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Repellent activities of ethanolic extract of *Hyptis suaveolens* Linn (Lamiaceae) and *Azadirachta indica* (Meliaceae) leaves against *Aedes aegypti* mosquitoes in Lokossa district in south-western Benin, West Africa

Nazaire Aïzoun [1,*], Eloi Honvoh [1], Sylvestre Codjia [1] and Daniel Chougourou [2]

[1] *Department of Sciences and Agricultural Techniques, Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) of Abomey, P.O. Box 133 Lokossa, Cotonou, Benin.*
[2] *Department of Environment Genius, Polytechnic School of Abomey-Calavi (EPAC), University of Abomey-Calavi(UAC), Cotonou, Benin.*

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Abstract

The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. The current study was aimed to evaluate the repellent activities of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves against *Aedes aegypti* adult mosquitoes in dengue vector control in Lokossa district in south-western Republic of Benin. Larvae of *Aedes aegypti* mosquitoes were collected from breeding sites using the dipping method from March to July 2024 during the great rainy season in Lokossa district of Mono department. Then, these larvae were reared in insectary until became adult mosquitoes. Repellent activities of ethanolic extract of both plant leaves were measured on adult mosquitoes with different concentrations of 1mg/cm^2, 2mg/cm^2, 3mg/cm^2, 4mg/cm^2 and 5mg/cm^2. The results showed that the leaves of both trees had repellent effects against *Aedes aegypti* adult mosquitoes. However, the repellent effect of *Azadirachta indica* leaves was higher than that of *Hyptis suaveolens* Linn leaves. Both ethanolic extracts of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves are found to be effective in the repellency against the bites of *Aedes aegypti* adult mosquitoes.

Keywords: *Hyptis suaveolens* Linn; *Azadirachta indica*; *Aedes aegypti*; Benin

1. Introduction

Arthropod bites remain a major portal for vector-borne disease transmission. These bites can cause local or systematic effects that may be infectious or inflammatory in nature. Arthropods, notably insects and arachnids, are vectors of potentially serious ailments including malaria, West Nile virus, dengue, and Lyme disease. Measures to curtail the impact of insect bites are important in the world wide public health efforts to safely protect patients and prevent the spread of disease [1]. Vector-borne diseases are among the most important public health problems and obstacles to socio-economic development of developing countries, particularly in the tropics, with malaria alone causing an estimated 1.5-2.7 million deaths and 300–500 million cases per year [2].

Prevention of mosquito bites is one of the main strategies to control or minimize incidence of many vector-borne diseases, such as dengue, transmitted to human through mosquito bites. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. It is not important only for local people in disease risk areas especially in tropical countries, but also for travelers who are vulnerable to diseases spread by mosquito vectors when they visit and seek leisure away from their home countries.

*Corresponding author: Nazaire Aïzoun

The adverse environmental effects from chemical insecticides, the spread of resistance to insecticide in the main vector species of human diseases could jeopardize these control tools. These vector speciesare becoming more and more resistant to synthetic insecticides [3-4].

The potential of plants as insecticide, repellent or fumigant sources against mosquitoes and other pest and vectors insects is well known [5-7]. Several ethnobotanical studies in East and West Africa have shown that human populations use local plants, mostly aromatic plants, to protect themselves against mosquito bites [8-9].

Very few researches were published on the use of essential oils in *Aedes aegypti* adult repellency in Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to evaluate the repellent activities of ethanolic extracts of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves against *Aedes aegypti* adult mosquitoes in lymphatic dengue vector control in Lokossa district in south-western Republic of Benin.

2. Material and methods

2.1. Study area

Figure 1 Map of Republic of Benin showing Lokossa District Surveyed

The study area is located in Republic of Benin (West Africa) and includes the department of Mono. Mono department is located in the south-western Benin and the study was carried out more precisely in Lokossa district (Figure 1). The southern borders of this district are Athiémé and Houéyogbé districts. The northern border is Dogbo district. The eastern border is Bopa district and the western border is Togo republic. Lokossa district covered 260km^2. The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites and peasant practices to control farming pests. We took these factors into account to evaluate the repellent activities of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves against *Aedes aegypti* adult mosquitoes in lymphatic filariasis vector control in Lokossa district in south-western Republic of Benin. Mono has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

2.2. Mosquito sampling

Aedes aegypti mosquitoes were collected from March to July 2024 during the great rainy season in Lokossa district of Mono department. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles. The samples were then carried out to the insectary of Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) in Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) located in Dogbo district.

2.3. Collection of the plant leaves

The leaves of *Hyptis suaveolens* Linn and *Azadirachta indica* were collected in their predilection areas in Lokossa district.

Figure 2 Tree of *Hyptis suaveolens* Linn

Figure 3 Tree of *Azadirachta indica*

2.4. Plant leaves extraction

To extract *Hyptis suaveolens* Linn and *Azadirachta indica* leaves, we collected fresh green leaves of both plants and we washed them with tap water. The leaves were dried outside of the laboratory at ambient temperature in a class room for a period of 3 days. Then, the dried leaves were crushed or grounded into powder with an electronic mix and a weight of 100grammes of the leave powder of each plant were extracted with 250 milliliters of ethanol for a period of 48 hours at temperature of 25°C.Each extract was then filtered with the aid of Whatman No. 1 filter paper. The mixture were dried and stored in some labeled bottles before being used in the preparing of concentrations of 1mg/cm^2, 2mg/cm^2, 3mg/cm^2, 4mg/cm^2 and 5 mg/cm^2. These concentrations were then used in the performing of repellent activities test of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves separately.

2.5. Performing of repellent activities test of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves

A batch of twenty five (25) *Aedes aegypti* adult mosquito aged 3 days old was put in a mosquito cage. These mosquitoes were obtained after collection from the breeding sites and rearing of larvae and pupae from Lokossa district in the insectary of the Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching

(ENSET). A volunteer whom before-arm is without trace of pomade, perfumed soap and so on, the day of the test was used. The left before-arm of this volunteer (handful closed) was coated with ethanol and served as control whereas his right before-arm was coated with 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5 mg/cm² of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves separately. The test was performed at night in laboratory (LaRPET) between 8 and 10 pm with each of prepared concentrations. The before-arm control and test were introduced simultaneously in the mosquito cage. The test was repeated during five (05) consecutive night in the same conditions of temperature (25+/-2) °C and relative humidity (80+/-2) % in laboratory (LaRPET).Both before-arm control and test were exposed to mosquitoes during one minute and retired after one minute to be introduced after four minutes of rest time. That was repeated during two hours which correspond to this test duration. The number of mosquitoes which went towards the before-arm control and test were registered just before the volunteer moving his before-arms in order to avoid that these mosquitoes took their meals on his body.

2.6. Statistical analysis

Correlation between different concentrations of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* and duration of protection against *Aedes aegypti* mosquitoes was established. The significance of the relationship at 5% level was determined using student t-test statistical application.

3. Results

3.1. Evaluation of repellent activity of *Hyptis suaveolens* Linn and *Azadirachta indica*

The analysis of figure 4 showed that *Aedes aegypti* female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Hyptis suaveolens* Linn leaves (test hand) with all tested concentrations. The repellent activities were detected after thirty (30) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm².

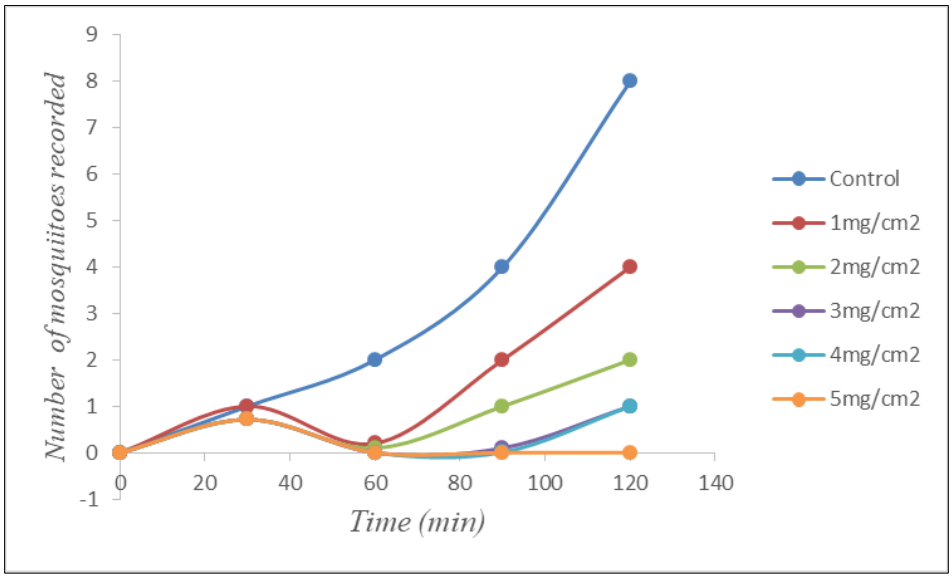

Figure 4 Repellent activity of *Hyptis suaveolens* Linn leaves on *Aedes aegypti* female adult mosquitoes

The analysis of figure 5 showed that *Aedes aegypti* female adult mosquitoes also preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Azadirachta indica* leaves (test hand) with all tested concentrations. The repellent activities were detected after twenty (20) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm².

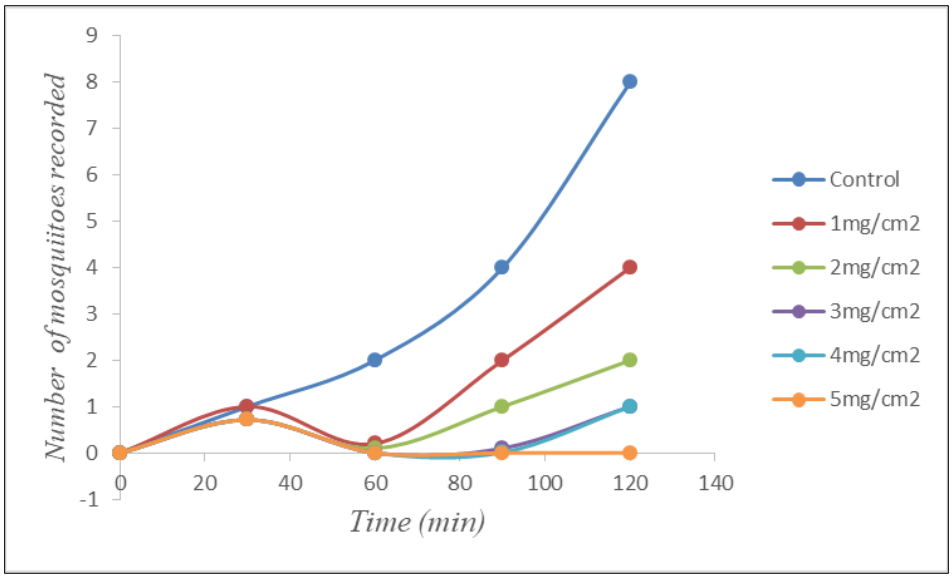

Figure 5 Repellent activity of *Azadirachta indica* leaves on *Aedes aegypti* female adult mosquitoes

The table 1 showed the advantages and disadvantages of the use of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves.

Table 1 Advantages and disadvantages of the use of ethanolic extract of plant leaves

Advantages	**Disadvantages**
Plants as *Hyptis suaveolens* Linn and *Azadirachta indica* are cultivated in many regions in Benin country Ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves is miscible with water after mixture Ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves is a cheap and easy method for larval control in their breeding sites Mosquitoes may not develop resistance to ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves Ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves is not toxic to most non-target organisms including mammals and fish.	Limited effectiveness of ethanolic extract *Hyptis suaveolens* Linn and *Azadirachta indica* leaves in the presence of vegetation and floating debris (is the main disadvantage)

4. Discussion

Aedes aegypti is a mosquito that carries dengue virus, yellow fever and other diseases transmitted to humans. Chemical larvicides are used to control the proliferation of this mosquito, which has generated a high degree of resistance; hence, new alternatives such as bio-larvicides formulated with plant extracts are of great interest.

Although the most common mosquito repellent currently available on the market, DEET (N,N-diethyl-3-methylbenzamide) has shown excellent protection from mosquito bites [10-12], there were reports of toxicity problems after application of DEET, range from mild effects such as contact urticaria [13]and skin eruption [14], to severe reactions, such as toxic encephalopathy [15-17]. To overcome these adverse effects, attempts to find and develop repellents derived from plant extracts is necessary. The development and use of locally available plants showing repellent activity avails an alternative strategy for the control or minimization of mosquito-borne diseases, especially in developing countries.

In the current study, *Aedes aegypti* female adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Hyptis suaveolens* Linn leaves (test hand) with all tested concentrations. The repellent activities were detected after thirty (30) minutes with all the concentrations tested of 1mg/cm^2, 2mg/cm^2, 3mg/cm^2, 4mg/cm^2 and 5mg/cm^2. These results showed that the ethanolic extract of *Hyptis*

suaveolens Linn leaves had repellent activities against *Aedes aegypti* female adult mosquitoes. Our results corroborated with those obtained by Conti *et al* [18] who had evaluated the main constituents *Hyptis suaveolens* for larvicidal and repellent activity against the Asian tiger mosquito, *Aedes albopictus* Skuse (Diptera: Culicidae), currently the most invasive mosquito worldwide, a mosquito of same specie as *Aedes aegypti*. *Hyptis suaveolens* EO had insecticidal activity against *A. albopictus* larvae and mortality was dosage dependent. At the highest dosages of 450 and 400 ppm, there were no significant differences on larval mortality, as mortality ranged between 98.33% and 93.33%, respectively. At dosages ranging from 250 to 350 ppm, mortality rates were lower and not significantly different from each other. Terpinolene was found to be the most effective pure compound. Efficacy protection from *H. suaveolens* EO, at dosages ranging from 0.03748 to 0.7496 [micro]g [cm.sup.-2] of skin, was evaluated during 150 min of observation. Results indicated that this EO had a significant repellent activity ([RD.sub.50]=0.00035 [micro]g [cm.sup.-2]; [RD.sub.90]= 0.00048 [micro]g [cm.sup.-2]), with differences in repellency rates, as a function of both concentration and observation time. Protection time ranged from 16 to 135 min. These results clearly evidenced that the larvicidal and repellent activity of *H. suaveolens* EO could be used for the development of new and safer products against A. albopictus. Another study was carried out by Tennyson [19] to study the mosquitocidal activity of *Hyptis suaveolens* (l.) poit (lamiaceae) extracts against *Aedes aegypti* (Diptera: culicidae) with good results. Lamiaceae have traditionally been used in developing countries for their insecticidal and repellent properties against several insect species.

In the current study, *Aedes aegypti* female adult mosquitoes also preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Azadirachta indica* leaves (test hand) with all tested concentrations. The repellent activities were detected after twenty (20) minutes with all the concentrations tested of 1mg/cm^2, 2mg/cm^2, 3mg/cm^2, 4mg/cm^2 and 5mg/cm^2. These results showed that the ethanolic extract of *Azadirachta indica* leaves had repellent activities against *Aedes aegypti* female adult mosquitoes. Our results corroborated with those obtained by Manzano *et al* [20] who had evaluated the ethanolic extract of *Azadirachta indica* leaves as a larvicide against *Aedes aegypti* and to determine the main compounds present in it by GC-MS. In the assay, three concentrations of ethanolic extract were used (10 mg L^{-1}, 20 mg L^{-1} and 50 mg L^{-1}). This was performed thrice against a positive control (commercial larvicide: spores and endotoxic crystals of *Bacillus thuringiensis* var. israelensis Serotype H-14) and negative control (water). After 72 hours of incubation, it was observed higher larval mortality (93%) in the ethanolic extract at a concentration of 50 mg L^{-1}; the extracts at 10 mg L^{-1} and 20 mg L^{-1} shown larval mortality of 47% and 70%, respectively. The majority compound determined by the GC-MS analysis was phytol (14.4% area). Their results demonstrated the larvicidal potential of the ethanolic extract of *A. indica* against larvae of *A. aegypti*. Another study carried out by Chatterjee *et al* [21] had evaluated the neem-based products as potential eco-friendly mosquito (including *Aedes aegypti*) control agents over conventional eco-toxic chemical pesticides with good results.In fact, mosquitoes cause serious health hazards for millions of people across the globe by acting as vectors of deadly communicable diseases like malaria, filariasis, dengue and yellow fever. Use of conventional chemical insecticides to control mosquito vectors has led to the development of biological resistance in them along with adverse environmental consequences. In this light, the recent years have witnessed enormous efforts of researchers to develop eco-friendly and cost-effective alternatives with special emphasis on plant-derived mosquitocidal compounds. Neem oil, derived from neem seeds (*Azadirachta indica* A. Juss, Meliaceae), has been proved to be an excellent candidate against a wide range of vectors of medical and veterinary importance including mosquitoes. It is environment-friendly, and target-specific at the same time. The active ingredients of neem oil include limonoids like azadirachtin A, nimbin, salannin and numerous other substances that are still waiting to be discovered. Of these, azadirachtin has been shown to be very effective and is mainly responsible for its toxic effects. The quality of the neem oil depends on its azadirachtin content which, in turn, depends on its manufacturing process. Neem oil can be used directly or as nanoemulsions or nanoparticles or even in the form of effervescent tablets. When added to natural breeding habitat waters they exert their mosquitocidal effects by acting as ovicides, larvicides, pupicides and/or oviposition repellents. The effects are generated by impairing the physiological pathways of the immature stages of mosquitoes or directly by causing physical deformities that impede their development. Neem oil when used directly has certain disadvantages mainly related to its disintegration under atmospheric conditions rendering it ineffective. However, many of its formulations have been reported to remain stable under environmental conditions retaining its efficiency for a long time. Similarly, neem seed cake has also been found to be effective against the mosquito vectors. The greatest advantage is that the target species do not develop resistance against neem-based products mainly because of the innumerable number of chemicals present in neem and their combinations. This makes neem-based products highly potential yet unexplored candidates of mosquito control agents. The current review helps to elucidate the roles of neem oil and its various derivatives on mosquito vectors of public health concern.

The use of ethanolic extract of both *Hyptis suaveolens* Linn and *Azadirachta indica* leaves presented many advantages and very few disadvantages. In fact, *Hyptis suaveolens* Linn and *Azadirachta indica* are cultivated in many regions in Benin country. Ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves is miscible with water after mixture. Ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves is a cheap and easy method for larval

control in their breeding sites. Mosquitoes may not develop resistance to ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves. Ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves is not toxic to most non-target organisms including mammals and fish. However, limited effectiveness of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves in the presence of vegetation and floating debris is the main disadvantage in its use.

5. Conclusion

Both ethanolic extracts of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves have repellent activities against dengue vectors. They are found to be effective against the bites of *Aedes aegypti* adult in the current study. However, the repellent effect of *Azadirachta indica* leaves was higher than that of *Hyptis suaveolens* Linn leaves. More effort must be done in order to explore the potentiality of these plant parts available for botanical repellent preparing.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest regarding the publication of this paper.

Statement of ethical approval

The study follows proper ethical procedures.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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