

Repellent activities of ethanolic extract of *Cymbopogon citratus* (Poaceae) and *Ocimum basilicum* L. (Lamiaceae) leaves against *Aedes aegypti* mosquitoes in Dogbo district in south-western Benin, West Africa

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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 *Cymbopogon citratus* (Poaceae) and *Ocimum basilicum* L. (Lamiaceae) leaves against *Aedes aegypti* adult mosquitoes in dengue vector control in Dogbo district in south-western Republic of Benin. Larvae of *Aedes aegypti* mosquitoes were collected from breeding sites using the dipping method from September to November 2023 during the small rainy season in Dogbo district of Couffo 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/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². The results showed that the leaves of both plants had repellent effects against *Aedes aegypti* adult mosquitoes. However, the repellent activity of *Cymbopogon citratus* was higher than that of *Ocimum basilicum* L. Both ethanolic extracts of *Cymbopogon citratus* and *Ocimum basilicum* L leaves are found to be effective in the repellency against the bites of *Aedes aegypti* adult mosquitoes.

Keywords: *Cymbopogon Citratus*; *Ocimum Basilicum* L; *Aedes Aegypti*; Dengue Control; Benin

1. Introduction

Many mosquito-borne diseases, such as malaria, dengue fever (DF), dengue hemorrhagic fever (DHF) and filariasis, are serious public health problems in tropical regions, especially in Africa and Asia. These diseases are transmitted to human beings through mosquito bite only since there is no effective vaccine available for the control of these diseases. Prevention of mosquito bites is one of the main strategies to control or minimize incidence of these diseases. The use of insect repellants can provide practical and economical means of preventing mosquito-borne diseases. It is important not only for local people in disease risk areas especially in tropical countries, but also for travellers who are vulnerable to diseases spread by mosquito vectors when they visit and seek leisure away from their home countries.

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.

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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 species are becoming more and more resistant to synthetic insecticides [1-2].

Due to the detrimental effects of synthetic products used for decades to reduce the transmission of mosquito-borne diseases, coupled with current resistance problems that have made it difficult to eliminate the vector, there is a growing interest in exploring plant-derived products to control mosquito populations and prevent their bite. Therefore, it is crucial to develop control strategies that are safer, more effective, and more sustainable compared to traditional chemical interventions.

The potential of plants as insecticide, repellent or fumigant sources against mosquitoes and other pest and vectors insects is well known [3-5]. 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 [6-7].

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 *Cymbopogon citratus* and *Ocimum basilicum* L leaves against *Aedes aegypti* adult mosquitoes in dengue vector control in Dogbo district in south-western Republic of Benin.

2. Material and methods

2.1. Study area

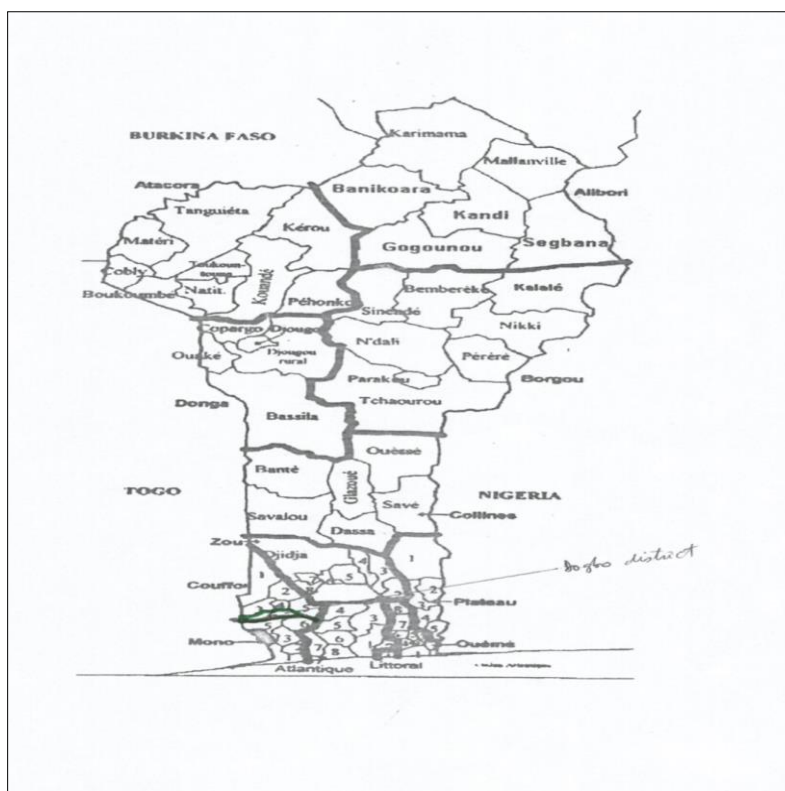


Figure 1 Map of Republic of Benin showing Dogbo District surveyed

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 (Figure 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 *Cymbopogon citratus* and *Ocimum basilicum* L leaves against *Aedes aegypti* adult mosquitoes in dengue 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.

2.2. Mosquito sampling

Aedes aegypti mosquitoes were collected from September to November 2023 during the small rainy season in Dogbo district of Couffo 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 *Cymbopogon citratus* and *Ocimum basilicum* L were collected in their predilection areas in Dogbo district.



Figure 2 Plant of *Ocimum basilicum* L



Figure 3 Plant of *Cymbopogon citratus*

2.4. Plant leaves extraction

To extract *Cymbopogon citratus* and *Ocimum basilicum* L 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 100 grammes 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 extract of *Cymbopogon citratus* and *Ocimum basilicum* L leaves separately.

2.5. Performing of repellent activities test of *Cymbopogon citratus* and *Ocimum basilicum* L 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 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 extract of

Cymbopogon citratus and *Ocimum basilicum* L 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 *Cymbopogon citratus* and *Ocimum basilicum* L leaves 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 *Ocimum basilicum* L. leaves

The analysis of figure 4 showed that *Aedes aegypti* adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Ocimum basilicum* L. leaves (test hand). The repellent activities were detected before one hour with the concentrations of 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm², but there was no repellent activity with the concentrations of 1mg/cm².

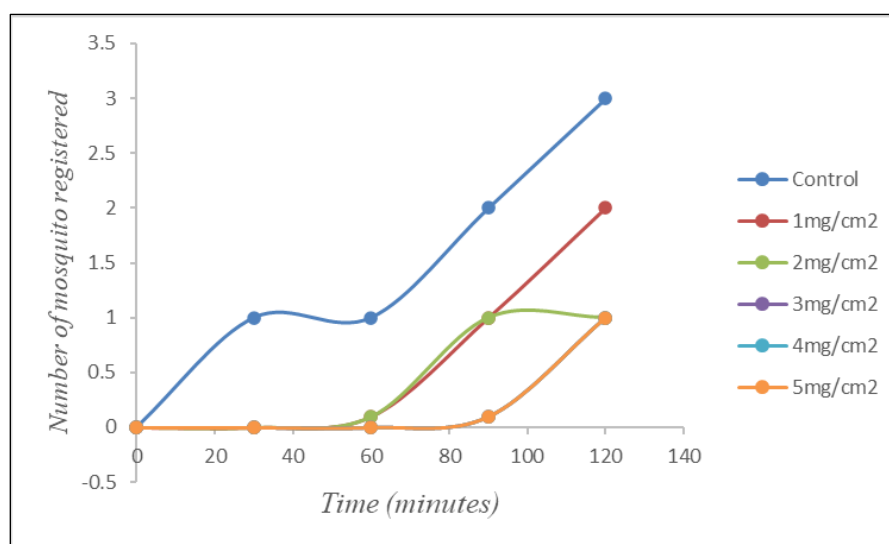


Figure 4 Repellent activity of *Ocimum basilicum* L. leaves on *Aedes aegypti* adult mosquitoes

3.2. Evaluation of repellent activity of *Cymbopogon citratus* leaves

The analysis of figure 5 showed that *Aedes aegypti* adult mosquitoes also preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Cymbopogon citratus* leaves (test hand) with all tested concentrations. The repellent activities were detected before forty five (45) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm².

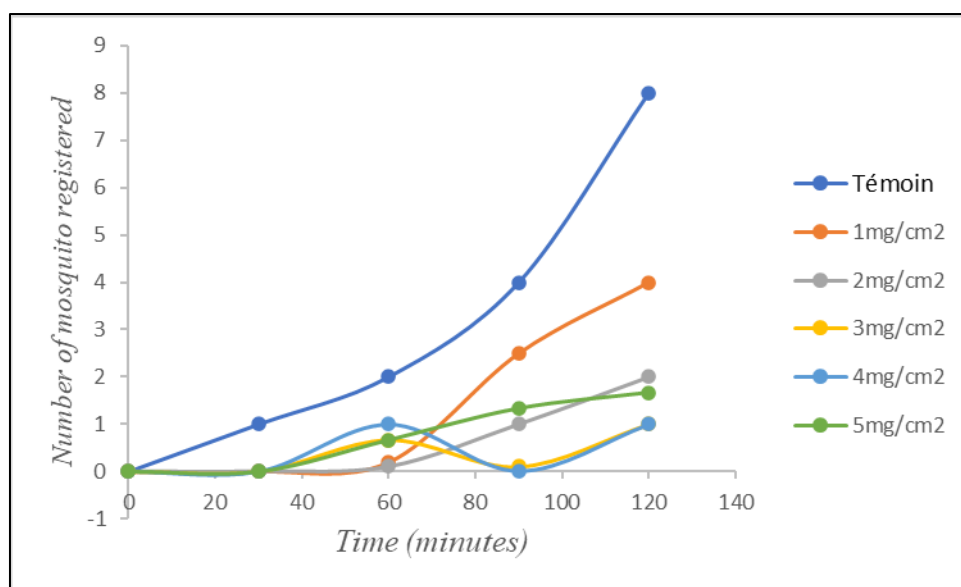


Figure 5 Repellent activity of *Cymbopogon citratus* leaves on *Aedes aegypti* adult mosquitoes

The table 1 showed the advantages and disadvantages of the use of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves.

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

Advantages	Disadvantages
Plants as <i>Cymbopogon citratus</i> and <i>Ocimum basilicum</i> L. are cultivated in many regions in Benin country	Limited effectiveness of ethanolic extract of <i>Cymbopogon citratus</i> and <i>Ocimum basilicum</i> L. leaves in the presence of vegetation and floating debris (is the main disadvantage)
Ethanolic extract of <i>Cymbopogon citratus</i> and <i>Ocimum basilicum</i> L. leaves is miscible with water after mixture	
Ethanolic extract of <i>Cymbopogon citratus</i> and <i>Ocimum basilicum</i> L. leaves is a cheap and easy method for larval control in their breeding sites	
Mosquitoes may not develop resistance to ethanolic extract of <i>Cymbopogon citratus</i> and <i>Ocimum basilicum</i> L. leaves	
Ethanolic extract of <i>Cymbopogon citratus</i> and <i>Ocimum basilicum</i> L. Leaves is not toxic to most non-target organisms including mammals and fish.	

4. Discussion

Dengue hemorrhagic fever is one of the most common infectious diseases. The vector of this disease is *Aedes aegypti*. Dengue hemorrhagic fever can be controlled by a few measures, one of them is using insecticide. However, frequent use of chemical insecticide could lead to resistance and is harmful to non-target organisms.

Although the most common mosquito repellent currently available on the market, DEET (N,N-diethyl-3-methylbenzamide) has shown excellent protection from mosquito bites [8-10], there were reports of toxicity problems after application of DEET, range from mild effects such as contact urticaria [11] and skin eruption [12], to severe reactions, such as toxic encephalopathy [13-15]. 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* adult mosquitoes preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Ocimum basilicum* L. leaves (test hand). The repellent activities were detected before one hour with the concentrations of 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm², but there was no repellent activity with the concentrations of 1mg/cm². These results showed that the ethanolic extract of *Ocimum basilicum* L. leaves had repellent activities against *Aedes aegypti* adult mosquitoes. Our results corroborated with those obtained by Corzo-Gómez *et al.* [16] who have recently showed in Moxico that many plants were effective in the repellency of *Aedes aegypti* mosquitoes including Basil (*Ocimum basilicum*). Also, Muhammad *et al.* [17] had studied the phytochemical screening and larvicidal activity of extracts of *Ocimum basilicum* (lamiaceae) leaves against *Aedes aegypti* with good results. In fact, the results obtained by these authors could encourage the search for bioactive compounds that will provide an alternative to synthetic larvicides.

In the current study, *Aedes aegypti* adult mosquitoes also preferred go towards the hand coated with ethanol (control hand) than the hand coated with ethanolic extract of *Cymbopogon citratus* leaves (test hand) with all tested concentrations. The repellent activities were detected before forty five (45) minutes with all the concentrations tested of 1mg/cm², 2mg/cm², 3mg/cm², 4mg/cm² and 5mg/cm². These results showed that the ethanolic extract of *Cymbopogon citratus* leaves had repellent activities against *Aedes aegypti* adult mosquitoes. Our results corroborated with those obtained by Wiranatha *et al.* [18] who had used the extract of bioinsecticide derived from lemongrass (*Cymbopogon citratus*) leaves on the population of adult *Aedes aegypti* mosquitoes with good results. In fact, these authors had shown that *Cymbopogon citratus* leaves extract has bioinsecticidal effect on *Aedes aegypti* mosquitoes and the optimal result of mosquito mortality is achieved by using the extract in 20% concentration with duration of 12 hours. Also, Mustapa *et al.* [19] had analyzed the essential oils components from aromatic plants using headspace repellent method against *Aedes aegypti* mosquitoes with good results. In fact, these authors showed that for the essential oils made with *Cymbopogon citratus*, the activity test showed that the average repellent power of 10% essential oils was 85%. So, it can be concluded that the chemical compounds contained in this plant have the potential to be environmentally friendly repellents in controlling and preventing *Aedes aegypti* mosquitoes.

The use of ethanolic extract of both *Cymbopogon citratus* and *Ocimum basilicum* L. leaves presented many advantages and very few disadvantages. In fact, *Cymbopogon citratus* and *Ocimum basilicum* L. are cultivated in many regions in Benin country. Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves is miscible with water after mixture. Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves is a cheap and easy method for larval control in their breeding sites. Mosquitoes may not develop resistance to ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves. Ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves is not toxic to most non-target organisms including mammals and fish. However, limited effectiveness of ethanolic extract of *Cymbopogon citratus* and *Ocimum basilicum* L. leaves in the presence of vegetation and floating debris is the main disadvantage in its use.

5. Conclusion

Both ethanolic extracts of *Cymbopogon citratus* and *Ocimum basilicum* L. 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 activity of *Cymbopogon citratus* was higher than that of *Ocimum basilicum* L. 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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