



REPELLENT ACTIVITIES OF ETHANOLIC EXTRACT OF *HYPTIS SUAVEOLENS* LINN. (LAMIACEAE) AND *AZADIRACHTA INDICA* (MELIACEAE) LEAVES IN MALARIA VECTOR CONTROL IN DOGBO DISTRICT IN SOUTH-WESTERN BENIN, WEST AFRICA

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ABSTRACT

Background: The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. **Objective:** The current study was aimed to evaluate the repellent activities of ethanolic extract of *Hyptis suaveolens* Linn. (Lamiaceae) and *Azadirachta indica* (Meliaceae) leaves on *Anopheles gambiae* s.l female adult mosquitoes in malaria vector control in Dogbo district in south-western Republic of Benin. **Methodology:** Larvae of *Anopheles gambiae* s.l mosquitoes were collected from breeding sites using the dipping method in June 2022 during the great rainy season in Dogbo district. Repellent activities of ethanolic extracts of both tree leaves were measured with different concentrations of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². **Results:** The results showed that the leaves of both trees have repellent effects on *Anopheles gambiae* sensu lato female adult mosquitoes. **Conclusion:** Both ethanolic extracts of *Hyptis suaveolens* Linn. and *Azadirachta indica* leaves are found to be effective in the repellency against the bites of *Anopheles gambiae* sensu lato female adult mosquitoes.

KEYWORDS: *Hyptis suaveolens* Linn, *Azadirachta indica*, malaria control, Benin.

INTRODUCTION

In year 2017, an estimated 219 million cases of malaria occurred worldwide.^[1] About 92% of these cases occurred in the World Health Organization (WHO) African Region. Children under the age of five are the most vulnerable with a child dying every 2 minutes from malaria infection.^[1]

Insecticide-treated nets (ITNs) and indoor residual spraying with insecticide (IRS) are the main vector control methods recommended by the World Health Organization for malaria control.^[2] The use of ITNs has yielded interesting results, including a reduction in malaria morbidity and mortality correlated with a reduction in vector biting and parasite inoculation rates.^[3-5]

Prevention of mosquito bites is one of the main strategies to control or minimize incidence of many vector-borne diseases, such as malaria, 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.

The adverse environmental effects from chemical insecticides, the spread of resistance to insecticide in the main malaria vector species could jeopardize these control tools. *Anopheles gambiae* is becoming more and more resistant to synthetic insecticides.^[6-7]

The potential of plants as insecticide, repellent or fumigant sources against mosquitoes and other pest and vectors insects is well known.^[8-10] 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.^[11-12]

Very few researches were published on the use of essential oils in *Anopheles gambiae* s.l. 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 extract of *Hyptis suaveolens* Linn. and *Azadirachta indica* leaves on *Anopheles gambiae* s.l. female adult mosquitoes in malaria vector control in Dogbo district in south-western Republic of Benin.

MATERIAL AND METHODS

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district (Fig.1). The southern borders of this district are Lokossa

and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km² and belongs to geographic region of ADJA. 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 on *Anopheles gambiae* s.l. female adult mosquitoes in malaria vector control in Dogbo district in south-western Republic of Benin. Couffo 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.

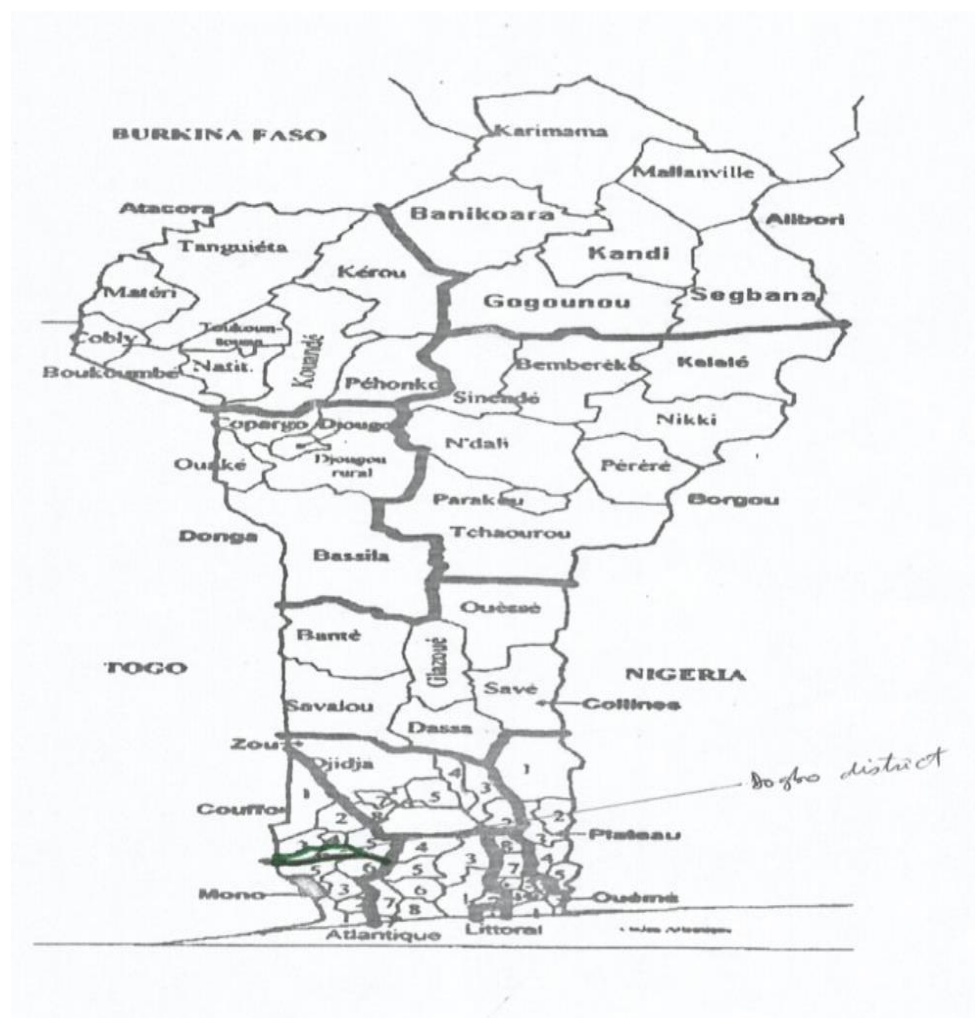


Fig. 1: Map of republic of benin showing dogbo district.

Mosquito sampling

Anopheles gambiae s.l. mosquitoes were collected in June 2022 during the great rainy season in Dogbo district. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles (Fig.2).

The samples were then carried out to the 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.



Fig. 2: Breeding site of *anopheles gambiae* s.l. larvae surveyed in dogbo district.

Collection of the plant leaves

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



Fig. 3: Tree of *hyptis suaveolens* linn.



Fig. 4: Tree of *azadirachta indica*.

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/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5 mg/cm². These concentrations were then used in the performing of repellent activities test of ethanolic extracts of *Hyptis suaveolens* Linn. and *Azadirachta indica* leaves separately.

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

A batch of twenty five (25) female adult mosquito *Anopheles gambiae* sensu lato 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 Dogbo 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 extracts of *Hyptis suaveolens* Linn. and *Azadirachta indica* 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.

Statistical analysis

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

RESULTS

The analysis of figure 5 showed that the female adult *Anopheles gambiae* s.l. mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Hyptis suaveolens* leaves (test hand), except that for the tests using concentrations of 4mg/cm² and 5mg/cm² of ethanolic extract of *Hyptis suaveolens* leaves, the repellent effect was detected after one hour exposure (after 85 minutes).

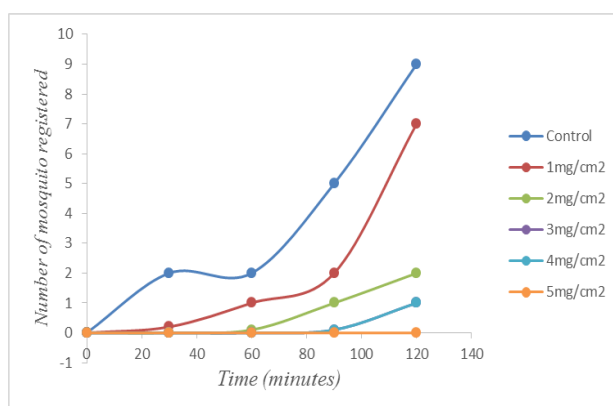


Fig. 5: Repellent activity of *Hyptis suaveolens* leaves on female adult *Anopheles gambiae* s.l. mosquitoes.

In the same way, the analysis of figure 6 showed that the female adult *Anopheles gambiae* s.l. mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Azadirachta indica* leaves (test hand), except

that for the tests using concentrations of 4mg/cm² and 5mg/cm² of ethanolic extract of *Azadirachta indica* leaves, the repellent effect was detected after one hour exposure (after 80 and 100 minutes respectively).

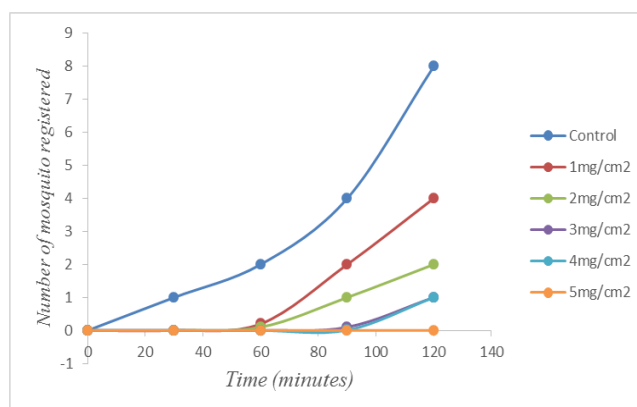


Fig. 6: Repellent activity of *Azadirachta indica* leaves on female adult *Anopheles gambiae* s.l. mosquitoes.

DISCUSSION

Although the most common mosquito repellent currently available on the market, deet (N,N-diethyl-3-methylbenzamide) has shown excellent protection from mosquito bites,^[13-15] there were reports of toxicity problems after application of deet, range from mild effects such as contact urticaria^[16] and skin eruption,^[17] to severe reactions, such as toxic encephalopathy.^[18-20] 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, the female adult *Anopheles gambiae* s.l. mosquitoes preferred go towards the hand coated with ethanol (Control hand) than the hand coated with ethanolic extract of *Hyptis suaveolens* leaves (test hand), except that for the tests using concentrations of 4mg/cm² and 5mg/cm² of ethanolic extract of *Hyptis suaveolens* leaves, the repellent effect was detected after one hour exposure (after 85 minutes). So, *Hyptis suaveolens* leaves have repellent activities on *Anopheles gambiae* sensu lato female adult mosquitoes. The results obtained in the current study corroborated with those obtained by Wangrawa et al.^[21] in Burkina-Faso who had shown that ethanolic extract of *Hyptis suaveolens* leaves possess repellent effects on adult *Anopheles gambiae* mosquitoes, malaria vectors. In fact, according to these authors, on four essential oils tested against *Anopheles gambiae* mosquito, *Hyptis suaveolens* oil was the most effective in mosquito repellency. Another study carried out by Ywaya et al.^[22] in western Kenya also confirmed our results and showed that essential oil of *Hyptis suaveolens* possess repellent properties against bites of *Anopheles gambiae* Sensu Stricto.

In the same way, In the current study, the female adult *Anopheles gambiae* s.l. mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Azadirachta indica* leaves (test hand), except that for the tests using concentrations of 4mg/cm² and 5mg/cm² of ethanolic extract of *Azadirachta indica* leaves, the repellent effect was detected after one hour exposure (after 80 and 100 minutes respectively). So, *Azadirachta indica* leaves have repellent activities on *Anopheles gambiae* sensu lato female adult mosquitoes. The results obtained in the current study corroborated with those obtained by Aremu et al.^[23] who had shown that ethanolic extract of *Azadirachta indica* leaves possess repellent effects on adult *Anopheles gambiae* mosquitoes, malaria vectors. In fact, these authors had shown the potential of neem seed oil cream as mosquito repellent particularly at higher concentrations of 7.5 and 10.0% w/w respectively. According to them, this finding may lead to new and more effective strategies for protection from mosquitoes and their control. Another study carried out by Ayinde et al.^[24] in Nigeria also confirmed our results and showed

that oil extracted from seeds of *Azadirachta indica* possess repellent properties against *Anopheles gambiae*.

CONCLUSION

Both ethanolic extracts of *Hyptis suaveolens* and *Azadirachta indica* leaves have repellent activities against malaria vectors. They are found to be effective against the bites of *Anopheles gambiae* sensu lato female adult in the current study. More effort must be done in order to explore the potentiality of these plant parts available for botanical repellent preparing.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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