 194–195.

Alekseev, A.N., Jensen, P.M., Dubinina, H.V., Smirnova, L.A., Makrouchina, N.A. and Zharkov, S.D., 2000. Peculiarities of behaviour of taiga (*Ixodes persulcatus*) and sheep (*Ixodes ricinus*) ticks (Acarina: Ixodidae) determined by different methods. Folia Parasitologica 47: 147–153.

Alexander, R.A. and Neitz, W.O., 1935. The transmission of louping ill by ticks (*Rhipicephalus appendiculatus*). Onderstepoon Journal of Veterinary Science and Animal Industry 5: 15–33.

Alfeev, N.I., 1946. The utilization of *Hunterellus hookeri* How. for the control of the ticks, *Ixodes rici nus* L. and *Ixodes persulcatus* P.Sch. with reference to peculiarities of their metamorphosis under conditions of the Province of Lenningrad. Review of Applied Entomology B 34: 108–109.

Alghali, M.A., 1992. Component technology development: An integrated tick management approach for livestock production in Africa. Insect Science and its Application 13: 609–612.

Al–Sharook, Z.K., Balan, Y., Jiang, Y. and Hein, Z., 1991. Insect growth inhibitors from two tropical Meliaceae. Effect of crude seed extracts on mosquito larvae. Journal of Applied Entomology 111: 425–430.

Amat, A.G., 1983. Pharmacological research for major taxons of Bonaerenses Compositae (in Spanish). Acta Farm Bonaerense 2: 23–36.

Amoo, O.J.A., 1992. Studies on the biology of African ticks: A review of the past and present with guides for the future. International Journal of Insect Science and its Application 13: 569–577.

Andrei, G.M., Coulombie, F.C., Courreges, M.C., deTorres, R.A. and Coto, C.E., 1990. Meliacine, an antiviral compound from *Melia azedarach* L., inhibits interferon production. Journal of Interferon Research 10: 469–476.

Anjaria, J.V., 1989. Herbal drugs: potential for industry and cash. In: (eds.). New crops for food and industry (ed. by Wickens, G.E. Haq, N. and Day, P.). Chapman and Hall, London, UK., pp. 84-92.

Anonymous, 1968. Food Composition Table for Use in Africa. U.S. Department of Health, Education, and Welfare, Public Health Service, Health Services and Mental Health Administration, National Center for Chronic Disease Control Nutrition Program, Bethesda, Maryland 20014 and Food Consumption and Planning Branch, Nutrition Division, Food and Agriculture Organization of the United Nations Rome, Italy, 1968. http://www.fao.org/docrep/003/X6877E/X6877E00.htm #TOC, as retrieved on 27 September 2003.

Anonymous, 1977. Posterior paralysis among sows kept on sawdust from the Azobe tree

(*Lophira alata* var. *procera*). Tijdschr Diergeneesk 102: 582.

Anonymous, 2001a. *Acacia sieberiana* DC. http://www.ildis.org/LegumeWeb/6.00/taxa/373.shtml, as retrieved on 20 October 2003.

Anonymous, 2001b. *Tylosema fassoglensis* (Schweinf.) Torre and Hillc. http://www.ildis.org/Legume Web/6.00/taxa/373.shtml, as retrieved on 20 October 2003.

Anonymous, 2001c. Structure and Data on Anthriscin, Deoxypodophyllotoxin, a natural product with anticancer activity isolated from *Bridelia scleroneura* [N11949, Q67B205] Korean. Molecular targets development programme, Center for Cancer Research at the National Cancer Institute. http://home.ncifcrf.gov/mtdp/ compounds/403148.html, as retrieved on 27 September 2003.

Anonymous, 2001d. Termite Control. HDRA–the organic organization. Ryton Organic Gardens, Coventry, UK. www.hdra.org.uk, as retrieved on 2 October 2003.

Anonymous, 2003a. *Fluegga virosa* (Roxb. ex Willd.) Voigt. Hong Gong Flora and vegetation. http://www.hkflora.com/index.htm, as retrieved on 1 October 2003.

Anonymous, 2003b. Management options for the Golden Apple Snail. http://www. applesnail.net/pestalert, as retrieved on 12 October 2003.

Anonymous, 2003c. On–farm technology testing. Sustainable Agriculture Extension Manual. UNDP SANE (Sustainable Agriculture Networking and Extension) Program, Berkeley, CA, USA. http://www.iirr.org/saem/contents.htm#top, as retrieved on 10 October 2003.

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

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

Araya, E.E., 2001. State of forest genetic resources in Eritrea. Sub–regional workshop of FAO/IPGRI/ICRAF on the conservation, management, sustainable utilization and enhancement of forest genetic resources in Sahelian and North–Sudanian Africa (Ouagadougou, Burkina Faso, 22–24 September, 1998). Forest Genetic Resources Working Papers FGR/23E. Forestry Department, FAO, Rome, Italy.

Arias, A.R., Schmeda–Hirschmann, G. and Falcão, A., 1992. Feeding deterrency and insecticidal effects of plant extracts on *Lutzomyia longipalpis*. Phytotherapy Research 6: 64–67.

Arthur, D.R., 1962. Ticks and Disease. International Monograph on Pure and Applied Biology, Vol. 9. Pergamon Press Ltd, Oxford, UK.

Assefa, F. and Williamson, S., 2001. Ethiopian farmers test alternatives to pesticides. Integrated Pest Management-Farmers Field School Project. Pesticides News No. 52, pp. 8–9. http://www.pan-uk.org/pestnews/pn52/pn52p8.htm, as retrieved on 9 October 2003.

Aubréville, A., 1936. La Flore Forestière de la Côte d'Ivoire. Paris, France.

Awumbila, B., 1996. Acaricides in tick control in Ghana and methods of application. Tropical Animal Health and Production 28: 50s–52s.

Ayensu, S.E., 1978. Medicinal plants of West Africa, 1st edn. Reference Publications, Algonac, MI, USA., pp. 1–45.

Backes, M.M., 1998. Indigenous Trees in Bukusuland. A selection of Useful Trees and Shrubs for Social Forestry in Bungoma. Promotion of Sustainable Forest Management (KEFRI/FD/GTZ). Excecutive printing works limited, Nairobi, Kenya.

Backes, M.M., 2001. The role of indigenous trees for the conservation of biocultural diversity in traditional agroforestry land use systems: The Bungoma case study: In-situ conservation of indigenous tree species Agroforestry Systems (in cooperation with ICRAF) 52: 119–132.

Baerts, M. and Lehmann, J., 1989. Guérisseurs et plantes médicinales de la région des crêtes Zaïre–Nil au Burundi. Musée royal de l'Afrique centrale, Tervuren, Belgique. Annals of Science Ecology 18: 214.

Baerts, M. and Lehmann, J., 1991. Plantes médicinales vétérinaires de la région des crêtes Zaïre-Nil au Burundi. Musée royal de l'Afrique centrale, Tervuren. Annals of Science Ecology 21: 133.

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

Bakheit, M.A. and Latif, A.A., 2002. The innate resistance of Kenana cattle to tropical theileriosis (*Theileria annulata* infection) in the Sudan. Annals of the New York Academy of Sciences 969: 159–163.

Balashov, Y.S., 1972. Bloodsucking ticks (Ixodoidea)–vectors of disease of man and animals. Miscellaneous Publications of the Entomological Society of America 8: 161 –376.

Baldwin, S. and Cervinskas, J., 1993. Community participation in research. Proceedings of a workshop held in Nairobi, Kenya, 23–27 September, 1991. Co–sponsored by the International Development Research Centre (IDRC) and the University of Nairobi. IDRC Publications, Nairobi, Kenya.

Bally, P.R.O., 1937. Native medicinal and poisonous plants of East Africa. Kew Bulletin 1: 10–25.

Bansal, P.R., Bahl, J.R., Garg, S.N., Naqvi, A.A., Sharma, S., Ram, M. and Kumar, S., 1999. Variation in quality of essential oil distilled from vegetative and reproductive stages of *Tagetes minuta* crop grown in North Indian plains. Journal of Essential Oil Research 11: 747–752.

Barbadine, 2003. Graines et semences tropicales/tropical seeds. http://www.barbadine.com/pages/tropical_seeds.htm, as retrieved on 3 November 2003.

Barnard, D.R., 1986. Density perturbation in populations of *Amblyomma americanum* Acari: Ixodidae) in beef cattle forage areas in response to two regimens of vegetation management. Journal of Economic Entomology 79: 122–127.

Batchelder, T., 2001. The Ethnobotany of Gastrointestinal Disorders: Mexican Asteraceae. Townsend Letter for Doctors and Patients. High Beam Encyclopeadia. The Gale Group, Farmington Hills, MI, USA. http://www.encyclopedia. com/doc/1G1-75178701.html, as retrieved on 25 March 2007.

Batcher, S.M., 2000. Element stewardship abstract for *Melia azedarach* Chinaberry, Umbrella tree. The Nature Conservancy's Wild land Invasive Species Team (ed. by Mandy, T., John M. and Randall, M.J.), University of California, Davis, USA. http://tncweeds.ucdavis.edu/esadocs/documnts/meliaze.html, as retrieved on 10 October 2003.

Bayer Healthcare, 2008. Advantix: Efficacy against ticks. http://www.advantix.info/ Questions-and-answers.1195.0.html, as retrieved on 17 October 2008.

Beentje, H.J., 1994. Kenya Trees, Shrubs and Lianas. National Museums of Kenya, Nairobi, Kenya.

Bekalo, I., Keengwe, M. Mathias, E. and Mundy, P., 1996. Ethnoveterinary medicine in Kenya. A field manual of traditional animal health care practice. Intermediate Technology Development Group and International Institute of Rural Reconstruction, Nairobi, Kenya.

Bekele, J. and Hassanali, A., 2001. Blend effects in the toxicity of the essential oil constituents of *Ocimum kilimandscharicum* and *Ocimum kenyense* (Labiateae) on two post-harvest insect pests. Phytochemistry 57: 385–391.

Bentley, M.D., Gaul, F., Rajab, S.M. and Hassanali, A., 1992. Tetranortriterpenes from *Turraea robusta* (Gurke). Journal of Natural Products 55: 84–87.

Berger, A., 1994. Using natural pesticides: current and future perspectives. A report for the Plant Protection Improvement Programme in Botswana, Zambia and Tanzania. Department of Entomology, Swedish University of Agricultural Sciences, Uppsala, Sweden. http://www.vaxteko.nu/html/sll/slu/rapport_vaxtsk_vetensk / RVX02/RVX02.htm, as retrieved on 12 October 2003.

Berger, J.M., Hoch, J., Neddermann, K.M., Bursuker, I., Mamber, S.W. and Kingston, D.G.I., 2002. A New Triterpene Saponin from *Pittosporum viridiflorum* from the Madagascar Rainforest. Journal of Natural Products 65: 11–15.

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

Berkvens, D.L. Geyscn, D.M., Chaka, G., Madder, M. and Brandt, J.R., 1998. A survey of the ixodid ticks parasitising cattle in the Eastern province of Zambia. Medical and Veterinary Entomology 12: 234–240.

Berlin, E.A. and Berlin, B., 1996. Medical Eohnabotany of the Highland Maya of Chiapas, Mexico. Princeton University Press, Princeton, NJ, USA.

Bertram, T.M., 1988. St. Benedict's Abbey, P.O. Peramiho, Tanzania.

Bhatt, D., Mitaliya, K., Pandya, N. and Baxi, U.S., 2001. Herbal therapy for jaundice. Advance in Plant Sciences 14: 123–125.

Biosafety News, 2002. Environment: Forests excision could turn Kenya into desert. Chester House, Picasso Productions, International Press Center, Nairobi, Kenya.

Blair, M. and Mansingh, A., 2002. Pesticidal potential of tropical plants 111: Acaricidal properties of few crude extracts and their fractions. Proceedings of the First International Symposium on Utilization of Natural Products in Developing Countries: Trends and Needs (ed. by Mansingh, A., Young, R., Yee, T., Delgoda, R., Robinson, E.D., Morrison, E. and Lowe, H.) held 10–14 July 2000 at the Natural Products Institute, University of the West Indies, Kingston, Jamaica.

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

Borges, L.M.F., Ferri, P.H., Silva, W.J, Silva, W.C. and Silva, J.G., 2003. *In vitro* efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. Medical and Veterinary Entomology 17: 228–231.

Botanical South Africa, 2003. Medicinal uses of botanicals of South Africa. http://library.thinkquestafrica.org/TQA00198/treemedical.htm. as retrieved on 27 October 2003.

Bowen, C.R. and Hollinger, S.E., 2002. Alternative Crops Web Site, 2 July 2002, Illinois State Water Survey, Champaign IL, USA. http://www.sws.uiuc.edu/data/altcrops/, as retrieved on 27 September 2003.

Branagan, D., 1973. The development periods of the ixodid *R. appendiculatus* under laboratory conditions. Bulletin of Entomological Research 63: 155–168.

Brechelt, A., 2002. Alternative for natural plant protection in the Dominican Republic. Proceedings of the First International Symposium on Utilization of Natural Products in Developing Countries: Trends and Needs (ed. by Mansingh, A., Young, R., Yee, T., Delgoda, R., Robinson, E.D., Morrison, E. and Lowe, H.) held 10–14 July, 2000 at the Natural Products Institute, University of the West Indies, Mona, Jamaica.

Broussalis, A.M., Ferraro, G.E., Martino, V.S., Pinzón, R., Coussio, J.D., Alvarez, C.J., 1999. Argentine plants as potential source of insecticidal compounds. Journal of Ethnopharmacology 67: 219–223.

Browner, C.H., de Montellano, O.B.R. and Rubel, A.J., 1988. A methodology for cross-cultural ethnomedical research. Current Anthropology 29: 681–702.

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

Bugyaki, L., 1955. La 'maladie de Kisenyi' du mouton due a un virus filtrable et transmise par des tiques. Bulletin Agricole du Congo Belge 46: 1455–1462.

Buisch, W.W., Hyde, L.J. and Mebus, A.C., 1998. Foreign pests and vectors of arthropod-borne diseases (vector-borne diseases and arthropod vectors). Foreign Animal Diseases, The Gray Book, 6th edn. (ed. by Buisch, W.W., Hyde, L.J. and Mebus, A.C.), Part IV. United States Animal Health Association. Pat Campbell and Associates and Carter Printing Company, Richmond, VA, USA.

Burke, G.S., Wikel, S.K., Spielman, A., Telford, S.R., McKay, K., Krause, P.J., and the Tick–Borne Infection Study group, 2005. Hypersensitivity to ticks and Lyme disease risk. Emerging Infectious Diseases 11: 36–41.

Buteyo, W.F.G., 2004. Chairman and Co–ordinator, Bungoma Indigenous Trees Conservation Club (BITCC), Bungoma, western Kenya.

Cabral, M.M.O., Garcia, E.S., Rembold, H., De Simone, S.G. and Kelecon, A., 1996. Anti –moulting activity in Brazilian *Melia azedarach*. Memórias Do Instituto Oswaldo Cruz 91: 117–118.

Cabrera, A.L., 1971. Compositae. Flora Patagónica (ed. by Correa, N.M.) Coleccion Cientifica del INTA, Buenos Aires, Argentina.

Camm, E.L., Towers, G.H.N. and Mitchell, J.C., 1975. UV-mediated antibiotic activity of some Compositeae species. Phytochemistry 14: 2007–2011.

Campbell, A., 1994. Community first: Landcare in Australia and London in UK:, International Institute for Environment and Development (IIED), Gatekeeper Series No. SA42.

Campin, J., 2005. Guide to plant relationships (for food allergy and intolerance identification). Version 13. http://www.purr.demon.co.uk/Food /RelatedPlantList.html, as retrieved on 15 May 2006.

Carroll, J.F. and Schmidtmann, E.T., 1996. Dispersal of blacklegged tick (Acari: Ixodidae) at the woods–pasture interface. Journal of Medical Entomology 33: 554–558.

Carroll, J.F., Klun, J.A. and Debboun, M., 2005. Repellency of deet and SS220 applied to skin involves olfactory sensing by two species of ticks. Medical and Veterinary Entomology 19: 101–106.

Carroll, J.F., Maradufu, A. and Warthen Jr., J.D., 1989. An extract of *Commiphora erythraea*: a repellent and toxicant against ticks. Entomologia Experimentalis et Applicata 53: 111–116.

Carroll, J.F., Schmidtmann, E.T. and Klun, J.A., 1995. Evidence for kairomonal influence on selection of host–ambushing sites by *Ixodes scapularis* (Acari: Ixodidae). Journal of Medical Entomology 32: 119–125.

Carroll, J.F., Solberg, V.B., Klun, J.A., Kramer, M. and Debboun, M., 2003. Comparative activity of deet and AI3–37220 repellents against the ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in laboratory assays. Journal of Medical Entomology 41: 249–254.

Carroll, J.F., Solberg, V.B., Klun, J.A., Kramer, M. and Debboun, M., 2004. Comparative activity of deet and AI3–37220 repellents against the ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in laboratory bioassays. Journal of Medical Entomology 41: 249–254.

Carroll, S.P., 2007. Evaluation of topical insect repellents and factors that effect their performance. Insect repellents: Principles, methods and uses (ed. by Debboun, M., Frances, S. and Strickman, D.), pp. 245–259. CRC Press., Boca Raton, FL, USA.

Cassady, J.M., Baird, W.M. and Chang, C.J., 1990. Natural products as a source of potential cancer chemotherapeutic and chemopreventive agents. Journal of Natural Products 53: 23–41.

Catley, A. and Mariner, J., 2002. Where there is no data–participatory approaches to veterinary epidemiology in pastoral areas of the Horn of Africa Issue Paper 10. International Institute for Environment and Development, London, UK.

Cavanilles, A.J., 1802. Descripción de las plantas que D. Antonio Josef Cavanilles demostró en las lecciones públicas del año 1801, precedida de los principios elementales de la Botánica, Imprenta Real, Madrid, Spain.

Cental Bureau of Statistics, 1996. Kenya Population Census 1989. Analytical Report Volume 11 April, 1996. Ministry of Planning and National Development, Government of Kenya, Nairobi, Kenya.

Cevallos–Casals, B.A. and Cisneros–Zevallos, L.A., 2002. Bioactive and functional properties of purple sweet potato *Ipomoea batatas* (L.). Acta Hort. (ISHS) 583, 195–203. http://www.actahort.org/books/583/583_22.htm, as retrieved on 21 October 2003.

Chalchat, J.C., Garry, R.P., Muhayimana, A., 1995. Essential oil of *Tagetes minuta* from Rwanda and France: chemical composition according to harvesting, location, growth stage and part of plant extracted. Journal of Essential oil Research 7: 375–386.

Chan, G.F.Q., Towers, G.H.N. and Mitchell, J.C., 1975. Ultraviolet-mediated antibiotic activity of thiophene compounds of *Tagetes*. Phytochemistry 14: 2295–2296.

Chand, N., 1991. Electrical behaviour of sisal fiber. Indian Journal of Fibre and Textile Research 16: 287–288.

Chang, S.G. and Chen, C.Y., 1997. Qualitative and quantitative analyses of cannabinoids in Cannabis sativa, huo–ma–jen and *Agave sisalana*. Chinese Pharmaceutical Journal 49: 77–88.

Chauhan, K.R., Klun, J.A., Debboun, M., Kramer, M., 2005. Feeding deterrent effects of catnip oil components compared with two synthetic amides against *Aedes aegypti*. Journal of Medical Entomology 42: 643–646.

Chen, C.C., Chang, S.J., Cheng, L.L. and Hou. R.F., 1996. Deterrent effect of the chinaberry extract on oviposition of the diamondback moth, *Plutella xylostella* (L.) (Lep., Yponomeutidae). Journal of Applied Entomology 120: 165–169.

Cherpanov, S.K., 1981. Plantae Vasculares Navaka, Lenningrad, U.R.S.S.

Chiera, J.W., Newson, R.M. and Maradufu, A., 1977. Anti–tick properties of molasses grass *Melinis minutiflora*. International Centre of Insect Physiology and Ecology (ICIPE) Fifth Scientific Annual Report., Nairobi, Kenya.

Chiera, W.J., 1985. The distribution and climbing behaviour of *Rhipicephalus appendiculatus* Neumann on grass stems. Insect Science and its Application 6: 213–215.

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

Chou, J.T. Rossignol, P.A. and Ayres, J.W., 1997. Evaluation of commercial insect repellents on human skin against *Aedes aegypti* (Diptera: Culicidae), Journal of Economic Entomology 34: 624–630.

Chunge, R.N., 1986. A study of head lice among primary school children in Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene 80: 42–46.

Clevenger, J.F., 1928. Apparatus for the determination of volatile oil. Journal of the American Pharmaceutical Association 17: 346.

Clevinger, A.J., 2003. Economic and medicinal uses. Euphorbiaceae (The Spurge Family). http://csm.jmu.edu/biology/clevinja/bio486/plantfamilies/AnnSimpson/ Euphorbiaceae/euphorbiaceae.htm, as retrieved on 28 November 2003.

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

Coetzee, C., Jefthas, E. and Reinten, E., 1999. Indigenous plant genetic resources of South Africa. Perspectives on New Crops and New Uses (ed. by Janick, J.) ASHS Press, Alexandria, VA., pp. 160–163. http://www.hort.purdue.edu/newcrop/ proceedings1999/v4-160.html, as retrieved on 27 October 2003.

Collins, D.A., Brady, J.N. and Curtis, C.F., 1993. Assessment of the efficacy of Quwenling as a mosquito repellent. Phytotherapy Research 7: 17–20.

Conrad, P.A., Denham, D. and Brown, C.G.D., 1986. Intraerythrocytic multiplication of *Theileria parva in vitro*: An ultrastructural study. International Journal of Parasitology 16: 223–30.

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

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

Cook, M.S., Khan, R.Z. and Pickett, A.J., 2007. The use of push–pull strategies in integrated pest management. Annual Review of Entomology 52: 375–400.

Cooley, R.A. and Kohls, G.M., 1934. A summary of tick parasites. Proceedings of the 5th Pacific Science Congress 5: 3375–3381.

Coop, R.L. and Kyriazakis, I., 1999. Parasite-nutrition interaction. Veterinary Parasitology 84: 187–204.

Copeland, R.A., Walker, T.W., Robert, L.L., Githure, J.I., Wirtz, R.A. and Klein, T.A., 1995. Response of wild *Anopheles funestus* to repellent–protected volunteers is unaffected by malaria infection of the vector. The Journal of the American Mosquito Control Association 15: 342–347.

Corbitt, N.J. and Wanyama, E., 2004. Circumcision Rites Among the Babukusu People of Kenya: A local Christian Response to a Cultural Tradition. BuildaBridge International, Baptist College, Limuru, Kenya.

Core, E.L., 1981. Castor bean, *Ricinus communis*. CRC handbook of biosolar resources. vol. 11. Resource Materials (ed. by McClure, T.A. and Lipinsky, E.S.), pp. 193–197. CRC Press, Boca Raton, FL, USA.

Cos, P., Hermans, N., de Bruyne, T., Apers, S., Sindambiwe, J.B., Witvrouw, M., de Clercq, E., Vanden Berghe, D., Pieters, L., Vlietinck, A. J., 2002. Antiviral activity of Rwandan medicinal plants against human immunodeficiency virus type-1. Phytomedicine 9: 62–68.

Couto, J.T., 1994. Operation oxpecker. The Farmers 10–11.

Craveiro, C.C., Matos, A.J.F. Machado, L.I.M. and Alencar, W.J., 1988. Essential oils of *Tagetes minuta* from Brazil. Perfumery and Flavourist 13: 35–36.

Cremlyn, R., 1978. Pesticides, Preparation and Mode of Action. John Wiley and Sons, New York, NY, USA.

Croes, A.F., Aarts, A.N., Bosveld, M, Breteler, H., Wullems, G.J., 1989. Control of tiophone accumulation in calli of two *Tagetes* species. Physiologia Plantarum 76: 205–210.

CTA, 2008. Technical Centre for Agriculture and Rural Cooperation (CTA). Demanding Innovation–Knowledge for Development: Indigenous Knowledge Systems. http://knowledge.cta.int/en/content/view/full/924, as retrieved on 18 November 2008.

Cumming, G.S., 2000. Host use does not clarify the evolutionary history of African ticks (Acari: Ixodoidea). African Zoology 35: 43–50.

Cumming, S.G., 1998. Host preference in African ticks (Acari: Ixodida): a quantitative data set. Bulletin of Entomological Research 88: 379–406.

Cunningham, A.B. and Mbenkum, F., 1993. Sustainability of Harvesting *Prunus africana* Bark in Cameroon. A Medicinal Plant in International Trade. People and Plants Working Paper 2. UNESCO, Paris, France.

Cunningham, A.B., 1990. Man and Medicines: The Exploitation and Conservation of Traditional Zulu Medicinal Plants. Mitheilungen aus dem Institut fur Allegemeine Botanik, Hamburg, Germany.

Cunningham, A.B., 1995. African Medicinal Plants: Setting Priorities at the Interface between Conservation and Primary Healthcare. People and Plants Working Paper 1. http://www.rbgkew.org.uk/peopleplants/wp/wp1/content.htm, as retrieved on 8 November 2003.

Cupp, E.W., 1991. Biology of ticks. The Veterinary Clinics of North America Small Animal Practice 21: 1–26.

D'Haese, L., Penne, K., and Elyn, R., 1999. Economics of theileriosis control in Zambia. Tropical Medicine and International Health 4: 49–57.

Daghero, J., Mattea, M., Reverchon, E., Della Porta, G. and Senatore, F., 1999. Isolation of *Tagetes minuta* L. oil using supercritical CO_2 extraction. Acta Hortuculturae (ISHS), 503: 21–26.

Dagne, E., Alemu, M. and Sterner, O., 1993. Flavonoids from *Euclea divinorum*. Bulletin of the Chemical Society of Ethiopia 7: 87–92.

Dalziel, J.M., 1937. The Useful Plants of West Tropical Africa. Crown Agents, London, UK.

Dautel, H. and Cranna, R., 2006. Assessment of repellency and mortality of a Imidacloprid + Permethrin spot–on solution against *Ixodes holocyclus* using a moving object assay. Australian Veterinary Practices 36: 138–147.

Dautel, H., 2004. Test systems for tick repellents. International Journal of Medical Microbiology Supplement 37: 182–188.

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

Davey, B.R., Garza, J. and Thompson, D.G., 1982. Seasonal observations on the development and ovipositional capability of *B. annulatus* and *B. microplus* reared on bovines. Journal of Medical Entomology 19: 24–28.

Davis, E.E., 1985. Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 22: 237–243.

de Castro, J.J. 1987. Effects of tick infestation on Boran (*Bos indicus*) cattle with differing previous exposure to ticks. Ticks and Tick–borne Diseases (ed. by Sutherst, W.R.) ACIAR Proceedings, Vol. 17, Canberra, Australia.

de Castro, J.J., 1991. Resistance to ixodid ticks in cattle with an assessment of its role in tick control in Africa. Breeding for disease resistance in farm animals (ed. by Axford, R.F.E. and Owen, J.B.) CABI, Wallingford, UK.

de Feo, V., Della Porta, G., Urrunaga Soria, E., Urrunaga Soria, R. and Senatore, F., 1998. Composition of the essential oil of *Tagetes filifolia* Lag. Flavour and Fragrance Journal 13: 145–147.

de Haan, C., Steinfeld, H. and Blackburn, H., 1996. Livestock and the environment: Finding a balance. European Commission Directorate–General for Development: Development Policy, Sustainable Development and Natural Resources., pp. 115. http://www.fao.org/docrep/x5303e/x5303e00.htm#Contents, as retrieved on 20 November 2008.

de la Fuente, J. and Kocan, K.M., 2006. Strategies for development of vaccines for control of ixodid tick species. Parasite Immunology 28: 275–283.

Debboun, M., Strickman, A.D. and Klun, A.J., 2005. Repellents and the military: our first line of defense. Journal of the American Mosquito Control Association 21: 4–6.

Dethier, V.G., 1956. Repellents. Annual Review of Entomology 1: 181–202.

Dewees, A.P., 1995. Social and economic incentives for smallholder tree growing. A Case Study from Murang'a District, central Kenya. Community Forestry Case Study Series. FAO, Rome, Italy. http://www.fao.org/docrep/U8995E/u8995e00.htm#Contents, as retrieved on 28 September 2003.

Diehl, P.A., Guerin, P., Vliamant, M. and Steullet, P., 1991. Biosynthesis, production site and emission rates of aggregation–attachment pheromone in males of two *Amblyomma* ticks. Journal of Chemical Ecology 17: 833–847.

Ding, Y. and Chen, Y.Y., 1989. Steroidal saponins from a cultivated form of *Agave sisalana*. Phytochemistry 28: 2787–2792.

Ding, Y. and Tian, R.H., 1993. Two new steroidal saponins from dried fermented residues of leaf juices of *Agave sisalana* forma Dong No. 1. Chemical and Pharmaceutical Bulletin Tokyo 41: 557–560.

Dipeolu, O.O. and Ndungu, J.N., 1991. Acaricidal activity of Kupetaba, a group mixture of natural products, against *Rhipicephalus appendiculatus*. Veterinary Parasitology 38: 327–338.

Dipeolu, O.O., 1990. Research on ticks of livestock in Africa: review of the trends, advances and milestones in tick biology and ecology in the decade 1980–1989. Insect Science and its Application 10: 723–740.

Dipeolu, O.O., Mongi, A.O., Punyua, D.K., Latif, A.A., Amoo, A.O. and Odhiambo, T.R., 1992. Current concepts and approach to control of livestock ticks in Africa. Discovery and Innovation 4: 35–44.

Dixon, W.J., and Massey, F.F., 1969. Introduction to Statistical Analysis. McGraw–Hill, New York, NY, USA.

Dolan, M., McAllister, J., Panella, N., Dietrich, G. Piesman, J. and Karchesy, J., 2008. Natural Pesticides and Repellents from Plant Essential Oils. Centers for Disease Control and Prevention, USA. http://www.afpmb.org/pubs/dwfp/meetings/ AMCA2008/230-Dolan.ppt, as retrieved on 29 October 2008.

Dolan, T.T., 1999. Dogmas and misunderstandings in East Coast fever. Tropical Medicine and International Health 4: 3–11.

Dolan, T.T., Young, A.S., Leitch, B.L. and Stagg, D.A., 1984. Chemotherapy of East Coast fever. Parvaquone treatment of clinical disease induced by isolates of *Theileria.* Veterinary Parasitology 15: 103–116.

Doube, B.M. and Kemp, D.H., 1979. The influence of temperature, relative humidity and host factors on the attachment and survival of *Boophilus microplus* larva to skin slices. International Journal of Parasitology 9: 449–455.

Duke, A.J. and Bogenschutz, J.M., 2003. Tico Ethnobotanical Dictionary. Dr. Duke's Phytochemical and Ethnobotanical Databases. USDA, ARS, Genetic Resources. http://www.ars-grin.gov/duke/dictionary/tico/p.html, as retrieved on 10 November 2003.

Duke, A.J. and Wain, K.K., 1981. Medicinal Plants of the World. Computer index with more than 85,000 entries, vol. 3.

Duke, A.J., 1983. James A. Duke's Handbook of Energy Crops. http://newcrop.hort. purdue.edu/newcrop/duke_energy/Melinis_minutiflora.html, as retrieved on 17 October 2005.

Duke, A.J., 1983a. *Euphorbia tirucalli* L. (Euphorbiaceae; Petroleum plant, Aveloz, Milkbush). Handbook of Energy Crops. Center for New Crops and Plant Products (ed. by Duke, J.A.) Purdue University, West Lafayette, IN, USA.

Duke, A.J., 1983b. Handbook of Energy Crops. *Acacia nilotica* (L.) Del. (Syn. *Mimosa nilotica*) commonly called "motse", Egyptian mimosa, or thorn. http://www.hort. purdue.edu/newcrop/duke_energy/Acacia_nilotica.html, as retrieved on 16 September 2004.

Duke, A.J., 1983c. Handbook of Energy Crops. *Sorghum bicolor* (L.) Moench, http:// www.hort.purdue.edu/newcrop/duke_energy/Sorghum bicolor .html, as retrieved on 21 October 2003.

Duke, A.J., 2002. A mini–course in medical botany: African Medicinal Plants in World Trade, module 9. http://www.ars-grin.gov/duke/ syllabus/ index.html#warn, as retrieved on 6 October 2003.

Duke–Elder, S. and MacFaul, P.A., 1972. Chemical Injuries. System of Ophthalmology. Vol. XIV. Non–mechanical Injuries, 1st edn.(ed. by Duke–Elder, S. and MacFaul, P.A.) Henry Kimpton, London, UK.

Dyer, O., 2004. U.S. judge halts compulsory anthrax vaccination for soldiers. British Medical Journal 329: 1062.

East Africa, 2003. Tick modeling, management and control in eastern and southern Africa. www.ento.csiro.au/research/pestmgmt/ticks/east.htm-9k, as retrieved on 11 October 2004.

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

El Tahir, A., Ibrahim, A.M., Satti, G.M.H., Theander, T.G, Kharazmi, A. and Khalid, S.A., 1998. The potential antileishmanial activity of some Sudanese medicinal plants. Phytotherapy Research 12: 576–579.

El Tahir, A. and Satti, G.M.H., 1999. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Maytenus senegalensis* (Lam.) Exell. Journal of Ethnopharmacology 64: 227–233.

Elbl, A. and Anastos, G., 1966. The ixodid ticks (Acarina, Ixodidae) of Central Africa, Volume 111. Genus *Rhipicephalus.* Koch, 1844. Musee Royal de l' Afrique Centrale, Tervuren, Belgique, Annales Sciences Zoologiques 147: 53–57.

El–Deeb, S.K., Abbas, A.F., El Fishawy, A. and Mossa, S.J., 2004. Chemical composition of the essential oil of *Tagetes minuta* growing in Saudi Arabia. Saudi Pharmaceutical Journal 12: 51–53.

El–Siddig, K., Ebert, G., Ludders, P., 1999. Tamarind (*Tamarindus indica* L.): A review on a multipurpose tree with promising future in the Sudan. Angewandte Botanik, 73: 202–205.

Elufioye, T.O., Agbedahunsi, J.M., 2004a. 8-(2-methylbutanoyl)-3, 10- epoxy-3, 8-dihydroxyl-4, 11 (13)-germacradien-12, 6-olide, a new sesquiterpene lactone from *Tithonia diversifolia.* Nigerian Journal of Natural Products and Medicine 8: 74–76.

Elufioye, T.O., and Agbedahunsi, J.M., 2004b. Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crossopteryx febrifga* (Rubiaceae) on mice *in vivo.* Journal of Ethnopharmacology 93: 161–171.

Erah, O.P, Asonye, C.C., Augustine, O. and Okhamafe, O.A., 2003. Response of *Tripanosoma brucei brucei*–induced anaemia to a commercial herbal preparation. African Journals Online: African Journal of Biotechnology. Volume 2: 9, September 2003. http://www.inasp.org.uk/ajol/journals/ajb/vol2no9abs.html, as retrieved on 29 October 2003.

Erasmus, B.F.N., van Jaarsveld, A.S., Chown, S.L., Kshatriya, M. and Wessels, K.J., 2002. Vulnerability of South African animal taxa to climate change. Global Change Biology 8: 679–693.

Espinar, L.A., 1967. Las especias de *Tagetes* (Compositae) de la region central Argentina. Kurtziana 4: 51–71.

Essuman, S., Dipeolu, O.O. and Odhiambo, R.T., 1991. Immunization of cattle with a semi –purified fraction of solubilized membrane–bound antigens extracted from the midgut of the tick *Rhipicephalus appendiculatus*. Experimental and Applied Acarology 13: 65–73.

Etkin, N.L., 1993. Anthropological methods in ethnopharmacology. Journal of Ethnopharmacology 38: 93–104.

Fabry, W., Okemo, P. and Aansorg, R., 1996. Activity of East African medicinal plants against *Helicobacter pylori.* Chemotherapy 42: 315–317.

Faninger, A. A. and Markovic–Brisk, A., 1960. Occupational dermatosis caused by mouldy sisal. Srpski Arhiv za Celokupno Lekarstvo 88: 303–311.

Fanshawe, D. B., 1948. Forest products of British Guiana, part II. Minor Forest Products. Forestry Bulletin No. 2. Forestry Department, Georgetown, Guyana.

Fanshawe, D. B., 1953. Fish poisons of British Guiana. Kew Bulletin 2: 239–240.

FAO, 1983. Food and Fruit–Bearing Forest Species–Examples from East Africa. Forestry Paper 44/1. FAO, Rome, Italy.

FAO, 1993. Part two: Update of recent developments. Processing and utilization. Proceedings of the FAO Expert Consultation on the Role of Forestry in Combating Desertification, Saltillo, Mexico, 24–28 June 1985. FAO Rome, Italy. http://www.fao.org/docrep/T0115E/T0115E00. htm, as retrieved on 9 October 2003.

FAO., 1998. Caribbean *Amblyomma* Eradication Programme, CARICOM / FAO / IICA programme on Tropical Bont Tick (TBT). http://www.fao.org/WAICENT /fao Info/Agrcult/AGA/AGAH/PD/pages/cap01.html, as retrieved on 22 June 2001.

Fawcet, D.W., Young, A.S. and Leitch, B.L., 1985. Sporogony in Theileria (Apicomplexa: Piroplasmida): A comparative ultrastructural study. Journal of Submicroscopic Cytology 17: 299–314.

Fei, B., and Xin, H.J., 2007. N,N–diethyl–meta–toluamide–containing microcapsules for bio–cloth finishing. American Journal of Tropical Medicine and Hygiene 77: 52–57.

Fernandes, M.C.E., Oktingati, A. and Maghembe, J., 1985. The Chagga home gardens: A multi–storeyed agro–forestry cropping system on Mt. Kilimanjaro, northern Tanzania, The United Nations University Press, Food and Nutrition Bulletin Vol. 7: 3, September 1985. United Nations University, Tokyo, Japan.

Fernandes, M.C.E., Oktingati, A. and Maghembe, J., 1995. The Chagga home gardens: A multi–storeyed agroforestry cropping system on Mt. Kilimanjaro, northern Tanzania. International Council for Research in Agroforestry, Nairobi, Kenya.

Fernandez–Ruvalcaba, M., Torre, P.F., Cruz–Vazquez, C. and Garcia–Vazquez, Z., 2004. Anti-tick effects of *Melinis minutiflora* and *Andropogon gayanus* grasses on plots experimentally infested with *Boophilus microplus* larvae. Experimental and Applied Acarology 32: 293–299.

Fivaz, B.H., Norval, R.A.I. and Lawrence, J.A., 1989. Transmission of *Theileria parva bovis* (Boleni strain) to cattle resistant to the brown ear tick *Rhipicephalus appendiculatus* (Neumann). Tropical Animal Health and Production 21: 129–134.

Fox, F.W. and Norwood–Young, M.E., 1982. Food from the wild–edible plants of Southern Africa, Delta, Johannesburg, South Africa.

Fradin, M.S. and Dax, J.F., 2002. Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine 347: 13–18.

Fraga, A.B., Alencar, M.M., Figueiredo, L.A., Razook, A.G. and Cyrillo, J.N.S.G., 2003. nálise de fatores genéticos e ambientais que afetam a infestação de fêmeas bovinas da raça Caracu por carrapato (*Boophilus microplus*). Revista Brasileira de Zootecnia 32: 1578–1586.

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

Freiburghaus, F., A. Steck, A., Pfander, H. and Brun, R., 1998. Bioassay–guided isolation of a diastereoisomer of kolavenol from *Entada abyssinica* active on *Trypanosoma brucei rhodesiense*. Journal of Ethnopharmacology 61: 79–183.

Gachathi, F.N.M., 1989. Kikuyu botanical dictionary of plant names and uses. UNEP: Ref –Dic–Subj–B655. German Agency for Technical Co–operation (GTZ). The print shop, Nairobi, Kenya.

Garboui, S.S., 2008. Plant–derived chemicals as tick repellents. Doctoral thesis, Uppsala University, Department of Evolution, Genomics and Systematics, Systematic Zoology, Uppsala, Sweden.

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

Garcia, R., 1962. Carbon dioxide as an attractant for certain ticks. Annals of Entomological Society of America 55: 605.

Garneau, Francois–X., Gagnon, H., France, J.I., Koumaglou, H.K., Moudachirou, M. and Addae–Mensah, I., 1996. Les Chemophytes de *Lippia multiflora, Lelaleuca quiquenervia* et *Clausena anisata* naturels du Togo, Benin et Ghana. Produits Naturels d'Origine Vegetale (ed. by Guy Collin, G. and Garneau, F.X.). Actes du cologne de Saint–Jean sur Richelieu, 18 au 24 Octobre, 1995. Laseve, University of Quebec, Chicoutimi, Canada.

Gelfand, M., Mavi, S., Drummond, R.B. and Ndemera, B., 1985. The traditional medicinal practitioner in Zimbabwe. Mambo Press, Gweru, Zimbabwe.

George, T.S., Gregory, P.J., Robinson, J.S., Buresh, R.J., Jama, B., 2002a. Utilisation of soil organic P by agroforestry and crop species in the field, western Kenya. Plant

Soil 246: 53–63.

George, T.S., Gregory, T.J., Robinson, J.S. and Buresh, R.J., 2002b. Changes in phosphorus concentrations and pH in the rhizosphere of some agroforestry and crop species. Plant Soil 246: 65–73.

Getahun, A., 1976. Some common medicinal and poisonous plants used in Ethiopian folk medicine. Faculty of Science, Addis Ababa University, Addis Ababa, Ethiopia. http://ip.aaas.org/tekindex.nsf/0/99b535e7618170c485256ae100696f4c/Body/M1?OpenElement, as retrieved on 12 October 2003.

Ghosh, S. Azhahianambi, P. and Yadav, M.P., 2007. Upcoming and future strategies of tick control: a review. Journal of Vector Borne Diseases 44: 79–89.

Gibson, G. and Torr, J.S., 1999. Visual and olfactory responses of heamatophagous Diptera to host stimuli. Medical and Veterinary Entomology 13: 2–23.

Gikonyo, N.K., Hassanali, A., Njagi, P.G.N., Gitu, M.P. and Midiwo, O.J., 2002. Composition of preferred (buffalo and ox) and non–preferred (waterbuck) hosts of some savanna tsetse flies. Journal of Chemical Ecology 28: 969–981.

Gil, A., Ghersa, M.C. and Leicach, S., 2000. Essential oil yield and composition of *Tagetes minuta* accessions from Argentina. Biochemical Systematics and Ecology 28: 261 –274.

Gladney, W.J. and Drummond R.O., 1970. Migration of male lone star ticks on the host in relation to mating. Journal of Economic Entomology 63: 1214–1216.

Glover, P.E., Stewart, J. and Gwynne, M.D., 1966. Maasai and Kipsigis notes on East African plants, Part III–Medicinal uses of plants. East African Agricultural and Forestry Journal 32: 200–207.

Godfray, J.C.H., 1994. Parasitoids: Behavioral and Evolutionary Ecology. Monographs in Behavior and Ecology. Princeton University Press, Princeton, NJ, USA.

Goethe, R. and Neitz, W., 1985. Investigations into the participation of male pheromones of *Rhipicephalus evertsi evertsi* during infestation. Onderstepoort Journal of Veterinary Research 52: 67–70.

Goffin, E., Cunha, D.P.A., Ziemons, E., Tits, M., Angenot, L. and Frederich, M., 2003. Quantification of tagitinin C in *Tithonia diversifolia* by reversed-phase high-performance liquid chromatography. Phytochemical Analysis 14: 378–380.

Goffin, E., Ziemons, E., de Mol, P., Madureira, M.D.C., Martins, P.A., Cunha, D.P.A., Philippe, G., Tits, M., Angenot, L., Frederich, M., 2002, *In vitro* antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: tagitinin C. Planta Medica 68: 543–545.

Golob, P., Moss, C., Dales, M., Fidgen, A., Evans., J. and Gudrups, I., 1999. The use of spices and medicinals as bioactive protectants for grains. Natural Resources Institute Chatham Maritime Chatham, Kent, UK. Food and Agriculture Organization of the United Nations, Rome. FAO Agricultural Services Bulletin No. 137: M–15.

Graf, J.F., Gogolewski, R., Leach–bing, N., Sabatini,G.A., Molento, M.B., Bordin, E.L.and Arantes, G.J., 2004. Tick control: an industry point of view. Parasitology 129: s427–s442.

Graham, K., Graham, A. and Towers, G.H.N., 1980. Cercaricidal activity of phenylheptatriyne and alpha-tertienyl, naturally occuring compounds in species of the Asteraceae. Canadian Journal of Zoology 58: 1955–1958.

Grainge, M. and Ahmed. S., 1988. Handbook of plants with pest-control properties. Resource Systems Institute, East-West Center, John Wiley, NY, USA.

Graven, E.H, Webber, L., Benians, G., Venter, M., Gardner, J.B., 1991. Effect of soil type and nutrient status on the yield and composition of *Tagetes* oil (*Tagetes minuta*

L.). Journal of Essential Oil Research 3: 303–307.

Gray, J.S., 1985. A carbon dioxide trap for prolonged sampling of *I. ricinus* populations. Experimental and Applied Acarology 1: 35–44.

Grieve, M., 1931. A modern herbal: medicinal, culinary, cosmetic, and economic properties, cultivation, and folklore of herbs, grasses, fungi, shrubs, and trees with all their modern scientific uses. Hafner Press, New York, NY, USA.

Grossard, M., 1998. The use of vaccines and genetically resistant animals in tick control. Revue Scientifique et Technique Office International des Epizooties 17: 188–199.

Grover, G.S. and Rao, T.J., 1978. *In vitro* antimicrobial studies of the essential oil of *Tagetes erecta*. Perfumery and Flavourist 3: 28.

Gu, J.Q., Park, E.J., Totura, S., Riswan, S., Fong, H.H.S., Pezzuto, J.M., Kinghorn, A.D., 2002. Sesquiterpenoids from *Tithonia diversifolia* with potential cancer chemopreventive activity. Journal of Natural Products 65: 532–536.

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

Guèye, E.F., 2002. Newcastle disease in family poultry: Prospects for its control through ethnoveterinary medicine. Vol. 14: 5. Livestock Research for Rural Development. Proceedings of the 27th World Veterinary Congress, 25–29 September 2002, Tunis, Tunisia (ed. by Preston, R.T., Penh, P. and Sansoucy, R.) Centre for Investigation in Sustainable Systems of Agricultural Production (CIPAV Foundation) Cali, Colombia.

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

Gupta, R.K. and Rutledge, L.C., 1989. Laboratory evaluation of controlled-release repellent formulations on human volunteers under three climatic regimens. Journal of the American Mosquito Control Association 5: 52–55.

Gurumurthy, S.B. and Sreenivasa, M.N., 2000. Occurrence and distribution of VAM fungi in the rhizosphere of five tree species grown under agroforestry system. Environment and Ecology18: 500–502.

Hadani, A., Ziv, M. and Rechav, Y., 1977. A laboratory study of tick repellents. Entomologia Experimentalis et Applicata 22: 53–59.

Hamburger, M., 2003. Tracking bioactivity in plant extracts–new concepts and approaches. Society for Medicinal Plant Research, 50 Years: 1953–2003. A jubilee edition (ed. by Sprecher, E.), pp. 109–122. Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany,

Hamburger, M., Marston, A. and Hostettmann, K., 1991. Search for new drugs of plant origin. Advances in Drug Research 20: 167–215.

Hamilton, J.G.C. and Sonenshine, D.E., 1989. United States Patent Number: 8, 884, 361.

Hamilton, J.G.C., Papadopoulos, E., Harrison, S.J., Liyod, C.M. and Walker, A.R., 1994. Evidence for a mounting sex pheromone in the brown ear tick *Rhipicephalus appendiculatus*. Experimental and Applied Acarology 18: 331–338.

Harjula, R., 1980. Mirau and his practice. A study of the ethnomedicinal repertoire of a Tanzanian herbalist. Institute for cultural research, University of Turku, Finland. Tri–med Books, Finchley, London, UK.

Harrison, R.I., Palmer, H.B. and Wilmshurst, C.E., 1973. Chemical control of cattle ticks–resistance problems. Pesticide Science 4: 531–542.

Hart, B.L., 1997. Effects of hormones on behavioral defenses against parasites. Parasites and Pathogens: Effects on Host Hormones and Behavior (ed. by Beckage, N.), pp. 210–230. Chapman and Hall, New York, NY, USA.

Hart, B.L., 2000. Role of grooming in biological control of ticks. Annals of the New York Academy of Sciences 916: 565–569.

Hart, L.A., Hart, B.L. and Wilson, V., 1996. Grooming rates in klipspringer and steinbok reflect environmental exposure to ticks. African Journal of Ecology 34: 79–82.

Hassan, S.M., Dipeolu, O.O., Malonza, M.M., 1994. Natural attraction of livestock ticks by the leaves of a shrub. Tropical Animal Health and Production 26: 87–91.

Hassanain, M.A., El–Garhy, M.F., Abdel–Ghaffar, F.A., El–Sharaby, A. and Megeed, A.K.N., 1997. Biological control studies of soft and hard ticks in Egypt. I. The effect of *Bacillus thuringiensis* varieties on soft and hard ticks (Ixodidae). Parasitology Research 83: 209–213.

Hassanali, A., Herren, H., Khan, R.Z., Pickett, A.J. and Woodcock, M.C., 2007. Integrated pest management: the push–pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 611–621.

He, W–D., Van Puyvelde, L., Bosselaers, J., de Kimpe, N., van der Flaas, M., Roymans, A., Mathenge, S., Mudida, F. and Chalo Mutiso, P., 2000. Bioactive principles from plants of Kenya. IV. Study on the anti–fouling activity of *Ozoroa insignis* Del. Mededelingen van de Faculteit Landbouwwetenschappen, Universiteit Gent 65: 29–33.

He, W–D., Van Puyvelde, L., Bosselaers, J., de Kimpe, N., van der Flaas, M., Roymans, A., Mathenge, S., Mudida, F. and Chalo Mutiso, P., 2002. Activity of 6–pentadecylsalicylic a cid from *Ozoroa insignis* Del. against marine crustaceans. Pharmaceutical Biology 40: 74–76.

Heine, B. and Heine, I., 1988. Plant concepts and plant use. An ethnobotanical survey of the semi–arid and arid lands of East Africa. Part 1: Plants of the Chamus (Kenya), Band 6, Kolner Beiträge zur Entwicklungsländerforschung/Cologne Development Studies. Breitenbach Publishers, Saarbrücken, Germany.

Heine, B. and König, C., 1988. Plant concepts and plant use .An ethnobotanical survey of the semi-arid and arid lands of East Africa.Part 2: Plants of the So (Uganda). Band 7, Kolner Beiträge zur Entwicklungsländerforschung/Cologne Development Studies. Breitenbach Publishers, Saarbrücken, Germany.

Heinrich, M, Ankli, A, Frei, B, Weimann, C, and Sticher, O., 1998a. Medicinal plants in Mexico: Healers' consensus and cultural importance. Social Science and Medicine 47: 1859-1871.

Heinrich, M. and Gibbons, S., 2001. Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. Journal of Pharmacy and Pharmacology 53: 425–432.

Heinrich, M., 1996. Ethnobotany of Mexican Compositae: An analysis of historical and modern sources. Proceedings of the International Compositae Conference, Kew, 1994. Vol. 2: Biology and Utilization (ed. by Caligaria, S.D.R. and Hind, N.J.D.), pp. 475-503. Royal Botanical Gardens, Kew, Richmond, UK.

Heinrich, M., Rimpler, H. and Barrerra, N.A, 1992. Indigenous phytotherapy of gastrointestinal disorders in a lowland Mixe community (Oaxaca, Mexico): ethnopharmacological evaluation. Journal of Ethnopharmacology 38: 63–80.

Heinrich, M., Robles, M., West, J.E.,Ortiz de Montellano, B.R. and Rodriguez, E., 1998b. Ethnopharmacology of Mexican Asteraceae (Compositae). Annual Review of Pharmacology and Toxicology 38: 539–565.

Hempel, P., 2002. Aboriginal Traditional Knowledge and Environmental Management. The Science and Environment Bulletin, Environment Canada, No. 32, September/ October 2002.

Hentgen, A., 1985. Forage trees in India: a chance for the rearing of ruminants. Fourrages 101: 105–119.

HerbWeb, 2000. Global botanical exchange. http://www.herbweb.com, as retrieved on 27 September 2003.

Hernandez, L., Parra, D., and Ahumada, A., 1990. Repellent and acaricidal activity of the oil and some chromatographic fractions of the grass *Melinis minutiflora* against *Boophilus microplus*. Revista Colombiana de Ciencias Quimico–Farmaceuticas 17: 45–50.

Herrera, F.L., 1941. Sinopsis de la Flora del Cuzco. Vol. 1. Parte sistemática. Government of Peru, Lima, Peru.

Hethelyi, E., Danos, B. and Tetenyi, P., 1986a. GC-MS analysis of the esssential oils of four *Tagetes* species and the anti-microbial activity of *Tagetes minuta*. Flavour and Fragrance Journal 1: 169-173.

Héthelyi, É., Dános, B. and Tétényi, P., 1989. Phytochemical studies on the essential oils of species belonging to the Achillea genus, by GC/MS. Biomedical and Environmental Mass Spectrometry 18: 629-636.

Héthélyi, É., Danos, B., Tétényi, P. and Juhász, G., 1986c. Phytochemical studies on *Tagetes* species; infraspecific differences of the essential oil in *T. minuta* and *T. Tenuifolia*. Herba Hung 26: 145–158.

Héthélyi, É., Dános, B., Tétényi, P. and Koczka, I., 1986b. GC/MS analysis of the essential oils of some *Tagetes* species. Progress in Essential Oil Research (ed. by Brunke, S.E.), pp. 131–137. Walter de Gruyter, Berlin, Germany.

Héthélyi, É., Kaposi, P, Domonkos, J, Kernoczi, Z.S., 1987. GC/MS investigation of the essential oils *Rosmarinus oflcinalis* L. Acta Pharmacology Hungary 57: 159–169.

Hibbard, B.E. and Bjostad, L.B., 1989. Corn semiochemical and their effects on insecticide efficacy and insecticide repellency toward western corn rootworm larvae (Coleoptera: Chrysomelidae), Journal of Economic Entomology 82: 773–781.

Hillard, O.M., 1977. Compositae in Natal. University of Natal Press, Pietermaritzburg, South Africa.

Hines, D.A. and Eckman, K., 1993. Indigenous multipurpose trees of Tanzania: Uses and economic benefits for people. FAO Miscellaneous/93/9 Working Paper, FAO, Rome and Ottawa, Ontario, Canada, 1st edn. Cultural Survival Canada, Development Services Foundation of Tanzania and Tree Roots Canada. http://www.fao.org/docrep/X5327e/x5327e00.htm, as retrieved on 27 September 2003.

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

Hocking, B., 1963. The use of attractants and repellents in vector control. Bulletin of World Health Organization 29: 121–126.

Homer, K.A., Manji, F. and Beighton, D., 1990. Inhibition of protease activities of periodontopathic bacteria by extracts of plants used in Kenya as chewing sticks (mswaki). Archives in Oral Biology 35: 421–424.

Homer, K.A., Manji, F. and Beighton, D., 1992. Inhibition of peptidase and glycosidase activities of Porphyromonas gingivalis, Bacteroides intermedius and Treponema denticola by plant extracts. Journal of Clinical Periodontology 19: 305–310.

Hoogstraal, H., 1956a. African Ixodoidea. I. Ticks of the Sudan with Special Reference to

Equatorial Province and with Preliminary Reviews on the Genera *Boophilus*, *Margarapus* and *Hyalomma*. Research Report, Department of the Navy, Bureau of Medicine and Surgery, Washington DC, USA.

Hoogstraal, H., 1956b. Ticks of the Sudan. African Ixodoidea. Vol. 1., USA Navy, Washington DC, USA.

Hosaka, E.Y. and Thistle, A., 1954. Noxious plants of the Hawaiian ranges. Extension Bulletin of the University of Hawaii and USA Department of Agriculture 62.

Howard, R., Matsumura, F. and Coppel, C.H., 1976. Trail-following pheromones of the rhinotermitidae: Approaches to their authentication and specificity. Journal of Chemical Ecology 2: 147–166.

Howell, C.J., De Vos, A.J., Bezuidenhout, J.D., Potgieter, F.T. and Barrowman, P.R., 1981. The role of chemical tick eradication in the control or preventntion of tick transmitted diseases in cattle. Tick Biology and Control (ed. by Whitehead, B.G. and Gibson, D.J.), pp. 61–66. Proceedings of an International Conference held in Grahamstown, 27–29 January, 1981. Tick Research Unit, Rhodes University, Grahamstown, South Africa.

Howell, F.G., 1975. The roles of host-related stimuli in the behaviour of *Argas cooleyi.* Journal of Medical Entomology 11: 715–725.

Hu, R., Hyland, K.E. and Oliver Jr., H.J., 1998 A review on the use of *Ixodiphagus* wasps (Hymenoptera: Encyrtidae) as natural enemies for the control of ticks (Acari: Ixodidae). Systematic and Applied Acarology 3: 19–28.

Huang, J., Nuessly, G.S., McAuslane, H.J., Nagata, R.T., 2003. Effect of screening methods on expression of romaine lettuce resistance to adult banded cucumber beetle, *Diabrotica Balteata* (Coleoptera: Chrysomelidae). The Florida Entomologist 86: 194–198.

Humbert, H., 1923. Les Composees de Madagascar. E. Lanier, Caen, France.

Hussein, G., Miyashiva, H., Nakamura, N. and Hattori, M., 1999. Inhibitory effects of Sudanese plant extract on HIV–1 replication and HIV–1 Protease. Phytotherapy Research 13: 31–36.

Ibrahim, H., Abudurahman, M.E., Shock, M., Ilyas, N., Monday, S. and Chordima, W., 1999. Pharmacognostic studies of the stem of *Carrisa edulis*. Online. Nigerian Journal of Natural Products and Medicine. Vol. 3. http://www.inasp.info/ajol/journals/njnpm/vol3abs.html, as retrieved on 20 October 2003.

ICIPE Annual Report., 1998/99. The International Centre of Insect Physiology and Ecology (ICIPE), Animal Health Management (Ticks): Sustainable Methods for Integrated Tick Management (ed. by Ng'eny–Mengech, A., Sogo, D., Mwanycky, S., Ndung'u., A.C.) ICIPE Science Press, Nairobi, Kenya.

ICIPE, 1997. Turning off the tick time bomb. Animal Health Division. International Centre of Insect Physiology and Ecology (ICIPE). ICIPE Annual Report Highlights, ICIPE Science Press, Nairobi, Kenya.

ICIPE, 1998/99. Animal Health Division, International Centre of Insect Physiology and Ecology (ICIPE). The Fifth Scientific Annual Report Highlights, ICIPE Science Press, Nairobi, Kenya.

ICRAF, 1992. A Selection of Useful Trees and Shrubs for Kenya (ed. by Khatiwada, P. Y.). International Centre for Research in Agroforestry (ICRAF), Nairobi, Kenya.

IIRR, 1994. Ethnoveterinary medicine in Asia: An information kit on traditional animal health care practices. International Institute of Rural Reconstruction, Silang, Philippines.

IK and DM, 2004. Introduction to the Indigenous Knowledge and Development Monitor.

Indigenous Knowledge and Development Monitor (IK and DM). Nuffic/IK–Unit, The Hague, The Netherlands. http://www.nuffic.nl/ciran/ikdm/introduction.html, as retrieved on 17 August 2004.

Imbabi, E.S., Ibrahim, K.E., Ahmed, B.M., Abulfutuh, I.M. and Hulbert, P., 1992. Chemical characterization of tamarind bitter principle, tamarindineal [tamarindienal]. Fitoterapia 63: 537–538.

Ioffe–Uspensky, I., Mumguoglu, Y.K., Uspensky, I., Gulun, R., 1997. *Rhipicephalus sanguineus* and *R. turanicus* (Acari: Ixodidae): Closely related species with different biological characteristics. Journal of Medical Entomology 34: 74–81.

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

Irvin, A.D., McDermott, J.J. and Perry, B.D., 1996. Epidemiology of Ticks and Tick-borne Diseases in Eastern, Central and Southern Africa (ed. by Werehire, P. (Production editor and Irvin, A.D., McDermott, J.J. and Perry, B.D.). Proceedings of a Workshop held in Harare 12–13 March 1996. International Livestock Research Institute (ILRI), Nairobi, Kenya and Addis Ababa, Ethiopia.

Irvine, R.F., 1961. Woody Plants of Ghana with Special Reference to their Uses. Oxford University Press, London, UK.

Isman, M.B., Matsuura, H., MacKinnon, S., Durst, T., Towers, G.H.N. and Arnason, J.T., 1996. Phytochemistry of the meliaceae. So many terpenoids, so few insecticides. Phytochemical Diversity and Redundancy in Ecological Interactions (ed. by Romeo, J.T., Saunders, J.A. and Barbosa, P.), pp. 155-178. Plenum Press, New York, NY, USA.

ISW–TBE, 2008. Tick borne encephalitis (TBE): health and wellness. International Scientific Working Group on Tick–Borne Encephalitis (ISW–TBE) http://www. virtualvienna.net/main/modules.php?name=News&file=article&sid=116, as retrieved on 24 September 2008.

ITDG and IIRR, 1996. Ethnoveterinary medicine in Kenya: A Field Manual of Traditional Animal Health Care Practices. Intermediate Technology Development Group and International Institute of Rural Reconstruction, Nairobi, Kenya.

Izelle, T., 2002. Zulu traditional healers' brew works. MRC News. Science in Africa. Africa's first on–line science magazine, No. 29, September 2002. http:// www. scienceinafrica.co.za/2002/august/brew.htm, as retrieved on 29 September 2003.

Jacobson, M., 1990. Glossary of Plant Derived Insect Deterrents. CRC Press, Boca Raton, FL, USA.

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

Jaenson, T.G.T, Pålsson, K. and Bong–Karlson, K.A., 2005. Evaluation of extracts and oils of tick–repellent plants from Sweden. Medical and Veterinary Entomology 19: 345–352.

Jaenson, T.G.T., Garboui, S. and Pålsson, K., 2006. Repellency of oils of lemon eucalyptus (*Corymbia citriodora*), geranium (*Pelargonium graveolens*) and lavender (*Lavandula angustifolium*), and the mosquito repellent MyggA Natural® to the common tick *Ixodes ricinus* (L.) (Acari: Ixodidae) in the laboratory and field. Journal of Medical Entomology 43: 731–736.

Jama, B., Palm, C.A., Buresh, R.J.,Niang, A., Gachengo, C., Nziguheba, G. and Amadalo, B., 2000. *Tithonia diversifolia* as a green manure for soil fertility improvement in western Kenya: A review. Agroforestry Systems 49: 201–221.

Janet, P., 1986. Trees, plants and a rural community in the southern Sudan. An international journal of forestry and forest industries. FAO–Food and Agriculture Organization of the United Nations, Unasylva–No. 154–Agroforestry–pathways; Vol. 38, 1986/4. http://www.fao.org/docrep/50630E/50630e00.htm#Contents, as retrieved on 20 September 2003.

Jaussaud, R., Magy, N., Strady, A., Dupond, J.L., Deville, J.F., 2001. Tick–borne encephalitis. La Revue de Medecine Interne 22: 542–548.

Jayasekara, T.K., Belmain, S.R., Stevenson, P.C. and Hall, D.R., 2002. *Securidaca longepedunculata* (Fres.) as a control for stored product insect pests. Proceedings of the 8th International Working Conference on Stored–Product Protection. York, UK, 2002. http://bru.gmprc.ksu.edu/proj/iwcspp/, as retrieved on 4 November 2003.

Jembere, B., Obeng–Ofori, D., Hassanali, A. and Nyamasyo, N.N.G., 1995. Products derived from the leaves of *Ocimum kilimandscharicum* (Labiatae) as post–harvest grain protectants against the infestation of three major stored product insect pests. Bulletin of Entomological Research 85: 361–367.

Jensenius, M., Fournier, P. and Didier Raoult, D., 2004. Rickettsioses and the International Traveler. Clinical Infectious Diseases 39: 1493-1499.

Jimenez-Osornio, J. J., 1991. Ethnoecology of *Chenopodium ambrosoides*. American Journal of Botany 76: 139.

Johns, T., Kokwaro, J.O. and Kimanani, E.K., 1990. Herbal remedies of the Luo of Siaya District, Kenya: establishing quantitative criteria for consensus. Economic Botany 44: 369–381.

Jones, L.D. and Nutall, P.A., 1989. Non–viraemic transmission of *Thogoto virus*: influence of time and distance. Transactions of the Royal Society of Tropical Medicine and Hygiene 83: 712–714.

Jongejan, F., Zivkovic, D., Pegram, R.G., Tatchell, R.J., Fison, T., Latif, A.A. and Paine, G., 1987. Ticks (Acari: Ixodidae) of the Blue and White Nile ecosystems with particular reference to the *Rhipicephalus sanguineus* group. Experimental and Applied Acarology 3: 331–346.

Jongejans, F., 2006. Ticks, Diseases and Climate Change. Coordinator (Co–ordination Action Project): Newsletter on Ticks and Tick–borne Diseases of Livestock in the Tropics, June, 2006, Issue 30. Integrated Consortium on Ticks and Tick–borne Diseases (ICTTD–3): http://www.icttd.nl, as retrieved on 8 October 2008.

Jongejans, F., 2007. The global importance of ticks. Co–ordinator (Co–ordination Action Project): Newsletter on Ticks and Tick–borne Diseases of Livestock in the Tropics, February, 2007, Issue 32. Integrated Consortium on Ticks and Tick–borne Diseases (ICTTD–3): http://www.icttd.nl, as retrieved on 24 November 2008.

Jonsson, N.N., Mayer, D.G., Matschoss, A.L., Green P.E. and Ansell, J., 1998. Production effects of cattle tick (Boophilus microplus) infestation of high yielding dairy cows. Veterinary Parasitology 78: 65–77.

Jordano, D. and Ocana, M., 1955. Catalogo del herbario de los botanicos cordobeses Rafael de Leon y Galvez, Fr. Jose de Jesus Munoz Capilla, Rafael Entrenas, y Antonio Cabrera. Anales del Institute botanico AJ Cavanilles 14: 597–720.

Juarez Lagunes, F.I., Fox, D.G., Blake, R.W., and Pell, A.N., 1999. Evaluation of tropical grasses for milk production by dual-purpose cows in tropical Mexico. Journal of Dairy Science 82: 2136–2145.

Judd, G.J.R. and Gardiner, M.G.T., 2005. Towards eradication of codling moth in British

Columbia by complimentary actions of mating disruption, tree banding and sterile insect technique: five–year study in organic orchards. Crop Protection 24: 718–733.

Juliette de Ba Levy, 1991. The Complete Herbal Handbook for Farm and Stable, 3rd edn. Faber and Faber, London, UK.

Juma, C. 1989. Biological Diversity and Innovation: Conserving and Utilizing Genetic Resources in Kenya. ACTS Press, Nairobi, Kenya.

Kaaya, G.P., 1992. Non–chemical control agents and factors capable of regulating tick population in nature: A mini Review. Journal of Insect Science and its Application 13: 587–594.

Kaaya, P.G., Mwangi, N.E. and Malonza, M.M., 1995. Acaricidal activity of *Margaitaria discoidea* (Euphorbiaceae) plant extracts against the ticks *Rhipicephalus appendiculatus* and *Amblyomma variegatum* (ixodidae). International Journal of Acarology 21: 123–129.

Kaiser, N.M., Sutherst, W.R. and Bourne, S.A., 1982. Relationship between ticks and zebu cattle in southern Uganda. Tropical Animal Health and Production 14: 63–74.

Kambewa, D.M.B., Mfitilodze, W.M., Hüttner, K., Wollny, A.B.C. and Phoya, D.K.R., 1997. The use of indigenous veterinary remedies in Malawi. Ethnoveterinary Medicine: Alternatives for Livestock Development (ed. by Mathias–Mundy E., Rangnekar, V.D.and McCorkle, M.C.). Proceedings of an International Conference held in Pune, India, 4–6 November 1997. Volume 1. Validation of Ethnoveterinary Medicine. BAIF Development Research Foundation, Pune, India.

Kametani, S., Kojima-yuasa, A., Kikuzaki, H., Kennedy, O.D., Honzawa, M. and Matsui-yuasa, I., 2007. Chemical constituents of Cape *Aloe* and their synergistic growth-inhibiting effect on Ehrlich ascites tumor cells. Bioscience, Biotechnolology and Biochemistry 71: 1220–1229.

Kariuki, D.P., 1989. Theileriosis in Kenya. Theileriosis in Eastern, Central and Southern Africa (ed. by Dolan, T.T.). Proceedings of a Workshop on East Coast Fever Immunization held in Lilongwe, Malawi 20–22 September 1988. The International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya. Initiatives Ltd., Nairobi, Kenya.

Kariuki, P.D., 1996. Animal agricultural research and poverty eradication. Proceedings of the National Veterinary Research Centre (NVRC) Scientific Conference, Muguga, held 4–6 December 1996 (ed. by Wanyangu, S.W., Kariuki, D.P., Wamwayi, H.M. and Mbogo, S.K.) Kenya Agricultural Research Instituten (KARI), Rusa Investments Limited, Nairobi, Kenya.

Kasonia, K.M., Ansay, N. Basegere, P. Gustin, S. and Matamba, M., 1991. Note d'ethnopharmacologie vétérinaire en cas de verminoses, diarrhée, coprostase et météorisme au Kivu et Kibali–Ituri (Zaïre). Tropicultura 9: 169–172.

Katz, T.M., Miller, J.H., Hebert, A.A., 2008. Insect repellents: Historical perspectives and new developments. Journal of the American Academy of Dermatology 58: 865–871.

Kayonga, A. and Habiyaremye, F.X., 1987. Médecine traditionnelle et plantes médicinales rwandaises. Contribution aux études ethnobotaniques de la flore rwandaise. The National University of Rwanda, Centre universitaire de recherche sur la pharmacopée et la médecine traditionnelle, CURPHAMETRA, Rwanda.

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

Kendall, B., van Houten, H., 1997. Using the wild sunflower, *Tithonia*, in Kenya for soil fertility and crop yield improvement. International Centre for Research in Agroforestry (ICRAF). Signal Press Limited, Nairobi, Kenya.

Kennewell, P. D., 1990. Comprehensive Medicinal Chemistry. Pergamon Press, Oxford, U.K.

Kenya National Archives (KNA), 1954–1956. File No. DC/EN/3/3/2: Annual Abaluyia Land and Law Customs, The Republic of Kenya, Nairobi, Kenya.

Khan, Z.R., Pickett, J.A., Wadhams, L. and Muyekho, F., 2001. Habitat management strategies for the control of cereal stemborers and striga in maize in Kenya. Insect Science and its Application 21: 381–388.

Killip, E.P. and A.C. Smith, C.A., 1935. Some American plants used as fish poisons. Washington Bureau of Plant Industry and U.S. Department of Agriculture, New York Botanical Garden, New York, NY, USA.

Knight, A.L. and Norton, G.W., 1989. Economics of agricultural pesticide resistance In arthropods. Annual Review of Entomology 34: 293–313.

Kofi–Tsekpo, W.M. and Kioy, D.W., 1998. Newer direction of plant drug research: The need to examine and investigate traditional remedies for pharmacotherapy of animal and zoonotic diseases. African Journal of Health Sciences 5: 12–14.

Kokalis-Burelle, N. and Rodriguez-Kabana, R., 2006. Allelochemicals as biopesticides for management of plant-parasitic nematodes. Allelochemicals: Biological Control of Plant Pathogens and Diseases. Book Series: Disease Management of Fruits and Vegetables. Vol. 2., pp. 15-29. http://www.springerlink.com/content/l3m74x76n742105v/fulltext.pdf, as retrieved on 25 September 2008.

Kokwaro, J.O., 1993. Medicinal Plants of East Africa, 2nd edn. East African Literature Bureau, General Printers Limited, Kampala, Nairobi, Dar Es Salaam, Tanzania.

Kokwaro, J.O., 1994. Flowering Plant Families of East Africa. An Introduction to Plant Taxonomy. East African Educational Publishers, Nairobi, Kenya.

Konnai, S., Imamura, S., Nakajima, C., Witola, H.W., Yamada, S., Simuunza, M., Nambota, A., Yasuda, J., Ohashi, K. and Onuma, M., 2007b. Acquisition and transmission of *Theileria parva* by vector tick, *Rhipicephalus appendiculatus*. Acta Tropica 99: 34–41.

Konnai, S., Yamada, S., Imamura, S., Simuunza, M., Chembensof, M., Chota, A., Nambota, A., Ohashi, K. and Onuma, M., 2007a. Attachment duration required for *Rhipicephalus appendiculatus* to transmit *Theileria parva* to the host. Vector–Borne and Zoonotic Diseases 7: 241–248.

Korolkovas, A. and Burckhalter, J.H., 1976. Essentials of Medicinal Plant Chemistry. John Wiley, New York, NY, USA.

Koschier, H.E and Sedy, A.K., 2003. Labiate essential oils affecting host selection and acceptance of *Thrips tabaci* lindeman. Crop Protection 22: 929–934.

Koumaglou, K.H., Akagana, K., Glitho, A.I., Garneau, F.X., Gagnon, H., Jean, F.I, Moudachirou, M. and Addae–Mensah, I., 1996a. Geranial and neral. Major constituents of *Lippia multoflora* Moldenke leaf oil. Journal of Essential Oil Research 8: 237–240.

Koumaglou, H.K., Dotse, K., Glotho, A.I., Garneau, F.X., Moudachirou, M. and Addae–Mensah, I., 1996b. Essential oils of *Cympogon shoenanthus* and *Lippia multiflora* from Togo. Rivista Italiana Eppos 7: 680–691.

Kristensen, M. and Lykke, M.A., 2003. Informant–Based Valuation of Use and Conservation Preferences of Savanna Trees in Burkina Faso. Economic Botany 57: 203–217.

Kroschel, J., 1996. Studies on the use of chemicals, botanicals and *Bacillus thuringiensis* in the management of the potato tuber moth in potato stores. Crop Protection 15: 197–203.

Krueger, R.A., 1988. Focus groups: a practical guide for applied research. Focus Groups and Ethnography (ed. by Agar, M. and MacDonald, J.). Human Organization 54: 78–86.

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

Kumar, A., 2003. Ayurvedic Medicines: Some Potential Plants for Medicine from India. Energy Plantation Demonstration project and Biotechnology Center, Department of Botany, University of Rajasthan, Jaipur, India.

Kunle, O., Okogun, J., Egamana, E., Emojevwe, E. and Shok, M., 2003. Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* leaf extract. Phytomedicine 10: 59–61.

Kuo, Y.H. and Chen C.H., 1997. Diversifolol, a novel rearranged eudesmane sesquiterpene from the leaves of *Tithonia diversifolia*. Chemical and Pharmaceutical Bulletin 45: 1223–1224.

Kuroda, M., Yokosuka, A., Kobayashi, R., Jitsuno, M., Kando, H., Nosaka, K., Ishii, H., Yamori, T. and Mimaki, Y., 2007. Sesquiterpenoids and flavonoids from the aerial parts of *Tithonia diversifolia* and their cytotoxic activity. Chemical and Pharmaceutical Bulletin 55: 1240–1244.

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

Labuda, M, Trimnell, A.R., Ličková, M., Kazimírová, M., Davies, G.M., Lissina, O., Hails, S.R. and Nuttall, A.P., 2006. An anti-vector vaccine protects against a lethal vector-borne pathogen. PLoS Pathogens 2(4): e27 doi:10.1371/ journal. ppat.0020027.

Lamaty, G., Menut, C., Zollo, P. H.A., Kuiate, J.R., Bessiere, J.M., Kodou, J., 1991. Aromatic plants of tropical central Africa. III. Constituents of the essential oil of the leaves of *Tithonia diversifolia* (Hemsl) Gray from Cameroon. Journal of Essential Oil Research 3: 399–402.

Lans, C, 2001. Creole remedies–case studies of ethnoveterinary medicine in Trinidad and Tobago. PhD thesis, Wageningen University and Research Centre, Wageningen, The Netherlands.

Lans, C. and Brown, G., 1998. Observations on ethnoveterinary medicines in Trinidad and Tobago. Preventive Veterinary Medicine 35: 125–142.

Lans, C., Turner, N., Brauer, G., Lourenco, G., Georges, K., 2006. Ethnoveterinary medicines used for horses in Trinidad and in British Columbia, Canada. Journal of Ethnobology and Ethnomedicine 2: 31.

Lans, C., Turner, N., Khan, T., Brauer, G. and Boepple, W., 2007. Ethnoveterinary medicines used for ruminants in British Columbia,Canada. Journal of Ethnobology and Ethnomedicine 3: 11.

Larisa, V., 2001. Background information on the biology of ticks. Department of Entomology, University of California, Davis, USA. http://entomology.ucdavis.edu/ faculty/rbkimsey/tickbio.html, as retrieved on 18 July 2001.

Latif, A.A. and Pegram, R.G., 1992. Naturally acquired host–resistance in tick control in Africa. Insect Science and its Application 13: 505–513.

Latif, A.A., 1992. Sustainable control methods for ticks and tick–home diseases in Africa.

Future of livestock industries in East and southern Africa (ed. by Kategile, J.A. and Mubi, S.). Proceedings of a workshop held at Kadoma Ranch Hotel, Zimbabwe, 20–23 July 1992. International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia.

Latif, A.A., Punyua, D.K, Nokoe, S. and Capstick, P.B., 1991. Tick infestations on Zebu cattle in Western Kenya: individual host variation. Journal of Medical Entomology 28: 114–121.

Lawrence, B.M. and Reynolds, R., 1992. Progress in essential oils. Perfumery and Flavourist 17: 131–133.

Lawrence, B.M., 1985a. A review of the world production of essential oils. Perfumery and Flavourist 10: 1–16.

Lawrence, B.M., 1985b. Essential oils of the *Tagetes* genus. Perfumery and Flavourist 10: 73–82.

Lawrence, B.M., 1993. A planning scheme to evaluate new aromatic plants for the flavour and fragrance industries. New crops (ed. by Janick, J. and Simon, J.E.), pp. 620-627. John Wiley, New York, NY, USA.

Lawrence, B.M., 2000. Progress in essential oils, *Tagetes* oils. Perfumery and Flavourist 25: 38–42.

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

Lawrence, J.A., Foggin, C.M. and Norval, R.A.I., 1980. The effects of war on the control of diseases of livestock in Rhodesia (Zimbabwe). Veterinary Record 107: 82–85.

Le Houérou, H., 2003, FAO Species Description (*Acacia macrothyrsa* Harms.). Grassland and pasture crops. http://www.fao.org/ag/AGP/AGPC/doc/Gbase/DATA / Pf000534.HTM, as retrieved on 12 October 2003.

Le Strange, R., 1977. A History of Herbal Plants, 1st edn. Angus and Robertson Publishers, London, UK.

Lees, A. D. and Milne, A., 1951. The seasonal and diurnal activities of individual sheep ticks (*Ixodes ricinus* L.). Parasitology 41: 189–208.

Lemmens, G.J. and Fryer, P.J., 1917. Insecticides, sheep–dip, animal–wash and the like. United States patent. 1, 242, 954 and 1,242,955. Patented 16 October 1917.

Lemordant, D., 1971. Contribution à l'ethnobotanique éthiopienne. Journal d'Agriculture Tropicale et Botanique Appliquee 18: 142–179.

Lemos, A.M., Teodoro, R.L., Oliveira, G.P. and Madalena, F.E., 1985. Comparative performance of six Holstein–friesian x Guzera grade in Brasil. Burdens of *Boophilus microplus* under field condition. Animal Production 41: 187–191.

Lersten, N.R., 1983. Crystals of calcium compounds in Gramineae. New Phytologist 93: 633–638.

Leung, A.Y., 1980. Encyclopedia of Common Natural Ingredients. John Wiley, New York, NY, USA.

Lewis, A.R., 1981. The pathology of *Rhipicephalus appendiculatus* infestation of eland *Taurotragus oryx*. Proceedings of an International Conference on Tick biology and control held 27–29 January, 1981 (ed. by Whitehead, G.B. and Gibson, J.D.), pp. 15–20. Tick Research Unit, Rhodes University, Grahamstown, South Africa.

Lewis, M.C., Silva, M., Sanborn, J., Pfeifer, K., Schreider, P.J. and Helliker, E.P., 2000. N,N–diethyl–meta–toluamide (DEET): Risk characterization document. Medical Toxicology and Worker Health and Safety Branches: California Environmental Protection Agency, Pesticide Registration Branch, Sacramento, CA , USA. http://

www.cdpr.ca.gov/docs/ risk/rcd/ deet.pdf, as retrieved on 21 September 2005.

Lewis, R., 2001. The Institute for Genomic Research (TIGR) Tackles East Coast Fever. Sequencing *Theileria parva* may aid vaccination efforts. The Scientist 15: 16, 8 January 2001. http://www.the-scientist.com/yr2001/jan/research010108.html, as retrieved on 21 June 2001.

Li, J., Perdue, E.M., Pavlostathis, S.G. and Araujo, R., 1998. Physicochemical properties of selected monoterpenes. Environment International 24: 353–358.

Lightfoot, C.J. and Norval, R.A.I., 1981. Tick problems in wildlife in Zimbabwe. 1. The effects of tick parasitism on wild ungulates. South African Journal of Wildlife Research 11: 41–45.

Lin, L.F., 1984. Extraction of leaf protein concentrate from sisal (*Agave sisalana*). Journal of Agricultural Research of China 33: 159–168.

Lindsay, P.J. and Kaufman, W.R., 1988. The efficacy of azadirachtin on putative ecdysteroid–sensitive systems in the ixodid tick *Amblyomma americanun* L. Journal of Insect Physiology 34: 439–442.

Lindsay, R.S. and Hepper, F.N., 1978. Medicinal plants of Marakwet, Kenya. Kew, Royal Botanic Gardens, Richmond, UK.

Lister, P., 2002. Traditional Indigenous Knowledge. http://sites.uws.edu.au/vip/listerp / tek.htm, as retrieved on 1 October 2003.

Lodos, J., Boue, O., de la Fuente, J.A., 2000. Model to simulate the effect of vaccination against *Boophilus* ticks on cattle. Veterinary Parasitology 87: 315–326.

Loewe, H. 1974. Recent advances in the medicinal chemistry of anthelminthics. Medicinal Chemistry IV (ed. by Maas, J.), pp. 271-301. Elsevier, Amsterdam, The Netherlands.

Logan, J.G., Birkett, M.A., Clark, S.J., Powers, S., Seal, N.J., Wadhams, L.J., Mordue Luntz, A.J. and Pickett, J.A., 2008. Identification of human–derived volatile chemicals that interfere with attraction of *Aedes aegypti* mosquitoes. Journal of chemical Ecology 34: 308–322.

Londt, J.G.H., Horak, I.G. and De Villiers, I.L., 1979. Parasites of domestic and wild animals in SouthAfrica. XIII. The seasonal incidence of adult ticks (Acarina: Ixodidae) on cattle in the Northern Transvaal. Onderstepoort Journal of Veterinary Research 46: 31–39.

Lukwa, N., Mutambu, S.L., Makaza, N., Molgaard, P. and Furu, P., 2001. Perceptions about malaria transmission and control using anti–malaria plants in Mola, Kariba, Zimbabwe. African Journals Online. Nigerian Journal of Natural Products and Medicine. Volume 5, November 2001. http://www.inasp.info/ajol/journals/ njnpm /vol5abs.html, as retrieved on 27 October 2003.

Lwande, W., Ndakala, J.A., Hassanali, A., Moreka, L., Nyandat, E., Ndung'u, M., Amiani, H., Gitu, P.M., Malonza, M.M. and Punyua, K.D., 1999. *Gynandropsis gynandra* (L) Brig. essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry. 50: 401–405.

Machado, M.I.L., Silva, M.G.V., Matos, F.J.A., Craveiro, A.A. and Alencar, J.W., 1994. The presence of indole as a minor constituent of *Tagetes erecta* leaf oil. Journal of Essential oil Research 6: 203–205.

Madder, M., Speybroeck, N., Brandt, J., Tiny, L., llodek, I. and Berkvens, D., 2002. Geographic variation in diapause response in the African brown ear tick *Rhipicephalus appendiculatus* (Acari: Ixodidae). Experimental and Applied Acarology 27: 209–221.

Mahgoub, S.M. and Ahmed, S.M., 1996. *Ricinus communis* seed extract as protectants of wheat grains against the rice weevil Sitophilus oryzae L. Annals of Agricultural Science 41: 483–491.

Makala, H.L., Mangani, P., Fujisaki, K. and Nagasawa, H., 2003. The current status of major tick borne diseases in Zambia. Veterinary Research 34: 27–45.

Makila, F.E., 1978. An Outline History of Babukusu of Western Kenya. Kenya Literature Bureau, Nairobi, Kenya.

Malerich, P.J. and Trauner, D., 2003. Biomimetic synthesis of (+/-)-pinnatal and (+/-)-sterekunthal A. Journal of American Chemical Society 125: 9554–9555.

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

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

Mann, O. 2001. Medicinal Trees of Bukusuland. Council for Human Ecology, Kenya (CHECK). 1st edn. Annex Import, Nairobi, Kenya.

Mansingh, A. and Williams, L.A.D., 1998. Pesticidal Potential of Tropical Plants-II. Acaricidal Activity of Crude Extracts of Several Jamaican Plants. Insect Science and Applications 18: 149–155.

Mansingh, A. and Williams, L.A.D., 2002. Pesticidal Potential of Tropical Plants–VIII. Acute toxicity and inhibition of oogenesis in the ticks *Boophilus microplus* Canest. and *Amblyomma cajennense* Fab. by the extracts of *Azadirachta indica* A. Juss and *Artocarpus altilis* Park. Proceedings of the First International Symposium on "Utilization of Natural Products in Developing Countries: Trends and Needs" (ed. by Mansingh, A., Young, R., Yee, T., Delgoda, R., Robinson, E.D et al.) held 10–14 July 2000 at the University of the West Indies, Kingston, Jamaica.

Manson–Bahr, P.E.C. and Apted, F.I.C., 1982. Manson's Tropical Diseases, 18th edn. Baillière Tindall, London, UK.

Maradufu, A., 1981. Furanosesquiterpenoids of *Commiphora erythraea* and *C. myrrh*. Phytochemistry 21: 677–680.

Maradufu, A., Lubega, R. and Dorn, F., 1978. Isolation of (5E)-ocimenone, a mosquito larvicide from *Tagetes minuta*. Llyodia 41: 181–182.

Maranga, R.O., Hassanali, A., Kaaya, G.P. and Mueke J.M., 2003. Attraction of *Amblyomma variegatum* (ticks) to the attraction–aggregation–attachment–pheromone with or without carbon dioxide. Experimental and Applied Acarology 29: 121–130.

Marin, R., 1999. Collection of Medicinal Plant Samples from Africa and Elsewhere. World Botanical Associates. http://www.worldbotanical.com/WBAMED2.HTM, as retrieved on 1 October 2003.

Marina, M., Mathias, E. and McCorkle, M.C., 2001. Ethnoveterinary Medicine: An Annotated Bibliography of Community Animal Healthcare, 2nd edn. Practical Action, ITDG Publishing, London, UK.

Martin, H.M., Barnett, S.F. and Vidler, O.B., 1964. Cyclic Development and Longevity of *Theileria parva* in the Tick *Rhipicephalus appendiculatus*. Experimental and Applied Acarology 29: 355–365.

Martin, J.G., 1996. Ethnobotany: Conservation Manual. A 'People and Plants', 1st edn. Springer; London, UK.

Martin, J.G., 2004. Ethnobotany: A Methods Manual. A 'People and Plants', 2nd edn. Earthscan Publications Ltd, London, UK.

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

Martina, M.B., 1998. Indigenous Trees in Bukusuland. A Selection of Useful Trees and Shrubs for Social Forestry in Bungoma. Promotion for Sustainable Forest Management Project, Gesellschaft für Technische Zusammenarbeit (GTZ) and German Development Service (DED), Nairobi, Kenya. Excecutive Printing Works, Nairobi, Kenya.

Martina, M.B., 2001. The role of indigenous trees for the conservation of biocultural diversity in traditional agroforestry land use systems: The Bungoma case study: *In–situ* conservation of indigenous tree species. Agroforestry Systems (in cooperation with ICRAF) 52: 119–132.

Masinde, P.S., 1996. Medicinal plants of the Marachi people of Kenya. The Biodiversity of African Plants (ed. by van der Maesen, J.G., van der Burgt, X.M. and van Medenbach de Rooy, J.M.). Proceedings of the 14th AETFAT Congress. Wageningen, The Netherlands, 22–27 August 1994. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Mathewson, M.D., 1984. The future tick control: A review of the chemical and non-chemical options. Impact of disease on livestock production in the tropics (Developments in Animal and Veterinary Sciences) (ed. by Riemann, H.P. and Burridge, M.P.), pp. 559-568. Elsevier, Amsterdam, The Netherlands.

Mathewson, M.D., Hughes, G., Macpherson, S.I. and Bernard, P.C., 1981. Screening techniques for the evaluation of chemicals with activity as tick repellents. Pesticide Science 12: 455–462.

Mathias, E. and McCorkle, M.C., 2004. Traditional livestock healers. Scientific and Technical Review of the Office International des Epizooties 23: 277–284.

Mathias, E., 2004. Ethnoveterinary medicine: harnessing its potential. Veterinary Bulletin 74: 27N–37N.

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

Mathias–Mundy, E. and McCorckle, M.C., 1989. Ethnoveterinary Medicine: An Annotated Bibliography. Bibliographies in Technology and Social Change, No. 6, Technology and Social Change Programme (ed. by Mathias–Mundy, E. and McCorckle, M.C.) IOWA State University, Ames, IA, USA.

Matson, B.A., 1967. Theileriosis in Rhodesia: 1. A study of diagnostic specimens over two seasons. Journal of the South African Veterinary Medical Association 38: 93–101.

Matthysse, J.G. and Colbo, M.H., 1987. The ixodid ticks of Uganda. Entomological Society of America, College Park, MD, USA.

Matzigkeit, U., 1990. Natural Veterinary Medicine. Ectoparasites in the Tropics. Weikersheim, Josef Margraf Verlag, Weikersheim., Germany.

Maundu, P., Berger, D., Ole Saitabau, J., Nasieku, C., Kipelian, J., Mathenge, M., Morimoto, S.G. and Hoft, Y., 2001. Ethnobotany of the Loita Maasai: Towards Community Management of the forest of the Lost Child–Experiences from the Loita Ethnobotany Project. People and Plants working paper 8. UNESCO, Paris. http://www.rbgkew.org.uk/peopleplants/wp/wp8/index-html, as retrieved on 28 September 2003.

Mbogo, K.S., Kariuki, P.D., McHardy, N. and Payne, R., 1995. Training Manual for Veterinary staff on Immunization against East Coast fever using the ECFiM System.

Kenya Agricultural Research Institute and Overseas Development Administration of UK.

McCorkle, C.M. and Bazalar, H., 1996. Field trials in ethno veterinary R and D: lessons from the Andes. Ethnoveterinary research and development (ed. by McCorkle, M.C., Mathias, E. and T.W Schillhorn van Veen, W.T.), pp. 265–282. Intermediate Technology Publications, London, UK.

McCorkle, M.C., Rangnekar, V.D. and Mathias–Mundy, E., 1997. Introduction: Whence and Whither ER&D? Ethnoveterinary Medicine: Alternatives for Livestock Development (ed. by Mathias, E., Rangnekar, V.D., McCorkle, M.C., Martin, M.). Proceedings of an International Conference held in Pune, India, 4 –6 November 1997. Volume 1: Applied Studies of Ethnoveterinary Systems. BAIF Development Research Foundation, Pune, India.

McCosker, J.P., 1993. Ticks in a changing world. Infectious and Parasitic Diseases Group, Animal Production and Health Division, FAO, Rome, Italy.

McDowell, P.G. and Waladde, S.M., 1985. 2,6–dichlorophenol in the tick, *Rhipicephalus appendiculatus* Neumann, a reappraisal. Journal of Chemical Ecology 12: 69–81.

McIntyre, A., 2002. Ashwagandha: winter cherry–*Withania somnifera*. http://www. positivehealth.com/permit/Articles/Regular/mcintyre77.htm, as retrieved on 21 October 2003.

McKeever, D.J., 2001. Cellular immunity against *Theileria parva* and its influence on parasite diversity. Research in Veterinary Science 70: 77–81.

McLeod, J., 1935. *Ixodes ricinus* in relation to its physical environment. I. The influence of climate on development. Parasitology 25: 123–144.

McMahon, C., Kröber, T. and Guerin, M.P., 2003. *In vitro* assays for repellents and deterrents for ticks: differing effects of products when tested with attractant or arrestment stimuli. Medical and Veterinary Entomology 17: 370–378.

McVaugh, R., 1943. Botanical collections of the La Plata expedition of 1853-1855. Brittonia 5: 64–79.

Menendenz, R., 1924. El *Melinitus minutifloraay* la garrapata. Revista de Agricultura de Puerto Rico 4: 219–223.

Metcalf, R.L. and Metcalf, R.A., 1982. Attractants, repellents, and genetic control in pest management. Introduction to insect pest management (ed. by Metcalf, R. L. and Luckmann, W.H.), pp. 299–303. Wiley Interscience, New York, NY, USA.

Michael, H.M., 2003. Medicinal Uses of Succulents. http://www.succulents.co.za / Contact.shtml, as retrieved on 8 November 2003.

Miller, R., 2004. Integrated pest management of cattle ticks (*Boophilus microplus* and *B. annulatus*). Proceedings of the Third International Seminar of Animal Reproduction and the Production of Milk and Meat, 26–27 February, 2004, Mexico City, Mexico.

Miller, R.J. and Cowles, S.R., 1990. Stimulo-deterrent diversion: A concept and its possible application to onion maggot control. Journal of Chemical Ecology 16: 3197–3212.

Miller, R.J., Byford, R.L., Smith, G.S., Craig, M.E. and Vanleeuwen, D., 1995. Influence of snakeweed foliage on engorgement, fecundity and attachment of the lone star tick (Acari: Ixodidae). Journal of Agricultural Entomology 12: 137–143.

Minja, M.M.J., 1989. Collection of Tanzanian medicinal plants for biological activity studies. Proceedings of the 7th Tanzania veterinary association scientific conference. Tanzania Veterinary Association 7: 67–78.

Minja, M.M.J., 1994. Medicinal plants used in promotion of animal health in Tanzania.

Animal Diseases Research Institute, Department of Pharmacology and Toxicology, Dar Es Salaam, Tanzania. Métissages en santé animale de Madagascar à Haïti, pp. 335–364. Presses Universitaires de Namur, Belgium.

Minshull, J.I. and Norval, R.A.I., 1982. Factors influencing the distribution of *Rhipicephalus appendiculatus* in Kyle Recreational Park, Zimbabwe. South African Journal of Wildlife Research 12: 118–123.

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

Mitchell, S.M. and Ahmad, M.H., 2006. A Review of Medicinal Plant Research at at the University of the West Indies, Jamaica, 1948–2001. West Indian Medical Journal 55: 243–269.

Mizrahi, Y., Nerd, A. and Sitrit, Y., 2002. New fruits for arid climates. Trends in New Crops and New Uses (ed. by Janick, J. and Whipkey, A.) ASHS Press, Alexandria, VA., pp. 378–384.

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

Moghaddam, M., Omidbiagi, R. and Sefidkon, F., 2007. Chemical composition of the essential oil of *Tagetes minuta* L. Journal of Essential oil Research 19: 3–4.

Mohamed, M.A.H., Harris, P.J.C., Henderson, J. and Senatore, F., 2002. Effect of drought stress on the yield and composition of volatile oils of drought-tolerant and non-drought-tolerant clones of *Tagetes minuta*. Planta Medica 68: 472–474.

Mondy, N., Pracros, P., Fermaud, M. and Corio–Costet M.F., 1998. Olfactory and gustatory behaviour by larvae of *Lobesia botrana* in response to *Botrytis cinerea*. Entomologia Experimentalis et Applicata 88: 1–7.

Mooring, M.S. and Hart, B.L., 1995. Differential grooming rate and tick load of territorial male and female impala, *Aepyceres melampus*. Behavioural Ecology 6: 94–101.

Mooring, M.S., 1995. The effect of tick challenge on grooming rate by impala. Animal Behaviour 50: 377–392.

Mordue, B. and Mordue, J., 2003. Biting midge chemical ecology. Biologist 50: 159–162.

Morgan, D.L., 1988. Focus groups as qualitative research. Focus groups and ethnography (ed. by Agar, M. and MacDonald, J.) Human Organization 54: 78–86.

Moronkola, O.D., Ogunwande, A.I., Walker, M.T., Setzer, N.W., and Oyewole, O.I., 2007. Identification of the main volatile compounds in the leaf and flower of *Tithonia diversifolia* (Hemsl) Gray. Journal of Natural Medicines 61: 63–66.

Morton, J., 1987. Cape Gooseberry. Fruits of warm climates (ed. by Morton, J.F. and Miami, F.L.). http://www.hort.purdue.edu/newcrop/morton/cape_gooseberry. html, as retrieved on 3 November 2003.

Morton, J.F., 1977. Poisonous and injurious higher plants and fungi. Forensic Medicine, vol 3 (ed. by Tedeschi, C.G.), 1456-1567. Saunders, Philadelphia, PA, USA.

Morton, J.F., 1981. Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan. Charles C Thomas Publishers, Springfield, IL, USA.

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

Moyo, B. and Masika, P.J., 2008. Tick control methods used by resource–limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. Tropical Animal Health and Production, 10.1007/s11250–008–9216–4.

Mozuraitis R, Stranden M, Ramirez MI, Borg-Karlson A.K, Mustaparta H., 2002. (−)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. Chemical Senses 27: 505–509.

MTDP, 2003. Molecular Targets Development Program (MTDP). Center for Cancer Research at the National Cancer Institute. http://home.ncifcrf. gov/mtdp/index.html, as retrieved on 20 October 2003.

Muchenje, V., Dzama, K., Chimonyo, M., Raats, J.G. and Strydom, P.E., 2008. Tick susceptibility and its effects on growth performance and carcass characteristics of Nguni, Bonsmara, and Angus steers raised on natural pasture. Animal 2: 298–304.

Muhammad, I., Mossa, J.S., Mirza, H.H. and El–Feraly, F.S., 1999. A new modified 6,7-secolabdane diterpenoid from *Clutia richardiana* L. Phytochemistry 50: 1225–1227.

Mukabana, W.R., 2002. Differential attractiveness of humans to the African malaria vector *Anopheles gambiae* Giles, Effects of host characteristics and parasite infection. PhD Thesis, Wageningen University and Research Centre, Wageningen, The Netherlands.

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

Mukhebi, A.W., Curry, J., Perry, D.B., Reid, S.R., Thorpe, W., Laker, C.R., Onchoke, D., Hassan, Z., Kariuki, D.P., Munyombwe, T. and Ushewokunze–Obatolu, U., 1993. Economic impact of theileriosis and its control in Africa. ILRI links: Biosciences Programme: Epidemiology and disease control (project 7). http://www.cgiar.org/ilri/research/impacts/imtrh2.cfm, as retrieved on 3 July 2001.

Mukhebi, A.W., Perry, B.D. and Kruska, R., 1991. Estimated economics of theileriosis control in Africa. Preventive Veterinary Medicine 12: 73–85.

Mulholland, D.A Monkhe, T.V, Coombes, P.H. and Rajab, M., 1998. Limonoids from *Turraea holstii* and *Turraea floribunda*. Phytochemistry 49: 2585–2590.

Mulla, M.S. and Su, T., 1999. Activity of biological effects of neem products against arthropods of medical and veterinary importance. Journal of American Mosquito Control Association 15: 133–152.

Munyua, M.J.S., Mbai, M.K., Kariuki, I.D. and Chibeu, M.D., 1998. Indigenous knowledge and the use of medicinal plants in small ruminant theriogenology in arid and semi-arid areas of Kenya. Working Paper Series No. 17. Pastoral Information Network Programme, Department of Range Management, University of Nairobi, Kenya.

Mutangah, J.G., 1996. Forest quality indicators with reference to Kakamega forest, Kenya. Plant Conservation Programme, National Museums of Kenya. http://www. earthwatch.org/europe/limbe/upf.html#top, as retrieved on 18 October 2003.

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

Mwambi, H., Baumgärtner, J. and Hadeler, K., 2000. Ticks and tick–borne diseases: a vector–host interaction model for the brown ear tick (*Rhipicephalus appendiculatus*). Statistical Methods in Medical Research 9: 279–301.

Mwangi, E.N., 1996. The role of women in animal husbandry: The Kenyan perspective. Livestock production and diseases in the tropics: Livestock production and human welfare (ed. by Zessin, H.K.). Proceedings of the VIII International Conference of the Institutions of Tropical Veterinary Medicine held from 25-29 September 1995. Berlin, Germany. Volume II. Deutsche stiftung für Internationale Entwicklung, Zentralstelle für Ernährung und Landwirtschaft, Feldafing, Germany.

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

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

Mwangi, E.N., Sayer, D.P., Njanja, C.J. and Bell, F.J, 1985. tick survey on goats and sheep in Kenya. Tropical Animal Health and Production 17: 102–106.

Mwangi, J., Thoithi, G.N., Addae–Mensah, I., Achenbach, H., Lwande, W. and Hassanali, A., 1998. Aromatic plants of Kenya III: Volatile and some non–volatile constituents of *Croton sylvaticus* Hochst. East and Central African Journal of Pharmaceutical Sciences 1: 41–43.

Mwangi, J.W., Addae–Mensah, I., Lwande, L.W. and Munavu, R.M., 1991b. Essential oils of two *Lippia ukambensis* (Vatke) Chemophytes and *Lippia somalensis* (Vatke) in Kenya. Journal of Essential Oil Research 3: 413–417.

Mwangi, J.W., Addae–Mensah, I., Munavu, R.M. and Lwande, L., 1995. The potential for commercialization of three African *Lippia* species as sources of essential oil for perfumery and medicinal purposes. Volarisation de la Biomasse Vegetale Par les Produits Naturels (Actes du Colloque de Chicoutimi, 22-25 Aout 1993, Université du Quebec). Centre de Recherches Pour le Development International (CRDI/ IDRC), pp. 205–216. University of Quebec, Chicoutimi, Canada.

Mwangi, J.W., Addae–Mensah, I., Muriuki, G., Lwande, W. and Munavu, R.M., 1991a. Essenitial oils of Kenya *Lippia* species III. Flavour and Fragrance Journal 6: 221–224.

Mwangi, J.W., Muriuki, G., Addae–Mensah, I., Munavu, R.M., Lwande, W., Craviero, A.A. and Alencar, J.W., 1989. Essential oils of *Lippia wilmsii*. Ev. Latinoamer Ouim 20: 143–144.

Mwangi. J., Njonge, E.W., Addae–Mensah, I., Munavu, R.M. and Lwande, W., 1994. Antimicrobial activity of the Essential from *Lippia* species in Kenya. Discovery and Innovation 6: 58–60.

Mweseli, M., 2004. Art, Wit and Wisdom of African Proverbs–Selected 'Bukusu' Proverbs from Kenya, their translation into Swahili and English and their Meanings. International Society for Oral Literature in Africa (ISOLA) Fifth Conference of ISOLA, 15–17 July 2004, University of the Gambia, Banjul., Gambia.

Nagarajah, S. and Nizar, B.M., 1982. Wild sunflower as a green manure for rice in the mid -country wet zone. Tropical Agriculturist 138: 69–80.

Nangendo, S.M., 1994. Daughters of the Clay, Women of the Farm: Women, Agricultural Economic Development, and Ceramic Production in Bungoma District, Western Province, Kenya. Ph.D. Dissertation, Department of Anthropology, Bryn Mawr College, Bryn Mawr, PA, USA.

Nardo, E.A.B, Costa, A.S. and Lourencao, L.A., 1997. *Melia azedarach* extract as an antifeedant to *Bemisia tabaci* (Homoptera: *Aleyrodidae*). Florida Entomologist 80: 92–94.

Nasimiyu, R., 1985. The participation of women in Bukusu economy: the situation as at the end of nineteenth century. History and Culture in Western Kenya: The People of Bungoma District Through Time (ed. by Wandibba, S.), pp. 51–64. Gideon S. Were Press, Nairobi, Kenya.

Nchu, F, Magano, S.R, Eloff, J.N., 2005. *In vitro* investigation of the toxic effects of extracts of *Allium sativum* bulbs on adults of *Hyalomma marginatum rufipes* and *Rhipicephalus pulchellus*. Journal of the South African Veterinary Association-Tydskrif van die Suid–Afrikaanse Veterinere Vereniging 76: 99–103.

Nchu, F., 2005. Developing methods for the screening of ethnoveterinary plants for tick control. MSc. Thesis, Department of Biology, Faculty of sciences, University of Limpopo, South Africa.

Nchu, F., Magano, S R and Eloff, J.N., 2004. *In vitro* investigation of the repellent effects of essential oils of *Tagetes minuta* and *Lippia javanica* on *Hyalomma marginatum rufipes* adults. 33rd Annual Congress of the Parasitological Society of Southern Africa (PARSA), 12–15 September 2004, Manyane, Pilansberg National Park, South Africa.

Ndumu, P.A., George, J.B.D. and Choudhury, M.K., 1999. Toxicity of neem seed oil (*Azadiracta indica*) against the larvae of *Amblyomma variegatum* a three–host tick in cattle. Phytotherapy Research 13: 532–534.

Ndung'u, M.W., Chhabra, C.S. and Lwande, W., 1999. *Cleome hirta* essential oil as livestock tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellent. Fitoterapia 70: 514–516.

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

Neher, R. T., 1968. The ethnobotany of *Tagetes*. Economic Botany 22: 317–325.

Neitz, W.O., 1955. Corridor disease: A fatal form of bovine theileriosis encountered in Zululand. Bulletin of Epizootic Diseases of Africa 3: 121–123

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

Nentwig, G., Boeckh, J., Hoever, F.P., Krüger, B.W. and Röder, K., 2002. Bayrepel® (KBR 3023), a new mosquito repellent: from laboratory synthesis to a worldwide commercial product. Proceedings II European Conference on Travel Medicine, Florence, Italy.

Neupane, F.P., 1992. Insect pests associated with some fuelwood and multipurpose tree species in Nepal. Journal of Tropical Forest Science 5: 1–7.

New Agriculturist, 2003. Reporting Agriculture for the 21st Century. From maths matrix game to management tools. www.new-agri.co.uk/00-3/develop/dev04.html, as retrieved on 10 October 2003.

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

Ngan, V., 2008. Insect repellents: DermNet NZ. New Zealand Dermatological Society Incorporated (NZDS), the DermNet Development Group. http://dermnetnz.org/treatments/insect-repellents.html, as retrieved on 16 October 2008.

Niang, A., 1987. Contribution à l'étude de la Pharmacopée traditionnelle mauritanienne. Thése pour le doctorat en médecine vétérináire. Ecole Nationale de Médecine vétérináire, SIDI THABET, Tunisie, Tunisia.

Nixalite of America, 2005. Flea and Tick Granular Repellent: Safe, all natural, biodegradable. ABC/Nixalite, East Moline, IL, USA. http://www.nixalite.com /fleaand tick-repellent.aspx, as retrieved on 28 April 2006.

Njoroge, N.G. and Bussmann, W.R., 2006. Herbal usage and informant consensus in ethnoveterinary management of cattle diseases among the Kikuyus (Central Kenya).

Journal of Ethnopharmacology 108: 332–339.

Nobel, P.S. and Berry, W.L., 1985. Element responses of *Agave*. American Journal of Botany 72: 686–694.

Nokoe, S., 1992. Modelling for the management and control of ticks: special considerations and ICIPE's approach. Insect Science and its Application 13: 545–549.

Nokoe, S., Meena, H., Munyinyi, D., Punyua, D., Latif, A.A., and Okello, O.O., 1992. A computer simulated model for the biology and control of *Rhipicephalusi appendiculatus*. Insect Science and its Application 13: 69–77.

Nolan, J., 1990. Acaricide resistance in single and multi-host ticks and strategies for its control. Parasitologia 32: 145–153.

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

Norval, R.A.I. and Lightfoot, C.J., 1982. Tick problems in wildlife in Zimbabwe. Factors influencing the occurrence and abundance of *Rhipicephalus appendiculatus*. Zimbabwe Veterinary Journal 13: 11–20.

Norval, R.A.I., 1977. Tick problems in relation to land utilization in Rhodesia. Rhodesia Veterinary Journal 8: 33–38.

Norval, R.A.I., 1979. Tick infestations and tick–borne diseases in Zimbabwe Rhodesia. Journal of South African Veterinary Association 50: 289–292.

Norval, R.A.I., 1989. Tick control in relation to the epidemiology of theileriosis. Theileriosis in Eastern, Central and Southern Africa (ed. by Dolan, T.T.). Proceedings of a Workshop on East Coast fever Immunization, held in Lilongwe, Malawi 20–22 September, 1988. The International Laboratory for Research on Animal Diseases. English Press, Nairobi, Kenya.

Norval, R.A.I., Andrew, H.R. and Yunker, C.E., 1989a. Pheromone mediation of host-selection in bont ticks (*Amblyomma hebraeum*). Science 243: 364–365.

Norval, R.A.I., Butler, J.F. and Yunker, C.E., 1989b. Use of carbon dioxide and natural or synthetic aggregation-attachment pheromone of the Bont tick, *Amblyomma hebraeum,* to attract and trap unfed adults in the field. Experimental and Applied Acarology 7: 171–180.

Norval, R.A.I., Peter, T., Yunker, C.E., Sonenshine, D.E. and Burridge, M.J., 1991. Responses of ticks *Amlyomma hebraeum* and *A. variegatum* to know or potential components of the aggregation attachment pheromone. I. Long range attraction. Experimental and Applied Acarology 13: 11–18.

Norval, R.A.I., Sutherst, R.W., Kurki, J., Gibson, J.D. and Kerr, J.D., 1988. The effect of the brow ear–tick *Rhipicephalus appendiculatus* on the growth of Sanga and European breed cattle. Veterinary Parasitology 30: 149–164.

Norval, R.A.I., Sutherst, R.W., Kurki, J., Kerr, J.D. and Gibson, J.D., 1997. The effects of the brown-ear tick, *Rhipicephalus appendiculatus*, on milk production of Sanga cattle. Medical and Veterinary Entomology 11: 148–154.

Norval, R.A.I., Tebele, N., Short, N.J. and Clatworthy, J.N., 1983. A laboratory study on the control of economically important tick species with legumes of the genus *Stylosanthes.* Zimbabwe Veterinary Journal 14: 26–94.

Norval, R.A.I., Yunker, C.E. and Butler, J.F., 1987. Field sampling of unfed adults of *Amblyomma hebreaum* Koch. Experimental and Applied Acarology 3: 213–217.

Nyazema, Z.N., 2002. Evaluation of phytotherapy (Herbalism). Department of Clinical Pharmacology, Medical School, Harare, Zimbabwe. http://groups.yahoo.com/group/Phytomedica/message/1399?, as retrieved on 8 November 2003.

Oakley, A., 1981. Interviewing women: A contradiction in terms. Participatory Research and the Race to Save the Planet: Questions, Critique and Lessons from the Field (ed. by de Rocheleau, D.) Agriculture and Human Values 11: 4–25.

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

Ochanda, H., Young, A.S.,Wells, C., Medley, G.F. and Perry, B.D., 1996. Comparison of the transmission of *Theileria parva* between different instars of *Rhipicephalus appendiculatus*. Parasitology 113: 243–253.

Odalo, J.O., Omolo, M.O., Malebo, H., Angira, J., Njeru, P.M., Ndiege, I.O. and Hassanali, A., 2005. Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. Acta Tropica 95: 210–218.

Ogundaini, A., 1999. Antimicrobial agents from some Nigerian plants. African Journals Online: Nigerian Journal of Natural Products and Medicine. Vol. 3. http://www.inasp.info/ajol/journals/njnpm/vol3abs.html, as retrieved on 20 October 2003.

Okahl, O., 1996. Fatal attraction or how do we get tick bites? Infection 24: 394–395.

Okello-Onen, J., Hassan, S.M. and Essuman S., 1999. Taxonomy of African Ticks: An Identification Manual. ICIPE Science Press, Nairobi, Kenya.

Okioga, D.M. and Rajamannan, A.H., 1997. United States Patent 5662915. Pesticide product derived from the plant *Tagetes minuta*. http://www.freepatentsonline.com/5662915.html, as retrieved on 12 November 2003.

Okulova, N.M., 1978. The vertical and horizontal movement of Ixodidae ticks in the forest in depending from the temperature and air humidity. Ecologiya 2: 44–48.

Okwemba, A., 2002. Local importancy herb to flavour yoghurt. An AWC Feature. Horizon Magazine. http://www.nationaudio.com/News/DailyNation/Supplements /horizon/24102002/story2.htm, as retrieved on 17 October 2002.

Ole–Moiyoi, O.K., 1989. Theileria parva: An intracellular parasite that induces reversible lymphocyte transformation. Experimental Parasitology 69: 204–210.

Olusola, L., Zebulon, S.C. and Okoye, U.F., 1997. Effect of *Vitex doniana* stem bark on blood pressure. African Journals Online: Nigerian Journal of Natural Products And Medicine http://www.inasp.info/ajol/journals/njnpm/vol1abs.html, as retrieved on 8 November 2003.

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

Onge, St.J., 2002. Fish–poison use in the Americas. http://www.survival.com/fish.htm, as retrieved on 10 November 2003.

Oni, P.I., 2001. State of Forest Genetic Resources in the dry north of Nigeria. Sub–Regional Workshop FAO/IPGRI/ICRAF on the conservation, management, sustainable utilization and enhancement of forest genetic resources in Sahelian and North–Sudanian Africa (Ouagadougou, Burkina Faso, 22–24 September 1998). Forest Genetic Resources Working Paper FGR/16E, Forestry Department, FAO, Rome, Italy.

Owoyele. V.B., Wuraola, C.O., Soladoye, A.O., Olaleye, S.B., 2004. Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. Journal of Ethnopharmacology 90: 317–321.

Owuor, O.B. and Kisangau, P.D., 2006. Kenyan medicinal plants used as antivenin: a comparison of plant usage. Journal of ethnobiology and Ethnomedicine 2: 7.

Palgrave, K.C., 1988. Trees of Southern Africa. Revised Edition. New Holland Publishers Ltd, London, UK.

Pålsson, K., Jaenson, T.G.T., Bæckström, P. and Borg–Karlson, A., 2008. Tick repellent substances in the essential oil of *Tanacetum vulgare*. Journal of Medical Entomology 45: 88–93.

Panda, H., 2004. Aromatic Plants: Cultivation, Processing and Uses. Asia Pacific Business Press, New Delhi, India.

Pande, B.C., 1999. Zeetress–a promising anti–stress remedy and immuno–modulator. A review. Ethnoveterinary Medicine: Alternatives for Livestock Development (ed. by Mathias, E., Rangnekar, V.D. and McCorkle, M.C.). Proceedings of an International Conference held in Pune, India, 4–6 November, 1997. Volume 1: Selected Papers. BAIF Development Research Foundation, Pune, India.

Papachristos, D.P. and Stamopoulos, D.C., 2002. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Products Research 38: 117–128.

Park, A.M., 2008. Glossary. Introducing Anthropology: An Integrated Approach, 2nd edn. Central Connecticut State University, USA, http://highered.mcgraw-hill.com/sites/0072549238/student_view0/glossary.html, as retrieved on 21 November 2008.

Parodi, L.R., 1959. Enciclopedia Argentina de Agricultura y Jardineria. Editorial Acme S.A.C.I., Buenos Aires 1: 845.

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

Pegram, R.G., Lemche, J., Chizyuka, H.G.B., Sutherst, R.W., Floyd, R.B., Kerr, J.D. and McCosker, P.J., 1989b. Ecological aspects of cattle tick control in central Zambia. Medical and Veterinary Entomology 3: 307–312.

Pegram, R.G., Lemche, J., Chizyuka, H.G.B., Sutherst, R.W., Floyd, R.B., Kerr, J.D. and McCosker, P.J., 1989a. Effect of tick control on liveweight gain of cattle in central Zambia. Medical and Veterinary Entomology 3: 313–320.

Pennetier, C., Corbel, V., Boko, P., Odjo, A., N'Guessan, R., Lapied, B. and Hougard, J., 2007. Synergy between repellents and non–pyrethroid insecticides strongly extends the efficacy of treated nets against *Anopheles gambiae. Malaria Journal* 6: 38.

Perkins, J., 1912. Beitrage zur flora von Bolivia. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie. Leipzig, 49(1).

Perry, B.D., Lessard, P., Norval, R.A.I., Kundert, K. and Kruska, R., 1990. Climate, vegetation and the distribution of *Rhipicephalus appendiculatus* in Africa. Parasitology Today 6: 100–104.

Perry, L.M., 1980. Medicinal Plants of East and Southeast Asia. MIT Press, Cambridge, MA, USA.

Persson , J., 1986. Trees, plants and a rural community in the southern Sudan. International Journal of Forestry and Forest industries, 38 (154): Unasylva, FAO. http://www.fao.org/docrep/50630E/50630e00.htm#Contents, as retrieved on 10 November 2003.

Peter, R.J., Van den Bossche, P., Penzhorn, B.L. and Sharp, B., 2005. Tick, fly, and mosquito control-lessons from the past, solutions for future. Veterinary Parasitology 132: 205–215.

Petit, S., 2003. FAO Species Description (*Stereospermum kunthianum* Cham.). Grassland and pasture crops. http://www.fao.org/ag/AGP/AGPC/doc/Gbase/DATA/Pf0005 34.htm, as retrieved on 10 October 2003.

Philippe, F.S., 2003. Desert Tropicals. http://www.desert-tropicals.com, as retrieved on 24 September 2003.

Phillipson, P., 1995. A Leaf from the Herbarium. Notes from the Selmar Schonland herbarium. Grahamstown, South Africa. Issue No. 2, September 1995.

Pires, M.D.F.C. and Purchio, A., 1991. Mycological evaluation of filtrated juice of *Agave sisalana* Perrine's leaf. Revista De Microbiologia 22: 272–275.

Politz, J. and Lekeleley, J., 1988. The knowledge of the Samburu on animal diseases and their traditional methods of treatment. Unpublished Research Work, Turkana, Kenya.

Polygenis–Bigendako, M.J., 1990. Recherches ethnopharmacognosiques sur les plantes utilisées en médecine traditionnelle au Burundi occidental. Thèse présentée en vue de l'obtention du grade de Docteur en sciences, année acad. 1989–1990, Universite libre de Bruxelles, Faculty of Science Laboratory. de Botanique Systématique et de Phytosociologie, Brussels, Belgium.

Port, R., 2000. Some Local history of the Bungoma area. Kenya Friendship Programme. The Committee to Support Links between Bloomington, Indiana and Bungoma, western Province, Kenya. http://www.cs.indiana.edu/~port/kenyafriends/index.html, as retrieved on 19 May 2004.

Puckhaber, L.S., Stipanovic, R.D. and Bost, G.A., 2002. Analyses for flavonoid aglycones in fresh and preserved *Hibiscus* flowers. Trends in new crops and new uses (ed. by Janick, J. and Whipkey, A.) ASHS Press, Alexandria, VA., pp. 556–563. http://www.hort.purdue.edu/newcrop/ncnu02/v5-556.html, as retrieved on 21 October 2003.

Putt, S.N.H., Shaw, A.P.M., Woods, A.J., Tyler, L. and James, A.D., 1988. Veterinary epidemiology and economics in Africa–A manual for use in the design and appraisal of livestock health policy. Veterinary Epidemiology and Economics, Department of Agriculture, University of Reading, Reading, England and International Livestock Centre for Africa, Addis Ababa, Ethiopia.

Puyvelde (van) L., Geysen, D., Ayobangira, F.X., Hakizamungu, E., Nshimiyimana, A. and Kalisa, A., 1985. Screening of medicinal plants of Rwanda for acaricidal activity. Journal of Ethnopharmacology 13: 209–215.

Qui, H., Jun, H.W. and McCall, J.W, 1998. Pharmacokinetics, formulation and safety of insect repellent N,N–diethyl–3–methylbenzamide (DEET): a review, Journal of American Mosquito Control Association 14:12–27.

Rajab, S.M., Bentley, M.D., Hassanali, A. and Chapya, A., 1988. A new limonoid from *Turraea holstii*. Phytochmistry 27: 2353.

Rajab, S.M., Mulholland, A.D. and Monkhe, T.V., 1998. Triterpenoids from *Turraea holstii*. Phytochemistry 49: 2585.

Rajput, I.Z., Hu, S., Chen, W., Arijo, G.A. and Xiao, C., 2006. Importance of ticks and their chemical and immunological control in livestock. Journal of Zhejiang University. Science B 7: 912–921.

Randolph, S.E. and Rogers, D.J., 1997. A generic population model for the African tick *Rhipicephalus appendiculatus*. Parasitology 115: 165–179.

Rao, R.R., Chowdhery, P.K. Hajra, S., Kumar, P.C., Pant, B.D., Naithani, B.P. Uniyal, R.M. and Mamgain S. K, 1988. Flora Indicae Enumeratio-Asteraceae. Botanical Survey of India. Ser. 4. Government of India, New Delhi, India.

Rao, Y.S., Mathew, K.M. and Potty, S.N., 1999. Tamarind (*Tamarindus indica* L.) research–a review. Indian Journal of Areca nut, Spices and Medicinal Plants 1: 127 –145.

Rechav, Y. and Whitehead, G.B., 1978. Field trials with pheromone–acaricide mixtures for control of *Amblyomma hebreaum*. Journal of Economic Entomology 71: 149–151.

Rechav, Y., Whitehead, G.B. and Knight, M.M., 1976. Aggregation response of nymphs to pheromone (s) produced by males of tick *Amblyomma hebreaum*. Nature 259: 563 –564.

Rees, A., 1817. *Tagetes*. The Cyclopedia, 35 (1). (ed. by Rees, A.). London, UK.

Regassaa, A., 2000. The use of herbal preparations for tick control in western Ethiopia. Journal of South Africa Veterinary Association, 71: 240–243.

Reiche, C., 1903. Estudios criticos sobre la flora de Chile. Anales University Chile 112: 97 –179.

Roach, R.G., 2003. People Profile: The Luhya of Kenya. Baptist Mission of Kenya: Orville Boyd Jenkins. http://www.geocities.com/orvillejenkins/profiles/Luhya .html, as retrieved on 15 May 2004.

Robbins, P.J. and Cherniack, M.G., 1986. Review of the biodistribution and toxicity of the insect repellent N,N–diethyl–m–toluamide (DEET). Journal of Toxicology and *Environmental Health* 18: 503–525.

Roberts, J.A., 1971. Behaviour of larvae of the cattle tick *Boophilus microplus* on cattle of differing degrees of resistance. Journal of Parasitology 57: 651–656.

Roch, N., Epaulard, O., Pelloux, I., Pavese, P., Brion, J., Raoult, D. and Maurin, M., 2008. African Tick Bite Fever in Elderly Patients: 8 Cases in French Tourists Returning from South Africa. Clinical Infectious Diseases 47: e28–e35.

Rodriguez, E. and Mabry, T.J., 1977. Tageteae-chemical review. The Biology and Chemistry of the Compositeae (ed. by Heywood, V.H., Harborne, J.B. and Turner, B.L.) Academic Press, London, UK.

Rodriguez–Saona, C., Poland, M.T., Miller, R.J., Stelinski, L.L., Grant, G.G., de Groot, P., Buchan, L. and MacDonald, L., 2006. Behavioral and electrophysiological responses of the emerald ash borer, *Agrilus planipennis*, to induced volatiles of Manchurian ash, *Fraxinus mandshurica*. Chemoecology 16: 75–86.

Roe, M.R., Donohue, V.K., Jones, A. and Witting, E.B., 2006. Development of a novel all natural tick and insect repellent, BioUD, as a DEET replacement and for use on cotton fabric. Entomological Society of America Annual Meeting. Display Presentations, Section D., Medical and Veterinary Entomology, 13 December 2006.

Ross, S.A., El-Keltawi, N.E. and Megalla, S.E., 1981. Antimicrobial activity of some Egyptian aromatic plants. Fitoterapia 52: 201–205.

Rossi, I., de Vallière, S., Hatz, C., Rudin, W., 2007. Personal protective measures against mosquito and other arthropod bites. Revue Medicale de la Suisse Romande 16: 1241–1246.

RSCU, 1992. A Selection of Useful Trees and Shrubs for Tanzania. Regional Soil Conservation Unit (RSCU). Nairobi, Kenya.

Rukangira, E., 2001. The African herbal industry: Constraints and challenges. Erboristeria Domani. http://www.conserveafrica.org/herbal_industry.rtf, as retrieved on 4 November 2003.

Rukangira, E., 2003. Overview on the Importance and the Status of Medicinal Plants and Traditional Medicine in Africa. Sustainable Development International. http://www.sustdev.org/Features/04.09.01.shtml, as retrieved on 27 October 2003.

Rulangaranga, Z.K., 1989. Some Important Indigenous Medicinal and Aromatic Plants in the Wild Flora of Tanzania Mainland. Tropical Forestry Action Plan, Working Paper 24. Tanzania Ministry of Lands, Natural Resources and Tourism, Dar es Saalam, Tanzania.

Rüngeler, P., Lyb, G., Castro, V., Mora, G., Pahl, H.L., Merfort, L., 1998. Study of three sesquiterpene lactones from *Tithonia diversifolia* on their anti-inflammatory activity using the transcription factor NF–kB and enzymes of the arachidonic pathways as targets. Planta Medica 64: 588–593.

Rutz, D.A. and Patterson, R.S., 1990. Biocontrol of Arthropods Affecting Livestock and Poultry (Wiley Series in Ecological and Applied Microbiology). Westview Press Inc., Boulder, CO, USA.

Ryan, F.M., 2002. Plant chemicals in pest control. Insect Chemoreception: Fundamental and Applied, pp. 193–222. Kluwer Academic Publishers, New York, NY, USA.

SACRED Africa, 2007. Using *Tithonia* as an organic fertilizer. Sustainable Agriculture Center for Research, and Development in Africa, (SACRED Africa) Bungoma. http://www.farmingsolutions.org/successtories/stories.asp?id=131, as retrieved on 24 March 2007.

Saini, K.R. and Hassanali, A, 2002–2003. Enhancing the diffusion of new tsetse control technologies for improved livestock health and productivity in smallholder indigenous communities of sub-Saharan Africa. International Centre of Insect Physiology and Ecology (ICIPE). Tsetse Research Highlights of 2002–2003 activities.http://www.icipe.org/research_areas/animal_health/tsetse/highlights2002-2003.html, as retrieved on 5 May 2007.

Saka, J.D.K. and Msonthi, J.D., 1994. Nutritional value of indigenous wild trees in Malawi. Forest Ecology Management 64: 245–248.

Salit, R., 2007. Deer tick numbers skyrocket locally. Rhode Island news. http://www.projo.com/news/content/EB_EBTICKS31_07-31-07_286IDI7.366ef82.html., as retrieved on 5 June 2007.

Samish, M, Ginsberg, H, and Glazer, I., 2004. Biological control of ticks. Parasitology 129: S389–S403.

Samish, M. and Alekseev, E., 2001. Arthropods as predators of ticks (Ixodoidea). Journal of Medical Entomology 38: 1–11.

Samish, M., 2000. Biocontrol of Ticks. Annals of the New York Academy of Sciences 916: 172–178.

Samuelsson, G., Faraha, H.M., Per Claeson, M., Hagos, M., Thulin, O., Hedberg, A.M., Warfa, A.O., Hassan, A.H., Elmi, A.D., Abdurahman, A.S., Elmi, Y.A., Abdie, and Alin, M.H., 1992. Inventory of plants used in traditional medicine in Somalia. II. Plants of the families Combretaceae–Labiatae. Journal of Ethnopharmacology 37: 47–70.

Sanon, S., Azas, N., Gasquet, M., Ollivier, E., Mahiou, V., Barro, N., Cuzin-Ouattara, N., Traore, A.S., Esposito, F., Balansard, G. and Timon–David, P., 2003. Antiplasmodial activity of alkaloid extracts from *Pavetta crassipes* (K. Schum) and *Acanthospermum hispidum* (DC), two plants used in traditional medicine in Burkina Faso. Parasitology Research 90: 314–317.

SAS Institute Inc., 2002–2003. Proprietary Software Release 8.1 (TS1M0). SAS Institute, Cary, NC, USA.

Sauer, J.R., Hair, J.A. and Houts, M.S., 1974. Chemo–attraction in the lone star tick. 2. Responses to various concentrations of CO2. Annals of Entomological Society of America 67: 150–152.

Saxena, B.P. and Koul, O., 1982. Essential oils and insect control. Cultivation and utilization of aromatic plants. Council of Scientific Research (Atal, C.K. and Kapur, B.M.), pp. 766-776. Jammu-Tawi, India.

Saxena, B.P. and Srivastava, J.B., 1973. *Tagetes minuta* L. oil: A new source of juvenile hormone mimicking substance. Indian Journal of Experimental Biology 11: 56–58.

Sbarbati, A. and Osculati, F., 2006. Allelochemical communication in vertebrates: kairomones, allomones and synomones. Cells Tissues Organs 183: 206–219.

Schippmann, U., 2001. Medicinal plants significant trade. CITES Projekt S–109, Plants Committee Document PC9 9.1.3 (rev.). BFN Scripten–39, BFN–German Federal Agency for Nature Conservation., pp. 51–58.

Schmidt, G.H., Rembold, H., Ahmed, A.A.I. and Breuer, M., 1998. Effect of *Melia azedarach* fruit extract on juvenile hormone titer and protein content in the hemolymph of two species of noctuid lepidopteran larvae (Insecta: Lepidoptera: Noctuidae). Phytoparasitica 26: 283–292.

Schmidt, J.R., 2003a. Agavaceae (Agave or Century Plant family). Plant Families Index, Botanical Dermatology Database (BoDD) Index to Botanical Dermatology Reviews. http://BoDD.cf.ac.uk/BoDDHomePage.html, as retrieved on 9 October 2003.

Schmidt, J.R., 2003b. Euphorbiaceae (Spurge family). Plant Families Index, Botanical Dermatology Database (BoDD) Index to Botanical Dermatology Reviews. http://BoDD.cf.ac.uk/BoDDHomePage.html, as retrieved on 29 September 2003.

Schmidt, J.R., 2003c. Guttiferae (St John's Wort family).Plant Families Index, Botanical Dermatology Database (BoDD) Index to Botanical Dermatology Reviews. http://BoDD.cf.ac.uk/BoDDHomePage.html as retrieved on 29 October 2003.

Schmidt, J.R., 2003d. Leguminosae (Pea family). Plant Families Index, Botanical Dermatology Database (BoDD) Index to Botanical Dermatology Reviews. http://BoDD.cf.ac.uk/BoDDHomePage.html, as retrieved on 18 October 2003.

Schoni, R., Hess, E., Blum, W. and Ramstein, K., 1984. The aggregation-attachment pheromone of the tropical bont tick *Amlyomma variegatum.* Isolation, Identification and Action of its components. Journal of Insect Physiology 30: 27-30.

Schreck, C.E., 1977. Techniques for the evaluation of insect repellents: a critical review. Annual Review of Entomology 22: 101–119.

Schreck, C.E., Fish, D. and McGovern, T.P., 1995. Activity of repellents applied to skin for protection against *Amblyomma americanum* and *Ixodes scapularis* ticks (Acari: Ixodidae). Journal of the American Mosquito Control Association 11: 136 –140.

Schwantes, U., Dautel, H. and Jung, G., 2008. Prevention of infectious tick–borne diseases in humans: Comparative studies of the repellency of different dodecanoic acid-formulations against *Ixodes ricinus* ticks (Acari: Ixodidae). Parasites and vectors 1: 8.

Schwartz, L, Tulipan, L and Birmingham, D.J., 1957. Occupational Diseases of the Skin, 3rd edn. Lea and Febiger, Philadelphia, PA, USA., pp. 300–310.

Seifert, G.W., 1984. Selection of beef cattle in northern Australia for resistance to the cattle tick (*Boophilus microplus*): research and applications. Development in Animal and Veterinary Sciences, Vol. 15. Impact of diseases on livestock production in the tropics (ed. by Riemann, H.P. and Burridge, M.J.), pp. 553–558. Elsevier, Amsterdam, The Netherlands.

Senatore, F. and De Feo, V., 1999. Chemical composition of the essential oil of *Tagetes mandonii* Sch. Bip. (Asteraceae). Flavour and Fragrance Journal 14: 32–34.

Senatore, F., Napolitano, F., Mohamed, M.A.H, Harris, P.J.C., Mnkeni, P.N.S, Henderson, J., 2004. Antibacterial activity of *Tagetes minuta* L. (Asteraceae) essential oil with different chemical composition. Flavour and Fragrance Journal 19: 574–578.

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

Seo, Y., 2002. A new bioactive triterpenoid saponin from *Pittosporum viridiflorum* from the Madagascar Rainforest. Journal of Natural Products 65: 65–68.

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

Seyoum, A., Pålsson, K., Kung'a, S., Kabiru, E.W., Lwande, W, Killeen, G.F., Hassanali, A. and Knols, B.G.J., 2002. Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning. Transaction Royal Society of Tropical Medicine and Hygiene 96: 225–231.

Shaw, M.K., Tilney, L.G. and Musoke, A.J., 1991. The entry of *Theileria parva* sporozoites in bovine lymphocytes: Evidence for MHC class I involvement. Journal of Cellular Biology 113: 87–101.

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

Silva, M.A., Alencar, M.M., Regitano, A.C.L., Oliveira, S.C.M. and Júnior, B.W., 2007. Artificial infestation of *Boophilus microplus* in beef cattle heifers of four genetic groups. Genetics and Molecular Biology 30: 1150–1155.

Simon, F., 1998. Bioprospecting or biopiracy? Drug Discovery Today 3: 399–402.

Singh, B., Joshi, V.P., Ram, R., Sharma, A., Zaidi, A.A., 2002. United States Patent 6444458. Use of *Tagetes minuta* oil and its components as antiviral agents. http://www.freepatentsonline.com/6444458.html, as retrieved on 27 March 2007.

Singh, B., Sood, R.P. and Singh, V., 1992. Chemical composition of *Tagetes minuta* oil from Himachal Pradesh (India). Journal of Essential Oil Research 4: 525–528.

Singh, P.R., 2002. Strategies for the management of crop pests with neem (*Azadirachta indica* A. Juss.) and other plant–derived insecticides. Proceedings of the first international symposium on "Utilization of Natural Products in Developing Countries: Trends and Needs" (ed. by Mansingh, A., Young, R., Yee, T., Delgoda, R., Robinson, E.D., Morrison, E. and Lowe, H.), held between 10th–14 July, 2000 at the Natural Products Institute, University of the West Indies, Mona, Kingston 7, West Indies, Jamaica.

Singh, S. and Kumar, S., 1998. *Withania somnifera*: The Indian Ginseng Ashwagandha, Lucknow. http://www.vedamsbooks.com/no15185.htm, as retrieved on 21 October 2003.

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

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

Slingenbergh, J., Hendrickx, G., and Wint, W., 2002. Imbalance in global livestock development must be redressed to halt the spread of animal diseases, food safety hazards and other veterinary public health risks. In: Will the Livestock Revolution succeed? Agriculture World Vision 2: 31–33.

Smith, A.C., 1991. Flora Vitiensis nova: a new flora of Fiji, Vol. 5., National Tropical Botanical Garden, Lawai, Kauai, HI, USA.

Smith, C.N. and Cole, M.M., 1943. Studies of parasites of the American dog tick. Journal of Economic Entomology 36: 569–572.

Smith, F.P., 1969. Chinese Materia Medica: Vegetable Kingdom, 2nd edn (revised by Hei, P.D.). Ku T'ing Book House, Taipei, Taiwan.

Smith, M.W. 1975. Some aspects of the ecology and lifecycle of *Amblyomma cajennense* (Fabricius 1787) in Trinidad and their influence on tick control measures. Annals of Tropical Medicine and Parasitology 69: 121–129.

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

Solberg, V.B., Klein, T.A., McPherson, K.R., Bradford, B.A., Burge, J.R. and Wirtz, R.A., 1995. Field evaluation of DEET and a piperidine repellent (AI3–37220) against *Amblyomma americanum* (Acari: Ixodidae). Journal of Medical Entomology 32: 870–875.

Solomon, G. and Kaaya, G.P., 1996. Comparison of resistance in three breeds of cattle against African ixodid ticks. Experimental and Applied Acarology 20: 223–230.

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

Sonenshine, D.E., 1985. Pheromones and other Semiochemicals of the Acari. Annual Review of Entomology 30: 1–28.

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

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

Sonenshine, D.E., Hamilton J.G.C., James, S., Philips, K.P.E. and Norval, R.A.I., 1992. Innovative techniques for control of tick disease vectors using pheromones. Proceedings of the 1st International Conference of Tick–borne Pathogens at the Host –Vector–Pathogen Interface, Minnesota, USA.

Sonenshine, D.E., Khalil, G.M., Homsher, P.J. and Mason, S.N., 1982. *Dermacentor variabilis* and *D. andersoni*: Genital sex pheromones. Experimental Parasitology 54: 317–330.

Sonenshine, D.E., Taylor, D. and Carson, E., 1986. Chemically mediated behaviour in Acari: Adaptations for finding hosts and mates. Journal of Chemical Ecology 12: 1091–1108.

Sones, K, and Catley, A. 2003. Primary animal health care in the 21st Century: shaping the rules, policies and institutions. Proceedings of the International Conference held 15–18 October 2002, Mombasa, Kenya by African Union/InterAfrican Bureau for Animal Resources, Nairobi, Kenya.

Soule, J.A., 1993. *Tagetes minuta*: A potential new herb from South America. New Crops (ed. by Janick, J. and Simon, J.E.), pp. 649–654. John Wiley, New York, NY, USA.

Souza, C.A.S., de Avancini, C.A.M. and Wiest, J.M., 2000. Antimicrobial activity of *Tagetes minuta* L. - Compositae (Chinchilho) against gram-positive and gram-negative bacteria. Brazilian Journal of Veterinary Research and Animal Science, São Paulo 37: 6, 2000 http://www.scielo.br/scielo.php? script=sci_arttext&pid=S1413-95962000000600001 &lng=en& nrm=iso, as retrieved on 24 April 2006.

Space, J.C. and Flynn, T., 2000. Report to the Government of Niue on invasive plant species of environmental concern. USDA Forest Service, Honolulu, HI, USA.

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

Speybroeck, N., Madder, M., Thulke, H.H., Mtambo, J., Tirry, L., Chaka, G., Marcotty, T. and Berkvens, D., 2004. Variation in body size in the tick complex *Rhipicephalus appendiculatus/Rhipicephalus zambeziensis*. Journal of Vector Ecology 29: 347–354.

Stadler, J., Mungai, G. and Brandl., R., 1998. Weed invasion in East Africa: insights from herbarium Records. African Journal of Ecology 36: 15–22.

Staub, D., Debrunner, M. Amsler, L. and Steffen, R., 2002. Effectiveness of a repellent containing DEET and EBAAP for preventing tick bites. Wilderness and Environmental Medicine 13: 12–20.

Stedman, T.L., Wilcox, M.D. and Follett, Co., 1942. Stedman's Shorter Medical Dictionary. http://www.botanical.com/botanical/mgmh/n/nuxvom08.html, as retrieved on 22 August 2004.

Steidle, J.L.M. and van Loon, J.J.A., 2003. Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. Entomologia Experimentalis et Applicata 108: 133–148.

Stockbauer, B., 2003. Tree list for environmental nurseries. African Blackwood Conservation Project. http://www.blackwoodconservation.org/5-year_programtreelist.html, as retrieved on 18 October 2003.

Stojanova, A., Primova, T. and Anastassov, C., 2000. Effect of Mineral Fertilization on the Essential oil Composition of *Tagetes patula* L. from Bulgaria. Journal of Essential oil Research 12: 609–612.

Stoll, G., 1988. Natural plant protection in the tropics. AGRECOL, Margraf Publishers Scientific Books, Valag Josef Margraf, Welkersheim, Germany.

Stone, B.C., 1970. The flora of Guam. Micronesica 6: 1–659.

Strickman, D.A., Miller, M.E., Lee, K.W., Kim, H.C., Wirtz, R.A., Perich, M., Novakoski, W.L., Feighner, B.H., Roh, C.S., 2001. Successful entomological intervention against *Anopheles sinensis*, limiting transmission of *Plasmodium vivax* to American soldiers in the republic of Korea. Korean Journal of Entomology 31: 189–195.

Sutherst R.W., Jones R.J. and Schnitzerlinmg H.J., 1982. Tropical legumes of the genus: *Stylosanthes*.and their ability to immobilize and kill cattle ticks. Nature 295: 320–321.

Sutherst, R.W. 1987. Ectoparasites and herbivore nutrition. The Nutrition of Herbivores (ed. by Hacker, J.B. and Ternouth, J.H.), pp. 191–209. Academic Press, Sydney, Australia.

Sutherst, R.W. and Wilson, L.J., 1986. Tropical legumes and their ability to immobilize and kill cattle ticks. Insects and the Plants Surfaces (ed. by Juniper, E.B. and Southwood, E.R.T.) Edward Arnold, Baltimore, MD, USA.

Sutherst, R.W., Norton, G.A., Barlow, N.D., Conway, G.R., Birley, M. and Cummins, H.N., 1979. An analysis of management strategies for cattle tick (*Boophilus microplus*) control in Australia. Journal of Applied Ecology 16: 359–382.

Sutherst, R.W., Wharton, R.H. and Utech, K.B.W., 1978. Guides to studies on tick ecology, CSIRO, Division of Entomology, CSIRO Technical Paper Number 14. Australia.

Sutherst, R.W., Wilson, L.J., Reid, R. and Kerr, J.D., 1988. A survey of the ability of tropical legumes in the genus *Stylosanthes*. to trap larvae of the cattle tick. Boophilus microplus (Ixodidae). Australian Journal of Experimental Agriculture 28: 473–479.

Sutton, J.A. and Orr, B.D., 1991. The use of the school essay as an RRA technique: A case study from Bong County, Liberia. RRA Notes. Participatory Methods for Learning and Analysis. No. 14. IIED Sustainable Agriculture Program.

Syed, Z., and Leal, W.S., 2008. Mosquitoes smell and avoid the insect repellent DEET. Proceedings of the National Academy of Sciences of the United States of America 105: 13598–13603.

Sykes, A.R. 1987. Endoparasites and herbivore nutrition. The Nutrition of Herbivores (ed. by Hacker, J.B. and Ternouth, J.H.), pp. 211–232. Academic Press, Sydney, Australia.

Tadesse, D.,1994. Traditional use of some medicinal plants in Ethiopia. Proceedings of the 13th plenary meeting of AETFAT, Zomba, Malawi, (2–11 April, 1991) Vol. 1 and 2, (Seyani, H.J. and Chicuni, C.A.) National Herbarium and Botanic Gardens of Malawi, Zomba, Malawi.

Taiwo, B.L. and Makinde, O.J., 2005. Influence of water extract of Mexican sunflower (*Tithonia diversifolia*) on growth of cowpea (*Vigna unguiculata*). African Journal of Biotechnology 4: 355–360.

Takanashi, M., 1998. Composition for curing diabetes mellitus, processes for the preparation of same and usage of same. U.S. Patent 5, 773.

Takken, W. and Knols, B.G., 1999. Odor–mediated behavior of Afrotropical malaria mosquitoes. Annual Review of Entomology 44: 131–57.

Takken, W., 1991. The role of olfaction in host-seeking of mosquitoes: a review. Insect Science and its Application 12: 287–295.

Tatchell, R.J. and Easton, E., 1986. Tick ecological studies in Tanzania. Bulletin of Entomological Research 76: 229–246.

Taylor, F.W., 1986. The potential for utilization of indigenous plants in Botswana. Plants for Arid Lands (ed. by Wickens, G.E. Goodin, J.R. and Field, D.V.), pp. 231–242. George Allen and Unwin, London, UK.

Taylor, L., 2002. Herbal Secrets of the Rainforest 2nd edn. Published by Sage Press (ed by Taylor, L.). Tropical Plant Database File for: Picão Preto (*Bidens pilosa*). http://www.rain-tree.com/picaopreto.htm, as retrieved on 1 November 2003.

Tereschuk, M.L., Riera, M.V.Q., Castro, G.R. and Abdala, L.R., 1997. Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. Journal of Ethnopharmacology 56: 227–232.

Thadeu, A.M., Barros de, A. and Evans, E.D., 1989. Acao de gramineas forrageiras em larvas infestantes do carrapato dos bovidos *Boophilus microplus*. Pesquisa Veterinária Brasileira 9: 17–21.

Thapa, J.M. and Pathak, N.L., 2002. The medicinal and health food plants of Nepal. Proceedings of the first international symposium on utilization of natural products in developing countries: Trends and Needs (ed. by Mansingh, A., Young, R., Yee, T., Delgoda, R., Robinson, E.D., Morrison, E. and Lowe, H.), held 10–14 July 2000 at the Natural Products Institute, University of the West Indies, Kingston, Jamaica.

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

Thavara, U., Tawatsin, A., Chompoosri, J., Suwonkerd, W., Chansang, U. and Asavadachanukorn, P., 2001. Laboratory and field evaluations of the insect repellent 3535 (Ethyl Butylacetylaminopropionate) and Deet against mosquito vectors in Thailand. Journal of American Mosquito Control Association 17: 190–195.

Theiler, A., 1904. East coast fever. Transvaal Agricultural Journal 2: 421–438.

Thijssen, R, Murithi, F.M. and Nyaata, O.Z., 1993. Existing Hedges on Farms in the Coffee-based Land-use System of Embu District, Kenya. AFREENA Report No. 65. ICRAF, Nairobi, Kenya.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, R.S.U. and Schnitzler, J., 2006. Techniques for Molecular Analysis: Practical approaches to plant volatile analysis. The Plant Journal 45: 540–560.

Thompson, K.C., Roa, E.J. and Romero, N., 1978. Anti-tick grasses as the basis for developing practical tropical tick control packages. Tropical Animal Health and Production 10: 179–182.

Timberlake, J.R., 1987. Ethnobotany of the Pokot of Nothern Kenya, Unpublished report. East African Herbarium, Nairobi, Kenya and Center for Economic Botany, Royal Botanic Gardens. Kew, Richmond, UK.

Toledo, J.S., Ambrosio, S.R., Cerri, D., da Costa, F.B. and Cruz, A.K., 2003. Anti-leishmanial activity from *Tithonia diversifolia* leaves extract. Chemotherapy. Revista do Instituto de Medicina Tropical de São Paulo 45: 108–120.

Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., de Bruyne, T., Pieters, L., Totte, J. and Vlietinck, A.J., 1999. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasha, Congo. Phytomedicine 6: 59–66.

Tona, L., Kambu, K., Ngimbi, N., Cimanga, K. and Vlietnick, A.J., 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of Ethnopharmacology 61: 57–65.

Tona, L., Kambu, K., Ngimbi, N., Messia, K., Penge, O., Lusakibanza, M., Cimanga, K., De-Bruyne, T., Apers, S., Totte, J., Pieters, L. and Vlietincia, A.J., 2000 Anti-amoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasha, Congo. Phytomedicine 7: 31–38.

Tonbak, S., Aktas, M., Altay, K., Azkur, A.K., Kalkan, A., Bolat, Y., Dumanli, N. And Ozdarendeli, A., 2006. Crimean-Congo Haemorrhagic Fever virus: genetic analysis and tick survey in Turkey. Journal of Clinical Microbiology 44: 4120–4124.

Torto, B. and Hassanali, A., 1997. Progress in the search for anti–arthropod botanicals. Recent Research Development in Phytochemistry 1: 475–488.

Toyang, J.N., Nuwanyakpa, M., Ndi, C., Django, S. and Kinyuy, C.W., 1995. Ethnoveterinary medicine practices in the Northwest Province of Cameroon. IK Monitor, 3. http:// www.nuffic.nl/ciran/ikdm/3-3/articles/toyang.html, as retrieved on 20 October 2003.

Trade Winds Fruit, 2003. http://www.tradewindsfruit.com/custservice.htm, as retrieved on 26 October 2003.

Tredgold, M.H., 1990. Food Plants of Zimbabwe, 2nd edn. Mambo Press, Harare, Zimbabwe.

Trimnell, A.R., Davies, G.M., Lissina, O., Hails, R.S. and Nuttall, P.A., 2005. A cross-reactive tick cement antigen is a candidate broad-spectrum tick vaccine. Vaccine 23: 4329–4341.

Trongtokit, Y., Rongsriyam, Y., Komalamisra, N., Krisadaphong, P., Apiwathnasorn, C., Krisadaphong, P. and Apiwathasorn, C., 2004. Laboratory and field trial of developing medicinal local Thai plant products against four species of mosquito vectors. Southeast Asia Journal of Tropical Medicine and Public Health 35: 325–333.

Uilenberg, G., 2006. Ticks, Diseases and climate change. Editor: Newsletter on Ticks and Tick-borne Diseases of Livestock in the Tropics, June, 2006, Issue 30. Integrated Consortium on Ticks and Tick–borne Diseases (ICTTD–3): http://www.icttd.nl, as retrieved on 14 October 2007.

UNEP World Conservation Monitoring Centre, 2003. Traditional Lifestyles and Biodiversity Use. Regional Report (UNEP/CBD/WG8J/3/INF/3): AFRICA. Composite Report on the Status and Trends Regarding the Knowledge, Innovations and Practices of Indigenous and Local Communities Relevant to the Conservation and Sustainable Use of Biodiversity.

Urzua, A., 2002. Monoterpenes and sesquiterpenes in the headspace volatiles from intact plants of *Pseudognaphalium vira vira, P. heterotrichium, P. cheiranthifolium* and *P. robustum*: their insect repellent function. Boletín de la Sociedad Chilena de Química 47: 99–104.

USDA, ARS, National Genetic Resources Program, 2004. Phytochemical and Ethnobotanical Databases: National Germplasm Resources Laboratory, Beltsville, MD, USA. http://www.ars-grin.gov:8080/npgsp ub/xsql /duke/pl_act.xsql?taxon=2160, as retrieved on 4 March 2004.

Utech, K.B.W. and Wharton, R.H., 1982. Breeding for resistance to *Boophilus microplus* in Australian Illawarra Shorthorn and Brahman X Australian Illawarra Shorthorn cattle. Australian Veterinary Journal 58: 41–46.

UWMG, 2003. Uganda Wildlife Medicinal Garden (UWMG). A list of some of over 500 herbs in Uganda Wildlife Medicinal Garden, with information about diseases they cure. http://www.uweczoo.org/Medicinal_plants_tree_tags.pdf, as retrieved on 9 November 2003.

Vale, G.A. 1980. Field studies of the responses of tse tse flies and other Diptera to carbon dioxide, acetone and other chemicals. Bulletin of Entomological Research 70: 563 –570.

Valladares, G., Defago, M.T., Palacios, S. and Carpinella, M.C., 1997. Laboratory evaluation of *Melia azedarach (Meliaceae)* extracts against the elm leaf beetle (Coleoptera: *Chrysomelidae*). Journal of Economic Entomology 90: 747–750.

van Andel, T., 2000. The Diverse Uses of Fish–Poison Plants in Northwest Guyana. Economic Botany 54: 500–512.

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

van Grinsven, M., Parkipuny, L.M. and Johns, T., 1999. *Euclea divinorum* (Ebenaceae) Bark is a High-Potential Tanning Material. Economic Botany 53: 220–221.

van Puyvelde L.M., Ngaboyisonga, P.C. and Rwangabo, S., 1977. Enquêtes ethnobotaniques sur la médecine traditionnelle rwandaise. Tome 1: Préfecture de Kibuye. Institut National de Recherches Scientifiques, Butare, Rwanda.

van Rijn, C.P. and Tanigoshi., K.L., 1999. The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. Acarology Bulletin 23: 281–296.

Vanden Berghe, D.A, and Vlictinck, A.J., 1991. Screening methods for antibacterial and antiviral agents from higher plants. Methods in Biochemistry, vol 6 (ed. by Hostettmann, K.), pp. 47-69. Academic Press, London, UK.

Vasudevan, P., Kashyap, S. and Sharma, S., 1997. *Tagetes:* A multipurpose plant. Bioresource Technology 62: 29–35.

Vellaikumar, S., 1997. Comparative investigations on Chinaberry (*Melia azaderach* L.) and Neem (*Azadirachta indica* A. Juss.) seeds as sources of insect antifeedants. MSc. Thesis, IARI, New Delhi, India.

Vet, M.E.L. and Dicke, M., 1992. Ecology of infochemical by natural enemies in a tritrophic context. Annual Reviews in Entomology 37: 141–172.

Viable Herbal Solutions, 2003. About Olive Leaf-*Olea europaea* L. http://www.viableherbal.com/herbdesc3/1olive.htm, as retrieved on 27 October 2003.

Vial, L., Diatta, G., Tall, A., El Hadj, B., Bouganali, H., Durand, P., Sokhna, C., Rogier, C., Renaud, F. and Trape, J.F., 2006. Incidence of tick–borne relapsing fever in west Africa: longitudinal study. Lancet 368: 37–43.

VIE, 2002. Veterinary Informatics and Epidemiology at University of Glasgow (Department of Veterinary Medicine) and University of Strathclyde (Department of Statistics and Modelling Science and Department of Information Science). http://www.vie.gla.ac.uk/project/ecfxpert/ecfxpert.html, as retrieved on 3 September 2004.

Villamil, S.M., Alche, L., C.E. and Coto, C.E., 1995. Inhibition of herpes simplex virus type–1 multiplication by meliacine, a peptide of plant origin. Antiviral Chemistry and Chemotherapy 6: 239–244.

Visser, J.H., 1986. Host odour perception in phytophagous insect. Annual Reviews of Entomology 31: 121–144.

Volfová, A. and Patočka, J., 2003. Strychnin–historie a současnost. Vojenské Zdravotnické Listy 72: 110–113.

Wagner, W.L., Herbst, D.R. and Sohmer, S.H., 1999. Manual of the flowering plants of Hawaii. Revised edition. Bernice P. Bishop Museum special publication. University of Hawai'i Press, Bishop Museum Press, Honolulu, HI, USA.

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

Waladde, S.M. and Rice, M., 1982. The sensory basis of tick feeding behaviour. Physiology of Ticks (ed. by Obenchain, F.D. and Galun, R.), pp. 71–118. Pergamon Press, Oxford, UK.

Waladde, S.M., 1987. Receptors involved in host location and feeding in ticks. Insect Science and its Application 8: 643–647.

Waladde, S.M., Kemp, D.H. and Rice, M.I., 1979. Feeding electrograms and fluid uptake measurements of cattle tick, *Boophilus microplus* attached on artificial membranes. International journal of Parasitology 9: 89–95.

Waladde, S.M., Ochieng, S.A. and Gichuhi, P.M., 1991. Artificial-membrane feeding of the ixodid tick *R. appendiculatus*, to repletion. Experimental and Applied Acarology 11: 297–306.

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

Walker, R.A., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G. and Preston, P.M., 2003. Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Bioscience Reports, University of Edinburgh, Edinburgh, UK.

Wall, R. and Shearer, D., 1997. Ticks. Veterinary Entomology: Arthropod Ectoparasites of

Veterinary Importance, Kindle Edition (ed. by Wall, R. and Shearer, D.) Springer, London, UK and New York, NY, USA.

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

Wandibba, S., 1998. Who cares about the past? An example from western Kenya. World Archaeological Congress: The destruction and preservation of cultural property, held from 3–7 May 1998: Island of Brac: Croatia, at the University of Southampton.

Wanjau, S., Mukalama, B.J. and Thijssen, R., 1997. Biomass transfer-Harvesting free fertilizer: As part of the Kenya Woodfuel and Agroforestry Programme (KWAP) farmers in the Busia District of western Kenya taking part in an on-farm experiment in green manure. Research and Information Exchange in Ecologically Sound Agriculture (ILEIA), Newsletter 13: 25–27.

Wanjiru, J., 2003. Wild Sunflowers Enrich Fertility of African Farms. Environment News Service. Nairobi, Kenya. http://ens-news.com/ens/feb2003/2003-02-18-01.asp, as retrieved on 10 March 2007.

Wanyangu, S.W., Bain, R.K., Rugutt, M.K., Nginyi and Mugambi, J.M., 1996. Anthelmintic resitance amongst sheep and goats in Kenya. Preventive Veterinary Medicine 25: 285–290.

Wanzala, W., Sika, K.F.N., Gule, S. and Hassanali, A., 2004. Attractive and repellent host odours guide ticks to their respective feeding sites. Chemoecology 14: 229–232.

Wanzala, W., Zessin, K.H., Kyule, N.M., Baumann, M.P.O., Mathias, E. and Hassanali, A., 2005. Ethnoveterinary medicine: a critical review of its evolution, perception, understanding and the way forward. Livestock Research for Rural Development. Volume 17: 119. http://www.cipav.org.co/lrrd/lrrd17/11/wanz17119.htm, as retrieved on 16 August 2005.

Warry, W., 1992. The eleventh thesis: Applied anthropology as praxis. Human Organization 51: 155–163.

Waters–Bayer, A. and Bayer, W., 1994. Planning with pastoralists: PRA and more. A review of methods focused on Africa. Germany Agency for Technical Cooperation (GTZ), Eschborn, Germany.

Watt, J.M. and Breyer–Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd edn. E. and S. Livingstone, Edinburgh and London, UK.

Watt, M.D. and Walker, R.A., 2000. Pathological effects and reduced survival in *Rhipicephalus appendiculatus* ticks infected with *Theileria parva* protozoa. Parasitology Research 86: 207–214.

Webb, E.C. and David, M., 2002. The efficacy of neem seed extract (*Azadirachta indica*) to control tick infestation in Tswana, Simmentaler and Brahman cattle. South African Journal of Animal Science 32: 1–6.

Webb, L.J., 1948. Guide to medicinal and poisonous plants of Queensland. Council for Scientific and Industrial Research, Melbourne, Australia.

Wehmeyer, A.S., 1966. The nutrient composition of some edible wild fruit found in the Transvaal. South Africa Medical Journal 40: 1102–1104.

Weldon, P.J., Carroll, J.F., 2007. Vertebrate chemical defense: secreted and topically acquired deterrents of arthropods. Insect repellents: principles, methods, and uses (ed. by Debboun, M., Frances, S.P., and Strickman, D.), pp. 47-75. CRC Press, Boca Raton, FL, USA.

Were, G.S., 1967. A history of the Abaluyia of Western Kenya. c. 1500–1930. East African Literature Bureau, Publishing House, Nairobi, Kenya.

West, O., 1965. Fire in vegetation and its use in pasture management with special reference to tropical and subtropical Africa. In: Report of Commonwealth Bureau of Pastures and Field Crops, Berkshire. Commonwealth Agricultural Bureaux (CAB), Mimeographed publications 1/1965.

WHO, 1995. International travel and health vaccination requirement and health advice. World Health Organization, Geneva, Switzerland.

Whyte, W.F., 1981. Participatory Approaches to Agricultural Research and Development: A state–of–the–Art–Paper. Rural Development Committee, Centre for International Studies, Cornell University, Ithaca, NY, USA.

Wilcox, E.V. and McGeorge, W., 1912. Sisal and the utilisation of sisal waste. Bulletin of Hawaii Agriculture and Experiment Station (35), Hawaii, USA.

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

Wilkinson, P.R., 1957. The spelling of pastures in cattle tick control. Australian Journal of Agricultural Research 8: 414–423.

Willadsen, P. and Kemp, D.H., 1988. Vaccination with concealed antigens for tick control. Parasitology Today 4: 196–198.

Willadsen, P., 1997. Vaccines, genetics and chemicals in tick control: the Australian experience. Proceedings of the European Union International Symposium on Ticks and Tick–borne Diseases (ed. by Preston, P.M. and Hong, Y.) Tropical Animal Health and Production 29: 91s–94s.

Willadsen, P., Smith, D., Cobon, G. and Mckenna, R.V., 2006. Comparative vaccination of cattle against *Boophilus microplus* with recombinant antigen Bm86 alone or in combination with recombinant Bm91. Parasite Immunology. 18: 241–246.

William, N.S., 1999. Natural Products Drug Discovery. http://chemistry.uah.edu/Faculty/setzer/natprod.html, as retrieved on 8 October 2003.

Williams, L.A.D., 1993. Adverse effects of *Artocarpus altilis* Park, *Azadirachta indica* (A. Juss) on the reproductive physiology of the adult female tick, *Boophilus microplus* (Canest.). Invertebrate Reproduction and Development 23: 159–164.

Wilson, A. and Mansingh, A., 2002. Pesticidal Potential of Tropical Plants–VI. Toxic actions of ethanol extracts of selected plants on *Cylas formicarius elegantulus* Summer and *Boophilus microplus* Canestrinii. Proceedings of the first international symposium on Utilization of Natural Products in Developing Countries: Trends and Needs (ed. by Mansingh, A., Young, R., Yee, T., Delgoda, R., Robinson, E.D., Morrison, E. and Lowe, H.), held 10–14 July, 2000 at the Natural Products Institute, University of the West Indies, Kingston, Jamaica.

Wilson, L.J. and Sutherst, R.W., 1986. Acceptability of *Stylosanthes scabra* Vogel. as a substrate for larvae of the tick *Boophilus microplus* (Canestrini) (Acari: Ixodidae). Journal of the Australian Entomological Society 25: 353–358.

Wilson, L.J. and Sutherst, R.W., 1990. Oviposition sites of *Boophilus microplus* (Canestrini) (Acarina: Ixodidae) in *Stylosanthes* and grass pastures. Journal of the Australian Entomological Society 29: 101–105.

Wilson, L.J., Sutherst, R.W. and Kerr, J.D., 1989. Trapping of larvae of the cattle tick, *Boophilus microplus*, by *Stylosanthes scabra* under grazing conditions. Australian Journal of Agricultural Research 40: 1301–1308.

Won–Ja, L.E.E., Hoe–Sun, L.E.E., Young–Joon, A.H.N. and Dong–Kyu, L.E.E., 2007.

Laboratory evaluation of controlled–release repellent treated pulp fabric on human volunteers against mosquito vectors. Entomological Research 34: 37–42.

Won-Sik, C., Byeoung–Soo, P., Sae–Kwang, K. and Sung–Eun, L., 2002. Repellent activities of essential oils and monoterpenes against *Culex pipiens pallens*. Journal of the American Mosquito Control Association 18: 348–351.

Woodland, D., 1997. Contemporary Plant Systematics, 2nd edn. Andrews University Press, Berrien Springs, MI, USA.

Working Paper FGR/18E, 2001. State of Forest Genetic Resources in Kenya. FAO Sub–Saharan Africa Forest Genetic Resources Programme of the International Plant Genetic Resources Institute (IPGRI/SAFORGEN), Danish International Development Agency (DANIDA), DANIDA Forest Seed Centre (DFSC) and International Centre for Research in agroforestry (ICRAF), Forest Resources Division, FAO, Rome, Italy. http://www.fao.org/DOCREP/004/AB396 E/ab396e00.htm# Contents, as retrieved on 28 October 2003.

World Agroforestry Centre, 2003a. Agroforestry Database. The *Ekebergia capensis* http://www.worldagroforestrycetre.org/Sites/TreeDBS/Aft/speciesinfo.cfm?SpID=720, as retrieved on 6 October 2003.

World Agroforestry Centre, 2003b. The *Prunus* Net Database. http://www.world agroforestrycentre.org/Sites/TreeDBS/prunus/prunus.htm, as retrieved on 9 November 2003.

Wynn, S.G., 1999. Emerging Therapies: Using Herbs and Nutraceutical Supplements in Small Animals. AAHA Press, Boulder, CO, USA.

Xu, Z.Q., Xu, F.C. and Li, M.Q., 1993. Isolation and partial characterization of a protease from *Agave sisalana*. Acta Botanica Sinica 35: 171–178.

Yeoman, G.H. and Walker, J.B., 1967. The ixodid ticks of Tanzania. The Commonwealth Institute of Entomology, London, UK.

Yoder, J.A., Pollack, R.J. and Spielman, J.C., 1993. An anti–diversionary secretion of ticks: first demonstration of an acarine allomone. Insect Physiology 39: 429–436.

Young, A.S., de Castro, J.J., Burns, C.O.A. and Murphy, D.L., 1985. Potential of ear Tags impregnated with acaricides, for the control of the brown ear tick (*Rhipicephalus appendiculatus* Neumann 1901) infesting cattle. Parasitology 90: 391–399.

Young, A.S., Groocock, C.M. and Kariuki, D.P., 1988. Integrated control of ticks and tick–borne diseases in Africa. Parasitology 96: 403–432.

Young, A.S., Leitch, B.L. and Newson, R.M., 1981. The occurrence of a *Theileria parva* carrier state in cattle from an East Coast fever endemic area of Kenya. Proceedings of International Conference held at the International Laboratory for Research in Animal Diseases (ILRAD), Nairobi, Kenya (ed. by Irvin, A.D., Cunningham, M.P. and Young A.S.) Martinus Nijhoff Publishers, The Hague, The Netherlands., pp. 60–62.

Young, A.S., Leitch, B.L., Newson, R.M. and. Cunningham, M.P., 1986. Maintenance of *Theileria parva* infection in an endemic area of Kenya. Parasitology 93: 1–16.

Yumoto, T., Yamagiwa, J., Mwanza, N. and Maruhashi, T., 1994. List of Plant Species Identified in Kahuzi–Biega National Park, Zaire. Tropics 3: 295–308.

Zakir, U.R., Ahmad, S., Qureshi, S., Atiq, U.R. and Badar, Y., 1991. Toxicological studies of *Melia azedarach* L. (flowers and berries). Pakistan Journal of Pharmaceutical Sciences 4: 153–158.

Zar, J.H., 1996. Biostatistical Analysis, 3rd edn. Prentice Hall International Editions, Upper Saddle River, NJ, USA.

Zemede, A. and Tadesse, M., 2001. Prospects for sustainable use and development of wild food plants in Ethiopia. Economic Botany 55: 47–62.

Zimmerman, R.H., Garris, G.I. and Beaver, J.S., 1984. Potential of *Stylosanthes* plants as a component in an integrated pest management approach to tick control. Preventive Veterinary Medicine 2: 579–588.

Ziv, M., Sonenshine, D.E., Silverstein, R.M., West, Jr. and Gingher, K.H., 1981. Use of sex pheromone, 2, 6–dichlorophenol to disrupt mating by American dog tick, *Dermacentor variabilis*. Journal of Chemical Ecology 7: 829–840.

Zullo, M.A.T., Azzini, A., Salgado, A.L.B. and Ciaramello, D., 1989. Steroidal sapogenins in sisal. Bragantia, Campinas, v. 48: 21–26.

Zygadlo, J.A., Abburra, R.E., Maestri, D.M., Guzman, C.A., Grosso, N.R. and Espinar, L.A., 1993. Essential oil composition of *Tagetes terniflora* H.B.K. and *Tagetes laxa* Cabrera. Flavour and Fragrance Journal 8: 273–275.

Samenvatting

Dit proefschrift beschrijft de effecten van verscheidene etnobotanische plantenextracten op het gedrag van de teek *Rhipicephalus appendiculatus* (bruine oorteek), 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 klimgedrag 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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Although the journey to the PhD title was long, frustrating and tiresome, I can now look back on a long road of development of a scientific career with pride rather than with regrets. Kindly accept my apologies if I do not mention your kind contribution in this book, for the space is regulated and limited but I do really value your input to this work.

This work has been completed in the span of ten years and surely a great many people have contributed to its development in many special and different ways. I have interacted with many people either directly or indirectly discussing research ideas, funding problems, experimental design, results and their presentation, statistics etc. This teamwork mainly comprised, promotors (Profs. van Lenteren, Takken and Hassanali), coauthors, fellow PhD students, my students, workers at the Scholarship Network Centre, Nairobi, biostatisticians, Kenya government officials, ethnopractitioners and livestock farmers in Bungoma District, western Kenya, the Laboratory of Entomology stimulating family and Kenyans at Wageningen UR, my parents, my brothers, my sisters, my wife etc. To them all, I am very grateful for the fruitful interactions in a multi-trophic context.

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Curriculum vitae

Wycliffe Wanzala[1] 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 1st 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 1st 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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[1] correspondence: osundwa1@yahoo.com or wwanzala@hotmail.com

List of publications

Peer-reviewed publications

Kyule, M.N., Zessin, K.H., Baumann, M.P.O., **Wanzala, W.**, Sakwa, D. P. (2001) Survival analysis of the infective *haemonchus contortus* larvae (l_3) on pasture in central highlands in Kenya. In: Livestock Community and Environment. Proceedings of the 10th Conference of the Association of Institutions for Tropical Veterinary Medicine, Copenhagen, Denmark. http://www.aitvm.kvl.dk/E_periurban/E5Kyule.htm

Wanzala, W., Zessin, K.H., Kyule, M.N., Hassanali, A. and Mathias, E. (2001) The potential of traditional tick control practices used in livestock production. In: Newsletter, Berlin Alumni Network-BAN. Department of the International Animal Health, Freie University (FU), Berlin, Germany.

Wanzala, W., Onyango-Abuje, J.A, Kang'ethe, E.K., Ochanda, H., Harrison, L.J.S. (2002) Serodiagnosis of bovine cysticercosis by detecting live *Cysticercus bovis* using a monoclonal antibody-based antigen-ELISA. Journal of the South African Veterinary Association 74: 201-206.

Wanzala W., Saini, K. R., Hassanali, A., Ochanda, H.. Kabaru, J. M. (2002-2003) Evaluation and application of anti-tick natural products used by the Bukusu in livestock health management in Bungoma District. Animal Health, Ticks and Tick-borne Diseases, Highlights of 2002-2003 activities. International Centre of Insect Physiology and Ecology (ICIPE) Science Press, Nairobi, Kenya. http://www.icipe. org/home/62?task=view

Wanzala, W., Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H. and Harrison L.J.S. (2003) A comparison of meat inspection method and an Antigen-ELISA test in the diagnosis of bovine cysticercosis in Kenyan cattle. Journal of the Cameroon Academy of Sciences. 2: 265–277.

Wanzala, W., Zessin, K.H., Kyule, M.N., Baumann, M.P.O. Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H. and Harrison L.J.S. (2003) Analysis of post-mortem diagnosis of bovine cysticercosis in Kenyan cattle. Online Journal of Veterinary Research 1: 1-9.

Wanzala, W., Onyango-Abuje, J.A., Kang'ethe, E.K., Zessin, K.H., Kyule, N.M., Baumann, M.P.O., Ochanda, H. and Harrison, L.J.S. (2003) Control of Zoonotic parasite, *Taenia saginata* by post-mortem diagnosis of carcasses. Journal of African Health Sciences 3: 68–76.

Wanzala, W., Sika, K.F.N., Gule, S. and Hassanali, A. (2004) Attractive and repellent host odours guide ticks to their respective feeding sites. Chemoecology 14: 229-232.

Wanzala, W., Zessin, K.H, Kyule, N.M, Baumann, M.P.O, Mathias, E. and Hassanali, A., (2005) Ethnoveterinary medicine: a critical review of its evolution, perception, understanding and the way forward. Livestock Research for Rural Development. Volume 17, Article #119. As retrieved August 27, 2005 from http://www.cipav. org.co/lrrd/lrrd17/11/wanz17119.htm

Wanzala, W. Onyango-Abuje, J.A., Kang'ethe, E.K., Ochanda, H. and Harrison L.J.S. (2005) Distribution of *Cysticercus bovis* in bovine carcasses of naturally and artificially infected Kenyan cattle and its implication for routine meat inspection method. Online Journal of Veterinary Research 9: 66-73.

Wanzala, W. and Okanga, S. (2006) Ticks (Acari: Ixodidae) Associated with Wildlife and Vegetation of Haller Park along the Kenyan Coastline. Journal of Medical Entomology 43: 789-794.

Wanzala, W. Onyango-Abuje, J.A., Kang'ethe, E.K., K. H. Zessin, N. M. Kyule, M.P.O. Baumann, Ochanda, H., Harrison, L.J.S. (2007) Evaluation of an antigen-ELISA in the diagnosis of bovine cysticercosis in Kenyan cattle. Parasitology Research 100: 539-548.

Wanzala, W. (2008) Helping the Bukusu reclaim their 'hoofed' banks. In: Nurturing African Talents for Leadership in Insect Science: 25 years of the ARPPIS Experience. ISBN:9290642041. International Centre of Insect Physiology and Ecology (ICIPE) Science Press, Nairobi, Kenya.

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) Antifeedant 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://ismphpabstracts.blogspot. com/2008/04/oral-papers_22.html

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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)
- 5th 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