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Toxicity and repellent effects of leaf extracts of four Kenyan plants against nymphs and adults of Rhipicephalus appendiculatus (Neuman 1901)

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Abstract: *Ticks are important arthropod vectors of diseases of man and farm animals in Africa and in spite of the efforts to control them using synthetic acaricides, they continue to be amongst the leading vectors of disease-causing agents. Unfortunately, over-reliance on synthetic acaricides for tick control has led to problems including acaricide resistance, environmental pollution, and acaricide residues in products destined for human consumption. These problems highlight the need for alternative tick control methods that are environment friendly and yet effective. In the current study, the repellency and toxic effects of essential oils extracted from four Kenyan plants namely;Tagetes minuta L.(Asteraceae), Fuerstia. africana T.C.E. Friers (*Lamiaceae*), Tephrosia vogelii Hook. f. (Leguminosae) and Sphaeranthus ukambensis against the ixodid tick Rhipicephalus appendiculatu were determined. Essential oils were extracted by steam distillation from fresh plants using Clevenger apparatus. Host seeking nymphs (2-3 weeks old)and adult ticks were used and fumigant toxicity bioassay was done using four concentrations (0.1µ/ml, 0.01 µ/ml, 0.001 µ/ml, and 0.0001µ/ml) of the essential oils, each in four replicates and with ten ticks per replicate. In the control experiment, ticks were exposed to n-hexane. Mortality was recorded every 48 hours for 2 weeks. In repellency bioassay, a Y tube olfactometer and same concentrations as above were used. Treated paper discs were placed in one arm and control (discs treated with hexane) in the other. Ticks were introduced at the main arm and allowed to make choices on the direction of movement. Ticks on the two arms were counted after 30 minutes. The essential oils showed dose dependant mortality and repellency values. The highest repellency value for R. appendiculatus adults was observed with T. vogelii (93.8% at 0.01µ/ml) while for the nymphs, both S. ukambensis and T. vogelii recorded the highest repellency values of 100% and 95% respectively both at 0.1µ/ml concentration. Essential oils from Fuerstia. africana recorded the highest mortality values for nymph (97.8% at 0.1µ/ml) and adult (53.2 % 0.1µ/ml) Rhipicephalus appendiculatus . The present study has demonstrated that Tephrosia vogelii, Tagetes minuta and Sphaeranthus ukambensis have repellent and toxic effects on Rhipicephalus appendiculatus and therefore can be developed further for tick control and management.*

Keywords: *Essential oils, Fuerstia Africana, Repellency bioassay, Toxicity bioassay, Rhipicephalus appendiculatus*

I. Introduction

Rhipicephalus appendiculatus is a livestock pest of major economic importance in Africa where it is the chief vector of the pathogen Theileria parva (Apicomplexa: Theileridae) which causes East Coast fever [2] East coast fever leads to high mortality and morbidity rates, high costs of production due to costs involved in controlling the vector and high costs of chemotherapeutic drugs used to control it [3]. The disease is fatal in cattle and has been reported in 11 countries in East, Central and Southern Africa. The estimated direct economic loss associated with theileriosis is enormous with 1.1 million cattle deaths due to the disease annually [3].

The existing methods of tick control rely heavily on chemical acaricides and repellants. Although these methods have been effective in suppressing tick populations, their main disadvantages have been the high costs relative to the value of cattle and cattle products, the development of tick resistance to various ranges of acaricides among other challenges.

The problems associated with the use of synthetic acaricides has prompted a search for alternative methods of tick control that can be used alone or in combination with other tick control methods in an integrated tick management strategy [2,3].

Botanicals have been used in nature for millions of years without any adversative effects on the ecosystem. For instance repellency of plant material has been exploited for thousands of years by man by hanging bruised plants in houses, a practice that is still in wide use throughout the developing countries. Moreover, plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes which was first recorded in writings by ancient Greek, Roman and Indian scholars [9]. Pesticidal plants were widely used until the 1940s, when they were replaced by synthetic pesticides that were easier to handle and lasted longer. The consumption of synthetic pesticides in some of the developed countries is almost 3000 g/hectare. Overenthusiastic use of synthetic insecticides led to problems unforeseen at the time of their introduction. It was reported by the World Resources Institute that more than 500 insect and mite species are resistant to one or more. As pests become tolerant to insecticides, double and triple application rates are needed to maintain control [5]. Synthetic pesticides are generally persistent in nature. The World Health Organization (WHO) estimates that 200,000 people are killed worldwide, every year, as a direct result of pesticide poisoning. Moreover, the use of synthetic chemicals has been restricted because of their carcinogenicity, teratogenicity, high and acute residual toxicity, ability to create hormonal imbalance, spermatotoxicity, long degradation period, and result in toxic residues in food [6, 7, 8]. Repetitive use of synthetic pesticides has resulted in pesticide residue hazards, upsetting the balance of nature through disruption of natural enemies, pollinators and other wild life, extensive groundwater contamination, evolution of resistance and resurgence of treated populations, outbreaks of secondary pests, i.e. those normally kept under control by their natural enemies [6].

Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants. Steam distillation of aromatic plants yields essential oils, long used as fragrances and favoring in the perfume and food industries, respectively.

1.1 Mode of action of essential oils

Essential oils interfere with basic metabolic, biochemical, physiological, and behavioral functions of Arthropods. They inhale, ingest or absorb essential oils. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine [9] or GABA-gated chloride channels [8]Some essential oils have larvicidal effects and the capacity to delay development and suppress emergence of adults of insects of medical and veterinary importance [10, 11, , 12, 13, 14, 15].

In fact some of the essential oils such as thyme oil and monoterpenoids including thymol, anethole, eugenol, and citronellal combinations are being developed for cockroaches and the green peach aphid control and citronellal, cotronellol, citronellyl or a mixture of these are being developed for the human louse [16].

II. Materials And Methods

1.1. Plant materials

Fresh plants materials were collected in may 2009 from Machakos County, Kenya. The plants were authenticated by comparison with herbarium specimen. The specimen were given voucher numbers and the samples are kept at zoology laboratory (zoology department), Jomo Kenyatta University of agriculture and technology. The voucher numbers were given as indicated below:

Plant	**Voucher number**
Fuerstia africana	GKO/01
Tephrosia vogelii	GKO/02
Tagetes minuta	GKO/3
Sphaeranthus ukambensis	GKO/4

1.2. Isolation of oils

The essential oil extract was isolated from the plant materials by steam distillation using Clevenger apparatus [1] The condensing oils were collected in n-hexane solvent (Aldrich HPLC grade) and the solution was filtered using Whattmann grade 1 filter papers containing anhydrous sodium sulphate in a funnel to remove any remaining traces of water. Hexane was then removed by distillation at 60 ºC from 'Contes' Short Path distillation apparatus. When condensation stopped, the oil was collected and weighed into small amber coloured vials.

1.3. Preparation of the essential oil extracts

The stock solution was made by dissolving 100µl of the test essential oil extract in 1000 µl hexane to obtain a concentration of 0.1µl/µl. Subsequent lower concentrations (0.011µl/µl, 0.0011µl/µl, and 0.00011µl/µl) were made by diluting the stock solution 10, 100 and 1000 fold.

1.4. Preparation of the R. appendiculatus ticks for bioassays

The R. appendiculatus used were obtained from ICIPE in sectary laboratories. Prior to the bioassays the ticks were allowed to acclimatize to the ambient laboratory conditions for 48 hrs.

1.5. Fumigant toxicity bioassay

This method was used to test the bioactivity of essential oils as fumigants. Glass vials were used with each vial containing 10 unsexed R. appendiculatus adults and covered with a mesh. 40µ of different concentrations (0.1µl/µl, 0.01µl/µl, 0.001µl/µl, 0.0001µl/µl of the essential oil was applied on a 2 cm x 2 cm filter papers respectively. Each of these filter papers was placed in the vials containing the ten ticks. The fumes or the volatiles saturated the atmosphere of the vials. In the control filter papers without essential oils were placed in the glass vials. The set up was replicated 4 times and mortality recorded after every 24 hours for two weeks. The same set up was used in the bioassay for nymphs.

1.6. Repellency bioassay

The essential oil concentrations used were: 0.1µl/µl, 0.01µl/µl, 0.001µl/µl, and 0.0001µl/µl. A Y tube apparatus (olfactometer) was used. On one side of the arms of the tube a piece of filter paper soaked in 40µl of the crude extract was used? as the treatment. The other side of the tube was used as the control where a piece of filter paper was soaked in the solvent. The filter papers were soaked and allowed to dry before they were used. Ten unsexed newly hatched adult ticks were placed at point A of the Y tube. After 30 minutes the number of ticks on the treatment and control side were recorded. Those ticks which did not make any choice were also recorded. After two trials (replicates) the Y tube was thoroughly washed with clean water and then dried. Two more replicates were then done with the positions of the treated and control filter papers interchanged. The same set up was used to test repellency for nymphs.

The bioassays were conducted under the average laboratory conditions (25 ± 5˚C and 60 ± 10% RH and under a natural day/night regimen.

1.7. Statistical analysis

The positions of ticks were recorded after 30minutes. Ticks that were found on the control filter paper will be considered repelled. Those on the treated filter paper were considered not repelled. The ticks which did not make any choice were considered repelled.

Percent repellency (PR) values were computed using the formula

$$\left(\frac{NC-NT}{NC+NT}\right)\times 100$$

Where;
PR= percent repellency
NC=Number of ticks on the control
NT=Number of ticks on the treatment

PR data was arcsine transformed and analysed using one way analysis of variance (ANOVA). Negative values were treated as zero. LC was determined using probit analysis. All analyses were carried out using SAS software version 9.2[35].

1.8. Results

1.8.1. Toxicity

A significant dose dependent mortality was observed for the essential oils with bothadults and nymphs. There were significant differences in mortality in both cases at 95% confidence interval with Tukey grouping. In adults the highest mortality (53.2%) was achieved through the activity of essential oils from F. africana at a concentration of 0.1µ/ml. This was followed by T. minuta (41.6%). The lowest mortalities were recorded in S. ukambensis (25.9%) and T. vogelii (22%) at the same concentration (Table 1).

Among the nymphs, highest mortality was observed in F. Africana (97.7%) at the concentration of 0.1µ/ml (Table 2).

Overall, mortality in essential oils was higher in nymphs compared with adults. In both cases highest mortalities were observed in the highest concentration (0.1µ/ml). Among the essential oils from the four test plants, those from F. africana gave the highest tick mortality (Tables 1 and 2).

Mortality in this study was age dependent, showing higher vulnerability of the juvenile developmental stage (nymphs) compared with the adults.

Table1.: Adulticidal activity of essential oils against R. appendiculatus adults at 95% confidence interval

Plant	Concentration	Mean ± SE*	LC 50
Fuesrtia Africana	0.0001 µ/ml	1.8 ± 0.5^{cb}	0.096
	0.001 µ/ml	10.5 ± 1.7^{b}	
	0.01 µ/ml	1.4 ± 0.5^{c}	
	0.1 µ/ml	53.2 ± 4.5^{a}	
Tephrosia vogelii	0.0001 µ/ml	3.0 ± 1.1^{b}	0.201
	0.001 µ/ml	2.1 ± 0.8^{b}	
	0.01 µ/ml	18.6 ± 2.1^{a}	
	0.1 µ/ml	22 ± 2.9^{a}	
Tagetes minuta	0.0001 µ/ml	10.5 ± 1.7^{b}	0.121
	0.001 µ/ml	13.0 ± 1.8^{b}	
	0.01 µ/ml	11.4 ± 1.6^{b}	
	0.1 µ/ml	41.6 ± 2.9^{a}	
Sphaeranthus ukambensis	0.0001 µ/ml	12.9 ± 1.8^{c}	0.180
	0.001 µ/ml	3.4 ± 1.1^{c}	
	0.01 µ/ml	5.5 ± 1.0^{b}	
	0.1 µ/ml	25.9 ± 2.4^{a}	

SE*=Standard error

Table 2: Mean mortality of essential oils against R. appendiculatus nymphs at 95% confidence interval

Plant	Concentration	Mean ± SE*	LC 50
Fuesrtia Africana	0.0001 µ/ml	2.3 ± 0.6^{c}	0.041
	0.001 µ/ml	11.6 ± 1.7^{b}	
	0.01 µ/ml	15.18 ± 1.2^{b}	
	0.1 µ/ml	97.86 ± 1.1^{a}	
Tephrosia vogelii	0.0001 µ/ml	23.93 ± 2.0^{c}	0.035
	0.001 µ/ml	15.89 ± 1.7^{c}	
	0.01 µ/ml	41.25 ± 2.6^{b}	
	0.1 µ/ml	89.46 ± 2.7^{a}	
Tagetes minuta	0.0001 µ/ml	10.54 ± 1.0^{b}	0.053
	0.001 µ/ml	14.64 ± 1.2^{b}	
	0.01 µ/ml	14.46 ± 1.2^{b}	
	0.1 µ/ml	85.53 ± 2.6^{a}	
Sphaeranthus ukambensis	0.0001 µ/ml	15.18 ± 1.9^{b}	0.055
	0.001 µ/ml	16.25 ± 2.1^{b}	
	0.01 µ/ml	16.25 ± 2.2^{b}	
	0.1 µ/ml	80.89 ± 2.8^{a}	

SE*=Standard error

1.8.2. Repellency

A significant dose dependent repellency response was observed for the essential oils from all plants. In both nymphs and adults, highest percentage repellency was observed in concentration 0.1 µ/ml. (Tables 3 and 4). There were significant differences in repellency in both cases at $p<0.005$.

In adults highest percentage repellency was observed with essential oils from T. vogelii (93.8%) followed by S. ukambensis (60%). Percentage repellency values for essential oils from the remaining two plants was below 50%; F. africana (40.7%) and T. minuta (41.7%) (Table 3).

In nymphs the highest percentage repellency was observed in S. ukambensis (100%), followed by IR(95%) both at concentration 0.1 µ/ml. the least percentage repellency value was recorded with essential oils from F. Africana (65.7%). Overall, T. vogelii gave the highest percentage repellency value both in nymphs and adults.

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Table 3: Mean percentage repellency and SE values of essential oils after 30 minutes against R. appendiculatus adult

		Concentration			
plant	**0.1 µ/ml**	**0.01 µ/ml**	**0.001 µ/ml**	**0.0001 µ/ml**	**LC 50**
F. africana	40.7± 7.4 ba	11.1ba	77.8 a	23.7 ± 8.5ba	0.217
T. vogelii	93.8 ± 6.3a	25.3 ±5.9b	23.3 ±16.6b	37.2 ± 7.4b	0.029
T. minuta	41.7± 30.0a	17.8 ± 9.7a	43.9 ± 9.0a	15.6 ± 7.2a	0.029
S. ukambensis	60.0 ± 8.2a	41.7± 15.3a	30.1 ± 8.6a	33.6 ± 15.5a	0.157

Table 4: Mean percentage repellency and SE values of essential oils after 30 minutes against R. appendiculatus nymphs

	Concentration				
plant	**0.1 µ/ml**	**0.01 µ/ml**	**0.001 µ/ml**	**0.0001 µ/ml**	**LC 50**
F. africana	65.7± 5.7a	0	25a	12.5± 12.5a	0.075
T. vogelii	95.0± 5.0a	64.6± 20.5ba	22.9 ±7.9b	20.7± 9.6b	0.019
T. minuta	67.6± 22.2a	36.1 ±7.3a	22.9± 7.9a	13.3± 13.3a	0.061
S. ukambensis	100.0± 0.0a	30.0 ±12.9b	66.3± 19.7ba	49.5± 9.6ba	0.005

III. Discussion

Currently used fumigants including phosphine, methyl bromide, and DDVP (2, 2-dichlorovinyl dimethyl phosphate) provoke some safety concerns. Phosphine is the major cause of suicidal deaths in India. Methyl bromide has ozone-depleting potential and DDVP has a possible human carcinogen potential [17]. As a consequence, there is an increasing need for the development of safe alternatives that could replace the toxic fumigants for protection against pests. Insect resistance to phosphine is also a matter of serious concern [18, 6]. The present study demonstrated that essential oils from F. africana had more toxic effects on R. appendiculatus nymphs (97.7%) and adults (52.3%) compared with T. minuta (41.6%), T. vogelii (22%) and S. ukambensis (25.9%) at the highest concentration of o.1µ/ml. based on the results obtained from the GC-MS analysis of these essential oils, it is possible that ticks were killed by one or many of these compounds present.

Terpenes in the T. minuta oil have been reported to be responsible for the toxic effects reported in mosquitoes [6] Also, insecticidal activity of T. minuta oil has been reported against stored product pests [19,20] The T. minuta oil has been reported to have aphicidal properties [21] however none has been mentioned on ticks and especially R. appendiculatus. Findings from this study indicate that essential oils from T. minuta recorded a high mortality response with R. appendiculatus nymphs (85%) than adults (41. 6%) at the concentration of 0.1µ/ml.

Studies on essential oils have indicated toxic effects of essential oils on non tick pests. For instance, essential oils, such as clove, rosemary, thyme, eucalyptus, and various mint species have demonstrated contact and fumigant toxicity to a wide spectrum of insects, including human head lice [22]. Essential oils and their isolates have fumigant action, such as essential oil of Artemisia annua, Anethum sowa, Curcuma longa, Lippia alba and isolates like d-limonene, carvones and 1,8-cineole have been well documented as fumigants [23]

Some essential oils express bioactivity against stored product pests, such as oils of basil, citrus peel, eucalyptus, various mint species, lavender, and rosemary, but not all essential oils are active against all the insect pests [24]. Nutmeg oil has been determined to significantly impact both the maize weed, Sitophilus zeamais and the red-flour beetle, Tribolium castaneum and demonstrates both repellent and fumigant properties [25] The exact mode of action of these oils as fumigant is unknown, but the oils mainly act in the vapor phase via respiratory system [6, 14, 23].

Findings from the current study also have proven that essential oils from two (T. vogelii and S. ukambensis) of the four test plants can be used as repellents in the control of R. appendiculatus. This is due to the high repellency values in both adults essential oils from T. vogelii (93.8%) followed by S. ukambensis (60%). Percentage repellency values for essential oils from the remaining two plants was below 50%; F. africana (40.7%) and T. minuta (41.7%) (Table 4).

Essential oils extracted from other plants have been in use in pest control. For instance, essential oils of Cinnamomum camphora, C. cassia, and C. zeylanicum repel mosquitoes. Oils of soybean, lemongrass, cinnamon, and the compounds p-menthane-3,8-diol (from lemon eucalyptus), citronellal (from lemongrass), and 2-phenethylpropionate (from groundnut), are effective against mosquitoes [26]. Large number of essential oils repels arthropod species. Flea and tick control products for companion animals based on d limonene, a constituent of citrus peel oil, or oils of peppermint, cinnamon, clove, thyme, and lemongrass, have been introduced recently. Essential oils have pronounced in vitro and in vivo pediculicidal activity as the number of lice infesting water buffaloes in Egypt was significantly reduced 3, 6, 4, and 6 days after treatment with the essential oils of camphor (Cinnamomum camphora), peppermint (Mentha piperita), chamomile (Matricaria chamomilla), and onion (Allium cepa), respectively. The same oils repelled flies (Musca domestica, Stomoxys calcitrans, Haematobia irritans, and Hippobosca equine) infecting buffaloes for almost 6 days post-treatment. No adverse effects were noted on either animals or on pour-on operators after exposure to the applied oils [13].

A patented natural repellent based on nepetalactone and dihydronepetalactone obtained from Nepeta cataria that is effective against cockroaches, mosquitoes, mites, ticks, and other household insects [27, 28]. Nootkatone from Vetiver oil and its derivatives, tetrahydronootkatone and 1, 10-dihydronootkatone have been patented as repellent against mosquitoes, cockroaches, termites, and ants [29, 30].

Specific constituent compounds obtained from essential oils have proven activity against arthropod pests and ticks in particular. PMD (para-menthane 3, 8 diol) as a repellant has a lower vapour pressure than volatile monoterpenes found in most plant oils [37] it provides very high protection from a broad range of insect vectors for several hours [36] and has a proven safety to human health.

In addition, cineole, geraniol and piperidine found in bay leaves, Laurus nobilis, possess repellent properties towards cockroaches. d-Limonene is heavily used for controlling structural pests as termite in California, and other plant oils (clove, peppermint, etc.). Linalool is toxic to the eggs and larvae of insects. Limonene found in sour oranges (Citrus aurantium) is toxic to adult bean weevils (Callosobruchus phasecoli [34]. β -Ocimene is repellent to the leaf- cutter ant, Atta cephalotis, in both field and laboratory experiments [31].

Experiments with the aphid, Carvariella aegopodii, which feeds on the aromatic Apiaceae (Apiales) species in summer, indicate that the aphid are repelled by linalool [30,]

Several compounds that have proven repellence effects against R. appendiculatus were constituent compounds of the essential oils used in this study. The synergistic effects of these compounds in the essential oils might have contributed to the high repellency values observed. A synergistic phenomenon among metabolites of essential oils may result in a higher bioactivity as minor constituents found in low percentages may act as synergists, enhancing the effectiveness of the major constituents through a variety of mechanisms. For instance the repellent activity of the mixture of essential oils from Artemisia princeps and Cinnamomum camphora against the adult weevils, Sitophilus oryzae and Bruchus rufimanus was significantly higher than that elicited by individual oils [33].

The essential oils involved in the current study can be in the development of repellents that can be used commercially in the management of R. appendiculatus in Kenya and the rest of the world.

IV. Conclusion

It is concluded (from the data presented on plant volatile and their effects on ticks) that each plant species had its own chemical niche of activity against ticks.

Although the need to know the effect of each individual chemical constituent on ticks exists, the crude essential oil possessed increased bioactivity compared to its individual constituents. It is therefore reasonable to deduce that the repellent and toxic effects of the essential oil of the four plants used in this study were a result of the synergy among the chemical constituents.

In summary, the results of this study, further strengthens the view that T. vogelii, T. minuta and S. ukambensis are potential sources of anti-tick agents and also to some extent validates the traditional use of the plants for insect pest control by the farmers in livestock keeping areas in Kenya. However, it is important that future studies should include field trials in order to confirm laboratory results.

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