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Acaricidal Activities of The Essential Oils of Six Ethnoveterinary Plants Used by Fulani Herdsmen in Nigeria

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Ticks and tick-borne diseases cause devastating effects on livestock production. The misuse of synthetic acaricides in tick control causes increasing levels of acaricide resistance, environmental pollution and livestock byproducts residue accumulation. In the search for an effective and safer alternative, this study evaluated the acaricidal efficacies of essential oils (EOs) of six documented ethnoveterinary plants. The EOs were extracted by hydrodistillation, and acaricidal efficacies of six different concentrations of each plant were tested using adult immersion test. Amitraz and cypermethrin, and 2% dimethylsulphoxide served as positive and negative controls, respectively. Data was analyzed by the probit method, and lethal concentrations were determined using regression analysis. A p value ≤ 0.05 was considered significant. *Cymbopogon citratus, Hyptis suaveolens* and *Ocimum gratissimum* EOs (20, 10, 5%) produced 100% tick mortality while *Eucalyptus globulus* EO exhibited 100% mortality only at 20% concentration. The mortalities from *Balanites aegyptiaca* and *Citrus limon* EOs at 20% concentration were 90 and 47.5%, respectively. Lower concentrations caused tick mortalities of less than 50%. *Cymbopogon citratus/E. globulus* EO combination showed the highest synergistic acaricidal activity (LC_{50}- 0.63%; 100% inhibition of tick oviposition and hatchability). *Cymbopogon citratus* EO may be developed into an effective and eco-friendly botanical-based acaricide, and studies are ongoing to determine its safety profile/mechanism of action.

Keywords: Antitick, *Amblyomma variegatum,* Botanical-based, Essential oil.

Introduction

Livestock production, an integral part of agriculture, is vital to the socio-economic development of any country [1]. However, livestock growth globally has not kept pace with human population explosion due to many constraints; one of which is the devastating effects of ticks and tick-borne diseases on livestock's health and general well-being [2]. Ticks are obligate, haematophagous arthropods that are considered to be leading vectors of pathogens of veterinary and medical importance [3, 4]. Their ability to transmit bacterial diseases (e.g., Lyme disease), protozoal diseases (e.g., theileriosis and babesiosis), rickettsial diseases (e.g., anaplasmosis), and viral diseases (e.g. Louping ill) [5] cannot be overemphasized.

Amblyomma variegatum, commonly called tropical bont tick, is a very economically important tick of livestock. It is the main vector of *Ehrlichia*

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ruminatium which causes heartwater disease in ruminants [6]. Besides, it greatly facilitates, via immunosuppression, the development of dermatophilosis, a chronic and contagious skin disease caused by the Gram-positive bacterium, *Dermatophilus congolensis* [7]. This causes significant damage to hide and skin, and subsequent rejection of such hides by the leather industry [8]. *Amblyomma variegatum* has been reported to be very prevalent in Nigeria, and other parts of Africa, as well as the Caribbeans [9, 10].

At present, the application of conventional synthetic acaricides, using dipping tanks, pour-ons, spot-ons, hand-spraying, is the most used method for tick control globally [11]. Unfortunately, indiscriminate and unethical use has led to the development of resistant populations of ticks [12]. The non-degradable residues in these acaricides pollute animal by-products (milk and meat), and the environment, and have been proven to be harmful to human applicators [13]. In addition, the increasing cost of available acaricides poses a major challenge to the large population of African farmers that mainly practice subsistence farming [14].

Several unconventional approaches have been reported as alternative tick control methods, including the rearing of resistant hosts, the release of sterile males, pasture spelling, tick vaccines and the use of tick parasites [15, 16]. However, none of these methods have been successfully applied in large-scale control schemes. These problems coupled with the unsuitability of tick resistant cattle for all production systems make the current situation unsatisfactory [17]. The need for the exploration of other alternative measures, such as the use of plant extracts, essential oils (EOs) and nano emulsions, cannot be overstated [18, 19].

Essential oils are natural, highly volatile, mainly lipophilic, and complex compound mixtures, obtained from different plant parts [20]. They are defined by the International Organization for Standardization as “products obtained from natural raw material of plant origin, by steam distillation, by mechanical processes, or by dry distillation, after separation of the aqueous phase by physical processes” [21]. Their composition consists of varying amounts of terpenoids, coumarins, anthraquinones, alkaloids, flavonoids, sterols and fatty acids, and these determine their properties and biological activities [22]. Approximately 3 000 different EOs have been described, and 300 of them are used commercially in the pharmaceutical, agrochemical, food, sanitary, cosmetic and perfume industries [23].

Several literatures have reported the potential of plant EOs to replace or complement synthetic acaricides [18, 25 - 27]. They have the ability to penetrate faster through tick cuticle, producing acaricidal and repellant effects [28, 29], inhibitory effects on tick growth and development [30], and anti-feedant and arrestant effects [31]. In addition, EOs are easily biodegradable, making them less toxic to the environment and non-target species. Resistance may also develop slowly due to the presence of several compounds [32]. The use of EOs is continually increasing globally, as there is greater demand for pure natural ingredients in many sectors [33].

The complexity of the mixture of biological active compounds, which can synergistically, additively, and/or antagonistically interact, as well as the high variability of EOs, makes the identification of their target sites and putative mechanism(s) of action (MOA) extremely difficult [34], Nevertheless, many research works in the literature have elucidated the MOA of individual compounds and EOs [20, 35, 36]. The molecular diversity, wide spectrum of activity, the structure-activity relationships, and capability of targeting paradoxical responses triggered by different genes and pathways of EOs were also highlighted [35]. Because EOs have multi-target inhibitory effects on microorganisms and parasites, combining different EOs or EO and conventional drugs can enhance the activity of the drugs and delay the emergence of resistance [37 – 39].

In spite of the reported potentials of EOs and the vast vegetation of Nigeria, as in many African countries [40], there is a dearth of EO-based acaricidal products available on a commercial scale in the market, largely because their efficacy and safety are still poorly understood. This study, therefore seeks to evaluate the acaricidal efficacies of the EOs from selected Nigerian plants (*Balanites aegyptiaca, Citrus limon, Cymbopogon citratus, Eucalyptus globulus, Hyptis suaveolens* and *Ocimum gratissimum*) with documented ethnoveterinary use against ticks. This serves as a preliminary step to find leads for the development of new botanical-based acaricides which are readily available, eco-friendly, efficacious, and can overcome the problem of increasing tick resistance to currently available treatments.

Material and Methods

Ethical consideration

Ethical approval for this study was obtained from the Research Ethics Committee of the College of Veterinary Medicine (COLVET), Federal

University of Agriculture, Abeokuta (FUNAAB). With an approval number of FUNAAB/COLVET/CREC/ 2020/05/01.

Plant selection

The six plants and plant parts evaluated in this study were selected based on their high relative frequency of use by Fulani herdsmen, whose primary occupation is raising livestock. Two ethnobotanical surveys aimed at identifying plants used by Fulani herdsmen to control tick infestation in Ogun State, Southwest Nigeria and Taraba State, Northeast Nigeria, were conducted. An extensive literature search revealed that approximately 86% of the crude extracts of the plant species documented had demonstrated acaricidal and tick-repellent properties [41, 42]. There is, however, a dearth of information on the acaricidal properties of the plant EOs on the economically important three-host tick *A. variegatum*.

Plant collection and identification

Balanites aegyptiaca seeds, leaves of *Cymbopogon citratus, E. globulus, H. suaveolens*, and *O. gratissimum* were collected from their natural habitat in Wukari local government area (LGA), Taraba State while *Citrus limon* fruit peels were collected at Odeda LGA, Ogun State. The plants were identified and authenticated by Dr. B. Oche at the Nigeria Natural Medicine Development Agency (NNMDA), Lagos State. They were given voucher numbers; *B. aegyptiaca* (MPNH/2019/01320), *Citrus limon* (MPNH/2019/01323), *Cymbopogon citratus* (MNPH/ 2019/01321), *E. globulus* (MNPH/2019/01298b), *H. suaveolens* (MPNH/2019/01324), *O. gratissimum* (MPNH/2019/01322), and deposited at the NNMDA herbarium for reference purposes.

Tick collection

Adults of both sexes and engorged females of *A. variegatum* ticks were harvested from naturally infested cattle at the Directorate of Livestock Farms, FUNAAB. The herds had no history of any acaricide application in the last 60 days prior to collection. The ticks collected were put in labelled plastic jars with perforated lids and transported to the Department of Veterinary Parasitology and Entomology Laboratory, COLVET, FUNAAB. The ticks were first rinsed gently in distilled water, wiped with absorbent paper, and examined to ensure they are in good condition. The morpho-anatomical identification key of Ixodidae by Walker *et al*. [6] was used to identify the ticks. Ticks were used the same day they were collected.

Drugs and chemicals

Amitraz (Amitraz 20EC®) and cypermethrin (Cyperpharma®) were obtained from a reputable Veterinary Pharmacy in Lagos State. The distilled water and anhydrous sodium sulphate were of analytical grade and were purchased from a reputable supplier.

Extraction of essential oil

The collected plant materials were washed with clean tap water, weighed, and 500 g of each plant sample was packed into a 10-litre round bottom flask containing 4 litres of distilled water attached to a Clevenger-type apparatus and heated for three hours [43]. Water at 0 °C flowed counter currently through the condenser to condense the steam. The EO that was extracted was mixed with the water vapour at 100 °C and both passed through the condenser and condensed into liquid. Cooling was done using an ice block and volatilization of the EO was avoided. The condensate was collected in a 500 ml beaker, and then poured into a separating funnel. This formed two layers of oil and water. The EO was separated and immediately transferred into a 100 ml dark glass bottle and tightly closed. This was thereafter collected in an airtight container, dried over anhydrous sodium sulphate to remove any entrapped water and stored at 4 °C for further use. The volume of EO obtained for each plant sample was weighed and the percentage oil yield was calculated using the method described by Ranitha *et al*. [44].

Yield of EO=

(Amount of EO obtained / Amount of Plant raw materials used) x 100

***In vitro* acaricidal assays**

The method employed by Amante *et al*. [45] was used to screen for the acaricidal efficacy of the EO of the six plants against non-engorged adult male and female *A. variegatum* ticks. They were divided into six groups (Group I-VI) of 10 ticks each, and dipped in small plastic containers containing 10 millilitres of 0.625, 1.25, 2.5, 5, 10 and 20% concentration of EO dissolved in a ratio of 1:9 in 2% dimethylsulphoxide (DMSO), respectively. Amitraz (1 and 2%) and cypermethrin (2 and 4%) were used as the positive controls. Dimethylsulphoxide in distilled water (2%) was used as the negative control. After two minutes in the test solutions, the solutions were decanted and the treated ticks were dried with filter paper. They were examined singly immediately post immersion (PI) for their light response under a USB digital microscope (Unimake 2mp, China). The ticks were then dried on absorbent paper before transferred into clean, padded plastic bowls (10 ticks per bowl) with perforated lids. Three replicates of each concentration of the test solutions were used, and the experiment was performed in duplicate (making a total of 60 ticks per concentration). Treated ticks were kept in a tick-rearing chamber and maintained at 28.5 ^{o}C (±3 ^{o}C), 78% relative humidity and 14:10 hour light/dark cycle to ensure their survival [46].

The percentage mortality of the ticks was determined 24 hours PI by observing under the USB digital microscope. The ticks were further observed daily for mortality up to day 7 PI. Ticks were confirmed alive and active if they exhibited normal behaviour on exposure to carbon dioxide from human breath (host-associated stimulus), and confirmed dead if cuticular darkness, halted Malpighian tubules movement and haemorrhagic skin lesions are observed [47]. The scoring system described by Fouche *et al.* [48] was used to score the acaricidal activities of the EOs. Plant EO with very good acaricidal activity are those with 80% and above tick mortality, good acaricidal activity: 70-80%, moderate activity: 60-70%, poor activity: 40-60%, and very poor activity: less than 40%.

To determine if there is any synergistic effect, the EO with the highest acaricidal activity (*Cymbopogon citratus*) was combined at the ratio of 6:4: *Cymbopogon citratus*/*B. aegyptiaca*, *Cymbopogon citratus*/*Citrus limon, Cymbopogon citratus/E. globulus* and tested against adult *A. variegatum* ticks, using the method described above.

Data analysis

Data was recorded in Microsoft excel, and subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp, 2015). Results were presented as mean ± standard error of mean (SEM), and the lethal concentrations (LC_{50} and LC_{90}) were determined by applying regression analysis to the probit transformed data of mortality. P value ≤ 0.05 was considered to be statistically significant

Results

Percentage yield of essential oils

Balanites aegyptiaca seed gave the highest yield of 43.0%, followed by *Citrus limon* fruit peel (2.92%) and *Cymbopogon citratus* (2.64%). The yields of *Eucalyptus globulus, O. gratissimum* and *H. suaveolens* leaves were low - 1.6, 0.86, and 0.12%, respectively (Table 1).

Acaricidal activities of six plant essential oils

The EOs displayed a dose-dependent acaricidal activity against non-engorged adult *A. variegatum* ticks. *Cymbopogon citratus*, *H. suaveolens*, *O. gratissimum* EOs at 20, 10 and 5% concentrations produced mortality of 100% each within 24 hours. The standard drug cypermethrin, at 4 and 2% concentrations, also produced mortality of 100% within 24 hours (Figure 1). The activity of these three plant EOs was better than amitraz, another commonly used synthetic acaricide, which produced a mortality of 75 and 67.5% at 2 and 1% concentration, respectively, within 24 hours (Figure 1). *Eucalyptus globulus* EO produced a mortality of 100% at 20% concentration, while mortalities from the five lower concentrations tested were less than 100%. The mortality from EO of *B. aegyptiaca* at 20% concentration was 90%, while that of *Citrus limon* was 47.5%. *Balanites aegyptiaca* and *Citrus limon* EOs at 10, 5, 2.5, 1.25 and 0.625% showed poor acaricidal activities as they caused tick mortalities of less than 50% (Figure 1).

The LC_{50} of *Cymbopogon citratus* EO was the lowest at 1.12%, followed by *O. gratissimum,* with 1.24%, *E. globulus* was 1.82%, while *H. suaveolens* was 2.11%. *Balanites aegyptiaca* and *Citrus limon* had the highest LC_{50} of 9.02 and 29.03% respectively. Likewise, the LC_{90} of *Cymbopogon citratus* EO was the lowest at 2.64%, followed by *O. gratissimum* with 3.24%, *H. suaveolens* with 5.73% and *E. globulus* with 21.03%. *Balanites aegyptiaca* and *Citrus limon* recorded the highest LC_{90} of 32.89 and 135.06% respectively (Table 2; Figure 2A-F).

TABLE 1. Essential oil yield of six ethnoveterinary plants used by Fulani herdsmen in Nigeria

Plant species	Part used	Weight of material use for extraction (g)	Quantity of essential oil extracted (g)	Percent age oil yield (%)	Colour of essential oil
Balanites aegyptiaca	Seed	500	215	43.0	Golden yellow
Citrus limon	Fruit peel	500	14.6	2.92	Greenish yellow
Cymbopogon citratus	Leaves	500	13.2	2.64	Light yellow
Eucalyptus globulus	Leaves	500	8	1.6	Straw
Hyptis suaveolens	Leaves	500	0.6	0.12	Dark yellow
Ocimum gratissimum	Leaves	500	4.3	0.86	Light yellow

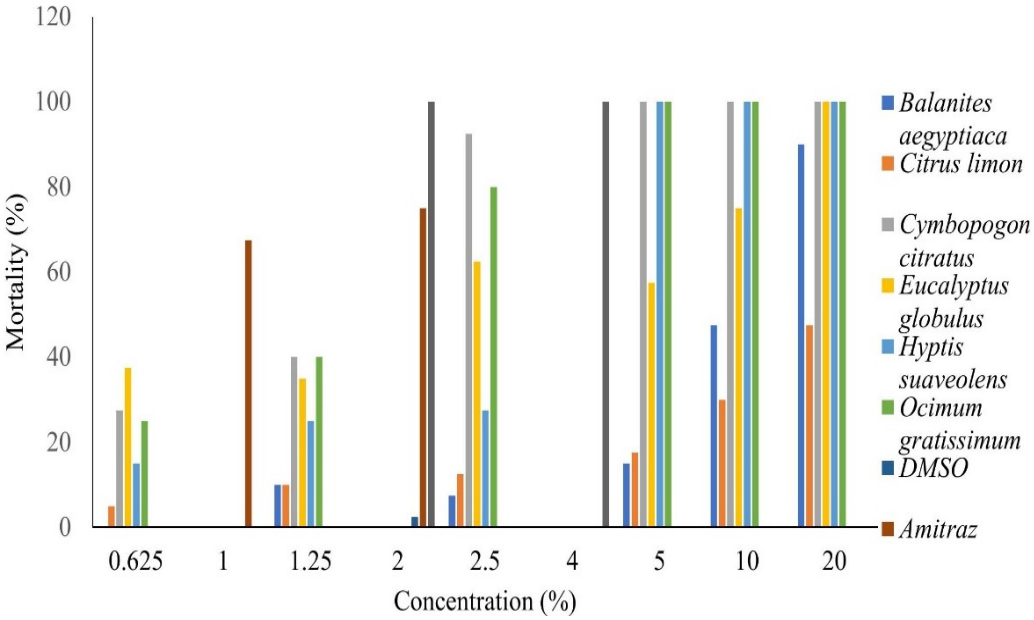

Fig. 1. Percentage of mortality of engorged female *Amblyomma variegatum* ticks exposed to various concentrations of plants essential oils with Dimethylsulphoxide (DMSO) as negative control

TABLE 2. The lethal concentrations of six plant essential oils against engorged female *Amblyomma variegatum* ticks

Essential oil	LC_{50} (%)	LC_{90} (%)
Balanites aegyptiaca	9.02	32.44
Citrus limon	29.03	135.07
Cymbopogon citratus	1.12	2.64
Eucalyptus globulus	1.82	21.03
Hyptis suaveolens	2.11	5.73
Ocimum gratissimum	1.24	3.24

95% Confidence Interval; LC - lethal concentration

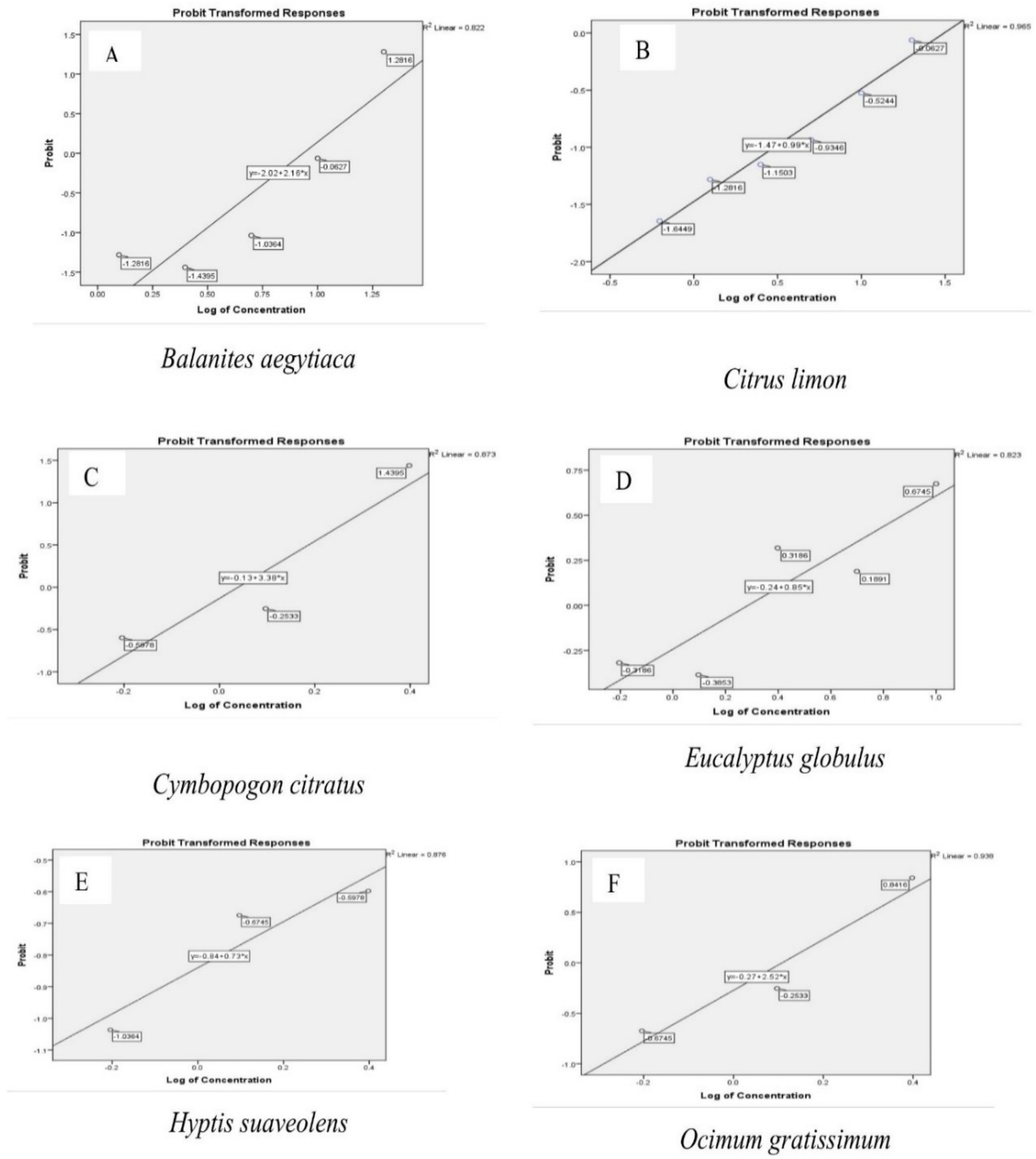

Fig. 2. A-F Lethal concentrations of the various acraricidal plant essential oil on engorged female *Amblyomma variegatum* ticks

Synergistic acaricidal activity of plant essential oil combinations

Cymbopogon citratus/E. globulus EO combination at the concentrations tested- 10, 5, 2.5 and 1.25%, all produced mortality of 100% within 24 hours. This was comparable to the positive control cypermethrin at 4% and 2%, which also produced 100% tick mortality within 24 hours. On the other hand, *Cymbopogon citratus/B. aegyptiaca* and *Cymbopogon citratus/Citrus limon* EO combinations only caused 10% tick mortality at 10% concentration (Figure 3).

The LC_{50} and LC_{90} of the EO combinations are reported as follows: *Cymbopogon citratus/E. globulus* - 0.63%, 1.12%; *Cymbopogon citratus/B. aegyptiaca* - 2.99%, 8.78%; *Cymbopogon citratus/Citrus limon* - 3.46%, 17.85%, respectively.

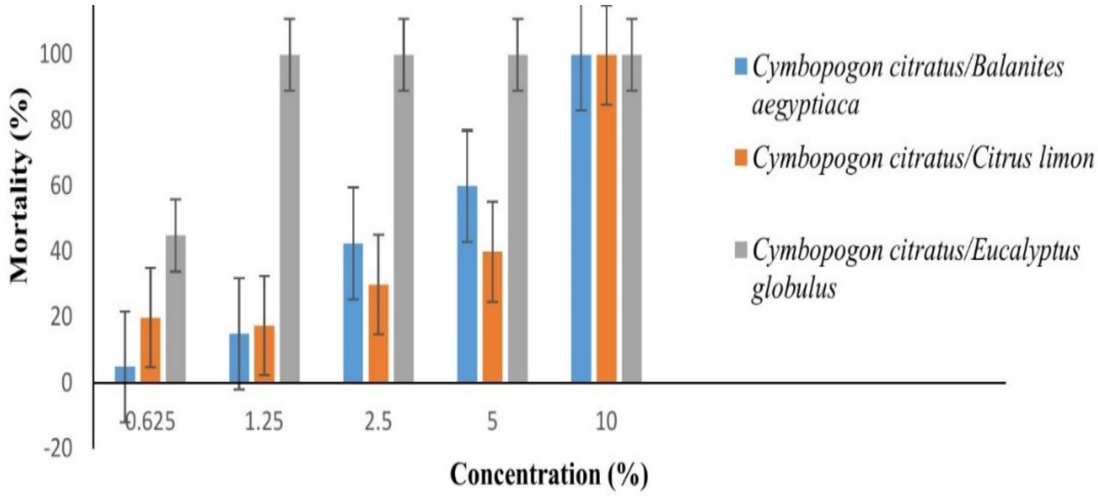

Fig. 3. Percentage of mortality of engorged female *Amblyomma variegatum* ticks exposed to various combinations of plants essential oils

Discussion

In this study, *B. aegyptiaca* seed obtained from Taraba State, Nigeria had a EO yield of 43.0%, which was the same with the yield (43.0%) of the plant reported by Ahmed and Ahmed [49] in Libya. This was, however, lower than the yield of 46.1% reported by Chapagain *et al.* [50] in Israel and 49.9% reported by Manji *et al*. [51] in Adamawa State, Northeast Nigeria. Obidah *et al*. [52] recorded an even lower yield of 29.6% in the same Adamawa State. These variations may be due to the different locations, season of the year when the seed were collected, or the level of dryness before extraction of EO. The EO yield of 2.92% from *Citrus limon* peel was similar to the yield of 3.0% reported in Tunisia by Hsouna *et al*. [53], and higher than the 1.5% yield reported in India by Sharma and Vashist [54], and 1.12% reported in Pakistan by Ahmad *et al*. [55]. This may also be due to the location or the technique used for extraction. For *Cymbopogon citratus,* the EO yield of 2.64% was almost the same as reported in Kaduna, Northwest Nigeria [56]. Ranitha et al. [44] in Malaysia, and Matasyoh et al. [57] in Kenya reported much lower yields of 1.46 and 0.8%, respectively.

The yield for *E. globulus* reported in this study was higher than that reported for the plant in Ethiopia (1.32%), Kaduna State, Northwest Nigeria (0.96%) and India (1.1%) [58 - 60]. However, it was lower than the yield reported in Bangladesh (3.5%), Brazil (3.1%) and Algeria (1.87%) [61 - 63]. Very low EO yield for *H. suaveolens* was reported from Tanzania (1.29%), Oyo State, Southwest Nigeria (0.29%), and Brazil (0.15%) [64 - 66]. *Ocimum gratissimum* grown in Niger State, North-central Nigeria produced EO yield of 0.92%, while yields of 1.40 and 1.66%, were reported from Mombasa, Kenya and the United States of America, respectively [67 - 69].

Variation in EO yield could be a result of the different extraction methods. Ibeh *et al.* [69] reported differences in EO yield from *O. gratissimum* when hydrodistillation, steam distillation and microwave distillation techniques were employed, with microwave distillation giving the highest EO yield. Variation in EO yield could also be influenced by the age at which the plants were collected. It has been reported that the EO yield from *H. suaveolens* leaves are highest during the flowering period [70]. Shiferaw *et al.* [60] reported the highest EO yield (1.32%) from *E. globulus* at three years old, and lowest yield (0.95%) in the same plant species at 100 years. Hanaa *et al*. [71] reported the effect of drying methods on *Cymbopogon citratus* EO yields, with oven drying yielding the highest (2.45%), followed by shade-drying (2.12%) and sun-drying (2.10%). Geographical location, soil composition and seasonal variations have also been found to influence EO yields [72].

Adult immersion test is the most commonly employed bioassay in evaluating plant extracts and EOs for tick repellent and acaricidal properties [73, 74], hence, the use for this study. Dimethylsulphoxide was used to dissolve the EOs as it has been reported to have a lesser toxicity profile than acetone, methanol and TWEEN®20 [75]. In their study of the role of dilution mediums in studies of insecticidal activity of EOs, the authors claim that there is no universally applicable solvent, although volatile organic solvents have promising properties. The excellent (100%) acaricidal activity of *Cymbopogon citratus* EO against adult *A. variegatum* ticks recorded in this study further supports its efficacy, as reported by Agwunobi *et al*. [72] and Yessinou *et al*. [76], where 100% mortality was reported for *Haemaphysalis longicornis* and *Rhipicephalus (Boophilus) microplus* ticks, respectively. The good acaricidal effect of *O. gratisimum* in this study is in agreement with the results of some other studies against *R.* (*B.*) *microplus* [29, 77]. Though there was less than 50% tick mortality within 24 hours, 20% concentration of *Citrus limon* exhibited 100% mortality on the fifth day. This is similar to the study conducted on *R. (B.) microplus* ticks in Brazil by Vinturelle *et al*. [78], where 100% mortality was recorded on the second day PI. This observation shows that *Citrus limon* may possess a residual acaricidal effect. Amitraz at 1 and 2% concentrations produced low acaricidal efficacy. This was similar with what was observed in a similar study against *R. (B.) annulatus* [73], and may be associated with the development of resistance species to this acaricide in our local populations in Nigeria, as reported by Akande *et al.* [12].

Balanites aegytiaca (Desert date) of the Family Zygophyllaceae, is one of the potent and neglected wild plant species. It is found mainly in African and South Asian deserts, and contains a number of bioactive compounds, including flavonoids, alkaloids, glycosides, phenols, steroids, saponins, furanocoumarins, and diosgenin [79 - 81]. *Citrus limon* (Lemon) is a flowing plant belonging to the Rutaceae family. Two dominant compounds present in the plant are limonene (39.74%) and β-pinene (25.44%) [82]. *Cymbopogon citratus* (Lemongrass), belonging to the Poaceae family, is widely distributed worldwide, and generates around 1000 tonnes of EO yearly [83]. The major active components present in lemongrass EO are limonene, β-myrcene, geraniol, citronellol, geranyl acetate, neral nerol, and citral, the main biologically active component [83]. 1,8 cineole (Eucalyptol) has been identified as the major secondary metabolite in *E. globulus* (Southern blue gum), a plant in the Myrtaceae family. It is generally considered as the main active ingredient responsible for its acaricidal activity [73]. Tripathi and Upadhyay [84] identified four major compounds namely sabinene (41.0%), terpinen-4-ol (12.31%), β-pinene (10.0%) and β-caryophyllene (8.0%), in the EO of *Hyptis suaveolens* (Pignut; Lamiaceae). *Ocimum gratissimum,* also known as African basil, is an aromatic herbaceous plant with three active components, namely, eugenol, myrcene, and 1,8-cineole [85].

In this study, the first point of contact of the EOs is the cuticle of the engorged female ticks. Their lipophilicity aided their crossing the ticks' integument to reach the haemolymph and act on the internal organs [86]**.** An important correlation between the digestive and reproductive systems of ticks has been reported, thus, digestive problems can inhibit reproductive capacity [87]**.** *Amblyomma variegatum* tick has a high biotic potential, so substances that act on their reproductive system are of great interest in their control [87]**.** In addition, neuro-morphological damage is capable of affecting nerve impulse transmission in ticks, impairing locomotion and feeding mechanisms. 1,8-cineole, which is mostly extracted from the EOs of plants of the Lamiaceae, Myrtaceae, and Zingiberaceae families [88], significantly altered the activities of superoxide dismutase (SOD), glutathione-s-transferases (GSTs), monoamine oxidase (MAO), nitric oxide synthase (NOS), and acetylcholinesterase (AChE). This may be explained as their mechanism of acaricidal action in mites and ticks [29, 89]. In addition, some plant EOs have immunomodulatory effects that could modify host-parasite immunobiology, and their lipid solubility might offer alternative, transcutaneous delivery routes [90].

Several studies have reported the synergistic effect of EO combinations against livestock pests [91 - 93]. In this study, the EO combinations were much more efficacious on adult *A. variegatum* ticks compared to when each was tested alone. This finding is similar to the reported 100% mortality recorded as a result of exposure of female mosquitoes (*Aedes aegypti*, *Aedes albopictus*), and houseflies (*Musca domestica*) to *Cymbopogon citratus*/*E. globulus* EO combination [95]. *Piper nigrum* and *Citrus limonum* EO combinations were also more effective than when they were tested individually on *R. (B.) microplus* [77]. A lower efficacy was, however, recorded for *E. camaldulensis/E. globulus* EO combination, compared to a higher toxic effect observed with *E. camaldulensis* alone [95]. The synergistic overall effect of compounds found in varied proportions could be responsible for the increased activity [96, 97]. This view reinforces the concept of a multitargeted approach as a therapeutic strategy, contributing to an integrated understanding of the biological activities of plant EOs [98, 99].

Limitations of the study

A major limitation to this study was the influence of abiotic factors such as climatic conditions (temperature, rainfall, humidity, light intensity, wind), soil conditions, agronomical practices (water supply, fertilization), and harvesting time on the EO yield. Ticks were also difficult to get during the dry season. The demise of one of the researchers, Dr Timothy Salihu, who handled the initial aspect of data collection and collation, further limited the study.

Recommendations

Physicochemical properties such as high volatility, low-appealing organoleptic properties, low bioavailability and instability, due to exposure to light, oxygen and high temperatures, limit the use of EOs in several formulations [100]. To overcome this, the use of colloidal carriers as delivery systems is recommended.

Conclusion

The six plant EOs in this study were efficacious against the three-host tick *A. variegatum*, validating their use in controlling ticks by Fulani herdsmen. With the recent increase in demand for organic livestock products, the most effective EO combination in this study (*Cymbopogon citratus/E. globulus*), has the potential to be developed as an eco-friendly acaricide. Further work is ongoing to determine the safety profile and mechanism of action of the EOs.

Conflict of interest

The authors declare no conflict of interest.

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