



Bioacaricidal effects of three volatile oils on cattle ticks

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Abstract

Use of synthetic chemicals in addressing livestock attacks by ticks has negative effects on animals, handlers and the environment. These problems necessitate search for potent alternatives in bioacaricides particularly volatile oils. Acaricidal effects of hydro-distilled volatile oils from the fresh leaves of *Eucalyptus citriodora*, *Ocimum gratissimum* (OG) and *Callistemon citrinus* were evaluated on ticks obtained from infested cattle using topical method at varying concentrations in acetone. For each concentration, ten ticks of similar species were placed in Petri dishes and administered 20µL of the mixture of volatile oil and acetone. Acetone (20µL) was used as control and the effects were observed for 24 h. Out of three tick species identified, *Rhipicephalus microplus* was most abundant and most sensitive to the oils. Others are *Amblyomma hebraeum* and *Hyalomma rufipes* (most resistant). The volatile oil of OG was the most potent with LC₅₀ of < 2 % (< 0.4 µL), < 2 % and 7.97% (1.59 µL) on the organisms respectively. The 5% of OG produced 100% mortality on *R. microplus* in 10-25 min while 20% concentration produced 100% mortality on *H. rufipes* in 24 h. Volatile oils of these plants particularly OG can serve as potent alternative bio-acaricidal agents to chemicals.

Keywords: Acaricidal agents; *Ocimum gratissimum*; *Rhipicephalus microplus*

INTRODUCTION

Ticks are blood-sucking ectoparasites whose infestation threatens the economic values of domestic animals like cattle, goat, sheep, camel etc. The problems caused by various species of ticks take different forms which sometimes extend to causing infections to man through human diseases vectored by the organisms. Ticks feeding on animals can result to devastating economic losses directly through the effects they inflict such as anemia, paralysis, toxicosis, reduced quality of leather and transmission of some diseases [1]. Aside these effects, many tick-borne diseases also do

pose daunting challenges to the wellbeing of the animals leading to multiple losses [2]. The organisms serve as vectors to some bacterial, protozoal, and viral diseases which also affect humans.

Africa has been identified as playing host to about 50 different species of ticks three of which have been reported to possess highest impact on the wellbeing, economic value and production of livestock [3]. The three species include *Amblyomma*, *Hyalomma*, and *Rhipicephalus* [4]. Literature reports have shown that *R. microplus* (*Boophilus microplus*) is the most dreaded ectoparasite of

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cattle with significant economic losses [5,6]. This parasite has been implicated to be a major vector of highly pathogenic *Anaplasma marginale*, *Babesia bovis* (and *B. bigemina*) which are the causes of bovine anaplasmosis and babesiosis respectively with high prevalence in some parts of Africa including Ghana[7]. Some cattle in Northern Nigeria, particularly the Sokoto and Zamfara States have recently been reported to high prevalence (44-76%) of three species of *Hyalomma* viz: *H. turanicum* (not previously reported in Nigeria), *H. rufipes* and *H. dromedarii*. DNA molecular analyses of the ticks revealed they harbor *Theileria annulata*, the causative agent of theileriosis [8]. All these are in addition to a plethora of tick-borne human diseases.

Aside inadequate and inefficient traditional methods of tick control, synthetic acaricidal agents have been found effective. However, demerits like tick resistance, high costs, unexpected toxic effects on man, non-target organisms and the environment have plagued their applications in many communities [9]. For instance, research reports have shown ivermectin-resistant strain of *R. microplus* in some countries [10]. Therefore, it becomes highly imperative to beam research search light on locally available medicinal plants particularly those that naturally contain volatile oils (essential oils) that are human and environment friendly with rapid action and biodegradation. Also, as there is dearth of information on the applications volatile oils in controlling ticks in Nigeria, this work was aimed at evaluating the acaricidal effects of volatile oils of *Ocimum gratissimum*, *Eucalyptus citriodora* and *Callistemon citrinus* fresh leaves.

EXPERIMENTAL METHODS

Collection and extraction of the plant materials. Fresh leaves of *Ocimum gratissimum* were obtained from Uselu markets, Benin-City while fresh leaves of *Eucalyptus citriodora* and *Callistemon citrinus*

were collected in April from University of Benin, Ugbowo Campus. The fresh leaves of each plant were separately extracted with Clevenger-type apparatus using steam distillation method. About 3303.71g of fresh leaves of *E. citriodora* were extracted in batches while 6112.4 and 1882.14g of fresh leaves of *C. citrinus* and *O. gratissimum* were also extracted in batches separately. As the volatile oil obtained from each plant is less dense than water, it floats and so the volume was read off from the calibration on the receiving arm of the apparatus. The volatile oils were collected separately using sample bottles and stored in the refrigerator maintained at 4°C.

Collection of the ectoparasites (ticks). With the assistance of Fulani cattle herdsman, the ticks were obtained from infected animals at the cattle markets located along Benin Technical College road and Aduwawa, Benin City. The ecto-parasites were collected by handpicking from the skin of the cattle and kept in aerated plastic containers. The ticks were separated into different species and their identities were established **with the use Tick Atlas** and further confirmed by Professor M A O Bamikole, Animal Scientist, Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City. Three different species were identified.

Preparation and administration of different concentrations of the volatile oil. Each of the volatile oils obtained from the plants was serially diluted into different concentrations of 2, 5, 10, and 20% in acetone. For example, to prepare a 20% concentration of the oil in 1mL, 0.2 mL of the volatile oil was added to 0.8 mL acetone. The solvent (acetone) was used because of its inertness and rapid evaporation. Ten (10) ticks of the same species were placed in a Petri dish and using a graduated micropipette, 20 µL of each of the concentrations was topically dispensed unto each of the ticks and observed for the subsequent effects. A volume of 20 µL of

acetone was used as control. The parasites were observed for 24 hours and the mortality rate was recorded along with the time it took each of the ticks to become completely motionless. The same procedure was carried out on the other species of ticks available and for all the three volatile oils in triplicates.

RESULTS

At the end of the steam distillation of the volatile oils with Clevenger-type apparatus, 3303.71 g of fresh leaves of *E. citriodora* produced 24.4 mL of the volatile oil corresponding to 0.74% while the fresh leaves of *C. citrinus* (6112.4g) and *O. gratissimum* (1882.14g) yielded 32.7 mL and 11.3 mL of the volatile oil respectively. These implied 0.53 and 0.6% respectively.

Identities of the ticks. The identities of the three species of ticks used were established by comparison with pictures of the species. These include *Rhipicephalus microplus* (Plates A and B); *Amblyomma hebraeum* (Plates C and D); and *Hyalomma rufipes* (Plates E and F). The *R. microplus* was observed to be most abundant in the population.

Acaricidal effects of the volatile oils on the ticks.

Eucalyptus citriodora. While no mortality was noticed in the ticks treated with acetone, the volatile oil of the plant was observed to exhibit lethal effects on the various species of ticks in a significantly ($P < 0.05$) concentration-dependent manner at varying times. At a concentration of 2% (containing 0.4 μL of the volatile oil), the volatile oil produced a mortality of $46.67 \pm 12.02\%$ on *R. microplus* in 24 h. This increased to $86.67 \pm 6.67\%$ with 5% (containing 1 μL of the volatile oil) concentration in 24 h. However, 100% mortality was attained at concentrations of 10% and 20% (2 and 4 μL of the volatile oil respectively) which occurred within 2-10 min. With *A. hebraeum*, at concentrations of 5 and 10%, the volatile oil produced 50 ± 5.77 and

$73.33 \pm 3.33\%$ mortalities respectively in 24 hours while 100% mortality was achieved with 20% concentration in 20-30 min. However, 20% concentration of the volatile oil produced only $55 \pm 5.00\%$ mortality on *H. rufipes* in 24 hours. Reapplication of the volatile oil yielded to 100% mortality in 2 h (Table 1).

Ocimum gratissimum. The volatile of the plant also produced significant ($P < 0.05$) increase in percentage mortalities with increase in concentration. Also, the ticks exhibited different susceptibilities to the constituents of the volatile. At a concentration of 2% (containing 0.4 μL of the volatile oil), a mortality of $86.67 \pm 6.67\%$ was produced by the volatile on *R. microplus* in 24 h. This was found to increase to 100% was on the ticks treated with 5 or 10% of the volatile in 20 μL acetone which was achieved in 5-25 min. Treating *A. hebraeum* with the 2 and 5% concentrations produced 56.67 ± 6.67 and $96.67 \pm 3.33\%$ respectively in 24 hours. Mortality of 100% was achieved in 30 min. with concentration of 10% (i.e. 2 μL of the oil). Also, at a concentration of 10%, the volatile oil produced $56.67 \pm 3.33\%$ in 24 hours on *H. rufipes* which on reapplication, produced 100% mortality within 20-30 minutes. The 20% concentration produced 100% mortality in 24 h. The ticks were not affected by treatment with 20 μL acetone used as control (Table 2).

Callistemon citrinus. The volatile oil of *C. citrinus* was observed to impact a non-significant but concentration dependent increase in mortality with increase in concentration of the volatile oil on *R. microplus*. At concentrations of 2, 5 and 10% the volatile oil produced 60 ± 0.00 , 80 ± 10.00 and $83.33 \pm 6.67\%$ mortalities respectively in 24 h after treatment. Reapplication of the respective concentrations produced 100% mortality in all the groups observed for another 24 h. However, 20% of the volatile oil gave 100% mortality within first 24 h of exposure.

Treating different groups of *A. hebraeum* with 2, 5, 10, and 20% concentrations of the oil produced 20 ± 5.77 , 36.67 ± 6.67 , 56.67 ± 3.33 and $73.33 \pm 3.33\%$ mortalities respectively within 24 h of application. Reapplication of the various concentrations led to 100% mortality in each the groups in 24 h. For *H. rufipes*, 20% concentration of the oil gave $70 \pm 0.00\%$ mortality in 24 h and 100% mortality after reapplication and observation for another 24h (Table 3).

The volatile oil of *E. citriodora* was observed to have LC_{50} of 2.46 % (0.49 μ L); 5 % (1 μ L) and 19.13 % (3.83 μ L) on *Rhipicephalus microplus*, *Amblyomma hebraeum* and *Hyalomma rufipes* respectively while the volatile oil *O. gratissimum* produced LC_{50} of < 2 % (i.e. < 0.4 μ L); < 2 % (< 0.4 μ L) and 7.97 % (i.e. 1.59 μ L) on the three ticks respectively. Also, the volatile oil of *C. citrinus* produced LC_{50} of < 2 % (i.e. < 0.4 μ L); 8.33 % (i.e. 7.67 μ L) and 17.11 % (i.e. 3.42 μ L) on the ticks (Table 4).



Plate: A



Plate: B



Plate: C



Plate: D



Plate: E

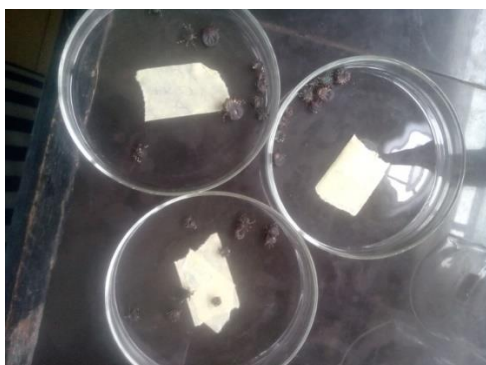
A= *Rhipicephalus microplus*C= *Amblyomma hebraeum*E= *Hyalomma rufipes*

Plate: F

B= *Rhipicephalus microplus* (from cattle ranch in Benin-City)D= *Amblyomma hebraeum* (from cattle ranch in Benin-City)F= *Hyalomma rufipes* (from cattle ranch in Benin-City)**Table 1:** The lethal effects of the volatile of *Eucalyptus citriodora* on the different species of ticks

% volatile oil in 20 μ L acetone	Mean % mortality (with time)		
	<i>Rhipicephalus microplus</i>	<i>Amblyomma hebraeum</i>	<i>Hyalomma rufipes</i>
2	46.67 \pm 12.02 (24 h)	NA	NA
5	86.67 \pm 6.67 (24 h)	50.00 \pm 5.77 (24 h)	NA
10	100 \pm 0.00 (10 min.)	73.33 \pm 3.33 (24 h)	NA
20	100 \pm 0.00 (2-7 min.)	100 \pm 0.00 (20-30 min.)	55 \pm 5.00 (24h) 100 \pm 0.00 (2h)*
Acetone (20 μ L)	0	0	0

NA= Not available * = Reapplication after 24 h

Table 2: Susceptibility of the ticks to volatile oil of *Ocimum gratissimum*

% volatile oil in 20 μ L acetone	Mean % mortality (with time)		
	<i>Rhipicephalus microplus</i>	<i>Amblyomma hebraeum</i>	<i>Hyalomma rufipes</i>
2	86.67 \pm 6.67 (24 h)	56.67 \pm 6.67 (24 h)	NA
5	100 \pm 0.00 (20-25 min.)	96.67 \pm 3.33 (24 h)	40 \pm 0.00 (24 h)
10	100 \pm 0.00 (5-10 min.)	100 \pm 0.00 (24 h)	56.67 \pm 3.33 (24 h) *100 \pm 0.00 (20- 30 min.)
20	NA	NA	100 \pm 0.00 (24 h)
Acetone (20 μ L)	0	0	0

* = after reapplication, NA = Not available

Table 3: Susceptibility of the ticks to volatile of *Callistemon citrinus*

% volatile oil in 20 μ L acetone	Mean % mortality (with time)		
	<i>Rhipicephalus microplus</i>	<i>Amblyomma hebraeum</i>	<i>Hyalomma rufipes</i>
2	60 \pm 0.00 (24 h)	20 \pm 5.77 (24 h)	NA
	*100 \pm 0.00 (24 h)	*100 \pm 0.00 (24 h)	
5	80 \pm 10.00 (24 h)	36.33 \pm 6.67 (24 h)	NA
	*100 \pm 0.00 (24 h)	*100 \pm 0.00 (24 h)	
10	83.33 \pm 6.67 (24 h)	56.67 \pm 3.33 (24 h)	NA
	*100 \pm 0.00 (24 h)	*100 \pm 0.00 (24 h)	
20	100 \pm 0.00 (24 h)	73.33 \pm 3.33 (24 h)	70 \pm 0.00 (24 h)
		*100 \pm 0.00 (24 h)	*100 \pm 0.00 (24 h)
Acetone (20 μ L)	0	0	0

* = after reapplication, NA = Not available

Table 4: The LC₅₀ of the volatile oils against the cattle ticks

Volatile oil	LC ₅₀ % (volatile oil concentration in 20 µL)		
	<i>Rhipicephalus microplus</i>	<i>Amblyomma hebraeum</i>	<i>Hyalomma rufipes</i>
<i>Eucalyptus citriodora</i>	2.46 (0.49 µL)	5 (1 µL)	19.13 (3.83 µL)
<i>Ocimum gratissimum</i>	< 2 (< 0.4 µL)	< 2 (< 0.4 µL)	7.97 (1.59 µL)
<i>Calistemon citrinus</i>	< 2 (< 0.4 µL)	8.33 (1.67 µL)	17.11 (3.42 µL)

DISCUSSION

The various shortcomings of synthetic acaricidal agents necessitate urgent and continuous search for human and environment friendly and available acaricide for the control of ticks infestation in cattle and other domestic animals. The volatile oils obtained from *Eucalyptus citriodora*, *Callistemon viridis*, and *Ocimum gratissimum* used in this work were potent against three the different species of ticks identified as *Rhipicephalus microplus*, *Amblyomma hebraeum* and *Hyalomma rufipes* at different concentrations. The volatile oils displayed varying acaricidal potentials which were largely dependent on the susceptibilities or resistance of the ticks and also on the composition and concentrations of the constituents.

R. microplus was observed to be more abundant in this study. The organism has been described the most common tick species of domestic cattle all over the world and its resistance to effective control has led to proliferation of many synthetic acaricides with attending side effects [11]. Earlier reports have indicated its rapid spread across the West African Sub region particularly with its ability to displace other species in the same genus, it is now found in other regions in Africa [12-14].

From the results obtained, the volatile oil obtained from *Eucalyptus citriodora* gave 100% mortality at 10% concentration for *Rhipicephalus microplus* within 10 minutes, 20% of this same oil was required to bring about 100% mortality for *Amblyomma hebraeum* within 20-30 minutes and a concentration higher than 20% will be required to bring about 100% mortality for *Hyalomma rufipes* if complete eradication is required within 24 h period. The differences in

concentration required to bring about 100% mortality for the different species of ticks used could be as a result of differences in their susceptibilities to the acaricidal effects of the oils due to their differences in structure. The pesticidal activity of *Eucalyptus* oil has been attributed to the component such as 1, 8-cineole, limonene, linalool, α -pinene, terpine, α -terpineol, allocimene and aromadendrene [15]. Among the various components of eucalyptus oil 1, 8-cineole (eucalyptol) is the most abundant. In fact, it has been reported to be a compound characteristic of the genus *Eucalyptus* which is largely responsible for a variety of its pesticidal and insecticidal effects [16]. It is possible this compound contributes substantially to the acaricidal effects of the volatile oil. There are corroborating reports on the acaricidal effects of *E. citriodora*, *E. globulus* and *E. staigeriana* on *Rhipicephalus microplus* (Syn *Boophilus maicroplus*) [17] and *H. scupense* [1].

All the four concentrations of *Ocimum gratissimum* tested in the study showed potential acaricidal effect on the three species of ticks used and the effects increased with the concentration of the essential oil along with duration of exposure. *Hyalomma rufipes*, which seemed to be more resistant to the lethal effects of volatile oil of *E. citriodora* produced 100% mortality within 24 h after treatment with the 20 % volatile oil of *O. gratissimum*. A 10% concentration was required to cause 100% mortality of *Amblyomma hebraeum* within 24 h while a 5% concentration was required to bring about the same effect (100%) for *Rhipicephalus microplus* within 25 minutes. The major constituent present in this essential oil is thymol. This compound is a highly selective chemical substance attacking

specific aspect of the endocrine system of insects thus inducing a toxic effect [18]. Its acaricidal effects on termites has been related to its ability to reduce drastically the activities of superoxide dismutase, monoamine oxidase, peroxidase and Ca^{2+} ATPase - the antioxidant enzymes necessary for the optimum performance of the cells [19]. It is possible this compound exerts similar effects on the ticks.

Volatile oil from *Callistemon citrinus* also showed acaricidal effect against cattle ticks probably owing to the presence of the 1,8-cineole and α -pinene which have been reported to be the major constituents [20].

The acaricidal effects of volatile oils have been attributed to the inhibition of arthropod's acetylcholinesterase; an enzyme essential for transmission of action potential [21,22]. Burgess [23] also reported that the hydrophobic nature of volatile oil may exert mechanical or structural damage on the parasite by disrupting the cuticular waxes and blocking the spiracles which leads to death by water stress and suffocation.

In order of efficacy, the volatile oil of *O. gratissimum* was the most potent against all the ticks, followed by *C. citrinus* and finally *E. citriodora*. This assertion is corroborated by the values of LC_{50} obtained for the volatile oils against the ticks in which the *O. gratissimum* displayed the lowest LC_{50} against all the organisms. Also, from the LC_{50} obtained, *R. microplus* was found to be most susceptible to the lethal effects of all the volatile oils while *H. rufipes* was the most resistant.

Acaricidal agents should be readily available; cheap; have rapid onset of action in order to ensure a fast knockdown of the target organisms while being human and environmentally friendly without forming toxic residues. The volatile oils used in this study, particularly those obtained from *O. gratissimum* and *E. citriodora* satisfied these qualities judging from the average time of death of the ticks especially *R. microplus* which died within minutes of treatments.

Thymol, the major constituent of *O. gratissimum* has been reported to reduce environmental contamination because of its low residue levels and has been approved as a save food additive by the FDA [24,25]. All the plants are readily available in abundance found in the wild or cultivates in many parts of the country. They are also known to possess pleasant aromatic odour.

The volatile oils of these plants can effectively serve as viable bioacaricidal alternatives to the synthetic acaricides particularly against the three species of ticks used in this work in order to enhance livestock production and improve the various quality and quantity of their products.

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