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Effectiveness of essential oils as single and combined repellents extracted from plants in Benin against malaria vectors *Anopheles gambiae* (Diptera: Culicidae)

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Abstract

This study focused on the six most widely used plants in the Benin community to control mosquito vectors, as reported in a recent study in Benin. We compared the repellent activity of the undiluted essential oils extracted from these six plants as single oil, as well as their combination against Anopheles gambiae using the arm-in-cage method to determine the time of protection. These six plants are *Syzygium aromaticum*, *Citrus aurantiifolia*, *Chromolaena odorata*, *Ocimum gratissimum*, *Hyptis suaveolens* and *Cymbopogon citratus*. *Chromolaena odorata* oil was the most effective against *An. gambiae* (210 min). Moreover, we tested binary combinations of these essential oils in order to assess the additional protective potential of the mixture. The combination (*Chromolaena odorata* + *Ocimum gratissimum*) exhibited the best performance with a protection time of up to 4 hours. This study demonstrates the potential for the combined essential oils of *Chromolaena odorata* and *Ocimum gratissimum* to be used as a botanical repellent to control malaria vector *An. gambiae*.

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Introduction

Mosquitoes have a major importance in public health due to their capacity to transmit numerous diseases such as malaria, chikungunya, dengue, Japanese encephalitis and filariasis, causing millions of sicknesses and deaths every year (Dengue, 2009; Sakulku *et al.*, 2009; Thavara *et al.*, 2009). Moreover, mosquito bites are also responsible of allergic responses causing local skin reactions such as urticarial.

Anopheles gambiae (Giles, 1902) complex are the predominant vectors of Plasmodium spp. transmission, particularly Plasmodium falciparum, which is the major protozoan parasite causing malaria infection in humans (Zoh *et al.*, 2020). According to WHO's latest world malaria report, there were an estimated 263 million cases and 597 000 deaths caused by malaria worldwide in 2023 (World Malaria Report 2024, s. d.).

Vector control is an essential component in malaria prevention interventions and has contributed to a significant reduction in malaria worldwide (Malaria Vectors, 2012; Otten *et al.*, 2009; Shargie *et al.*, 2010). It mainly relies on key interventions, including indoor residual spraying (IRS) insecticide treated nets (ITNs) which are based on the chemical insecticides. Unfortunately, chemical repellents may be unsafe for humans (Abdel-Rahman *et al.*, 2001; Das *et al.*, 2003; Kang, 2009).

Personal protection is recommended for preventing mosquito bites and reducing mosquito-borne diseases (Sakulku *et al.*, 2009; Senthilkumar and Venkatesalu, 2012; Tjahjani, 2008). Most common mosquito repellents currently available on the market contain a synthetic chemical N,N-diethyl-m-toluamide (DEET) that is effective against mosquitoes but can penetrate the skin and induce adverse health effects in human(Briassoulis *et al.*, 2001; Fradin and Day, 2002; Santhanam *et al.*, 2005).

Plant essential oils have been reported as major natural resources to repel mosquitoes (Abagli *et al.*, 2023; Benelli *et al.*, 2013; Melliou *et al.*, 2009; Pavela, 2009; Phasomkusolsil and Soonwera, 2011). The improvement of the efficacy of repellents have involved combination of essential oils for a synergistic or an additive effect(Santhanam *et al.*, 2005). In Benin, recent study has reported the use in combination of some plant resources against mosquitoes (Dossou *et al.*, 2025).

This study assessed the repellent activity of 6 essential oils extracted from Syzygium aromaticum, Citrus aurantiifolia, Ocimum gratissimum, Chromolaena odorata, Hyptis suaveolens and Cymbopogon citratus, as well as their binary combinations against Anopeles mosquito.

Materials and methods

Plant materials and essential oils

Six (06) essential oils were selected from the most reported recipes in the community using multiple plant species alone or in combination against mosquitoes as reported by the recent study in Benin from Dossou *et al.* Dossou *et al.*, (2025). Each plant material was extracted for essential oil by steam distillation (Table 1). All formulations were kept at room temperature before testing. Binary combinations (1:1 v/v) have been prepared base on recipes reported by Dossou *et al.*, (2025). We used ®Overtime Insect Repellent Lotion a chemical DEET repellent (Active Ingredient - N, N-Diethyl-meta-toluamide 25%) as positive control.

Mosquitoes tested

Wild larvae of Anopheles gambiae mosquitoes were collected in breeding sites using the dipping technique. Larvae and pupae were collected from various breeding sites in four (4) different zones (Agla, Houéyiho, Ladji and Tanto) in Cotonou city in Littoral department in southern Benin during the rainy season from September to November 2024 (Fig. 1). All larvae and pupae were kept in labelled bottles and then pooled together and reared until emergence. Newly emerged adults were transferred to screen cage (size 30 cm × 30cm × 30 cm) and continuously provided with 5% glucose solution in water soaked on cotton. Female of 4-5 days-old were used for repellency tests. Before starting the tests, the glucose solutions were removed from insect cage for 12h.

Table 1. List of plants used for essential oils extraction in the study

Scientific name	Common name	Family	Number of oil	Combinations	Essential oils combined
Syzygium aromaticum	Clou de girofle	Myrtaceae	Oi1	Comb1	Oi1 + Oi2
Citrus aurantiifolia	Citron vert des Antilles	Rutaceae	Oi2		
Chromolaena odorata	Herbe du Laos	Asteraceae	Oi3	Comb2	Oi3 + Oi4
Ocimum gratissimum	Faux basilic	Lamiaceae	Oi4		
Hyptis suaveolens	Feuille de curry	Lamiaceae	Oi5	Comb3	Oi5 + Oi6
Cymbopogon citratus	Citronnelle	Poaceae	Oi6		

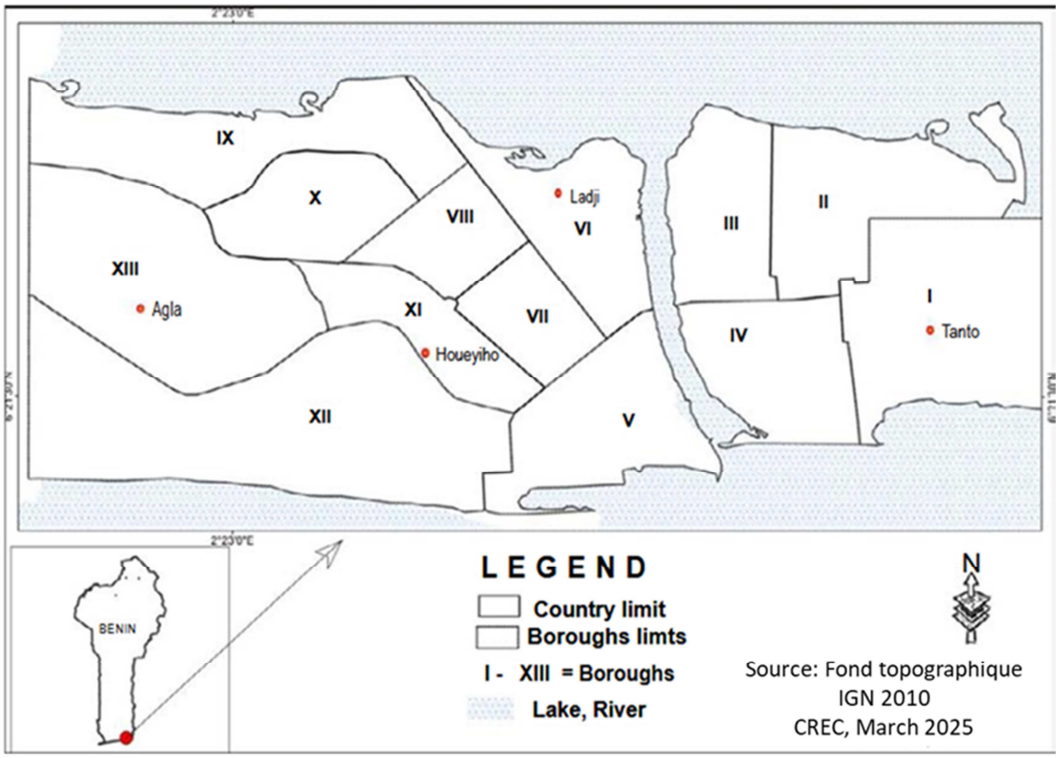

Fig. 1. Map showing the larvae collection area.

Test cage

A test cage (40 × 50 × 40 cm) was constructed with a metal frame to make decontamination easier. All sides were covered with an observable white net to allow viewing. A fabric sleeve was added to the front side of the test cage to allow access by a human forearm.

Volunteers for testing

Volunteers for testing are students of Cotonou Entomological Research Center. At total of six volunteers (three males and three females) between 25 and 37 years old, healthy, with no history of an allergic reaction to mosquito bites. Women who were pregnant were excluded.

Individuals who were sensitive to essential oils were also excluded. The volunteers did not use fragrance and repellent products for 12h before the tests. Volunteers were briefed in the methodology and approved an informed consent form.

Repellent activity

The arm-in-cage technique modified from the standard WHO method (Organization, 2009) and Junkum *et al.* (2021) has been used. Each undiluted essential oil was tested on volunteers at random. For the tests one volunteer was tested once per essential oil and once per day. The testing time was between 7pm to 11pm. Before application of the repellents, the arms of the volunteers were washed with distilled water. The arms of the human volunteer (the ventral part of forearm) were covered with a rubber sleeve with a 3 × 10 cm window. The left arm is treated and the right arm

served as a negative control and was exposed to mosquitoes without the application of essential oil. About fifty fasted aged 5–7 days An. gambiae were selected at random and placed in a standard cage (30 × 30 × 30 cm³) and rested for 2 h before the experiment. The test consists in exposing the control and tested forearms in the test cage containing mosquitoes. The control arm was exposed first before the treated arm. If at least 10 mosquitoes landed on the control arm within 3 min, the repellency test was continued.

After 30 min post application, the tested arm was exposed for 3 min, then withdrawn for 30 min, and then exposed again. The experiment was stopped when the second mosquitoes landed on the treated area and recorded as the complete-protection time for that subject (Junkum *et al.*, 2021). The protection time was estimated as the time between the application of essential oil and the second landed. A total of 0.1 mL of essential oil was applied to the treatment area of left forearm of each volunteer. The test was carried out 3 times per oil.

Statistical analysis

The complete protection time of each volunteer was recorded. The average protection time was used as a standard measure of the essential oils. Differences in significance were calculated by one-way analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DMRT) (Phasomkusolsil and Soonwera, 2010).

Results

Repellent activity of individual essential oils against An. gambiae

The repellency activity expressed in time of protection by the arm-in-cage method for six essential oils repellents and the positive control is shown in Fig. 2. Oils that provided protection for at least 120 min were considered highly effective. These were the essential oils of *Chromolaena odorata* (210 mn), *Ocimum gratissimum* (120 mn), *Citrus aurantiifolia* (120mn). The other oils had much lower median complete-protection times of 90 min (*Syzygium aromaticum*), 60 mn (*Hyptis suaveolens*) and 30 mn (*Cymbopogon citratus*).

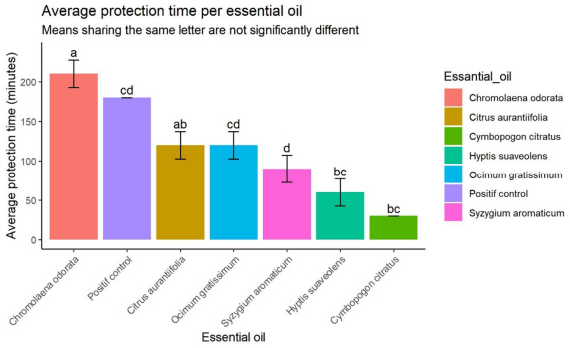

Fig. 2. Average protection times of six undiluted essential oils against wild *An. gambiae* mosquitoes

There were significant differences in repellency among essential oils and the positive control ($P<0.05$). Chromolaena odorata had the best efficiency in terms of protection time even higher than the chemical repellent (positive control) in which the protection time was 180 mn.

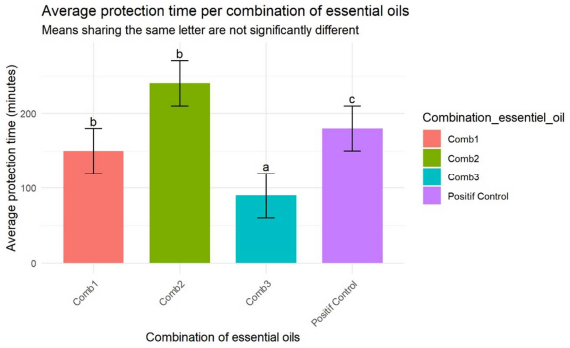

Fig. 3. Average protection times of essential oil combinations wild *An. gambiae* mosquitoes

Repellent activity of essential oil combinations

The six essential oils were mixed in three-oil combinations at a 1:1 (v/v) ratio to evaluate whether they exerced additive or synergistic effects (Fig. 3). Comb1 (*Syzygium aromaticum* + *Citrus aurantiifolia*) and comb2 (*Chromolaena odorata* + *Ocimum gratissimum*) provided more than the threshold of 120 min of protection (Fig. 3) with respectively 150 mn and 240 mn. Comb3 (*Hyptis suaveolens* + *Cymbopogon citratus*) presented a low protection effect, showing that even combined, these two essential oils combined provide a relatively short-term protection time of less than 120 minutes. Combination 2 exhibited the best performance of all, with a protection time of up to 4 hours.

Discussion

This study clearly assessed the repellent potential of six oils tested in isolation, as well as their combination based on the recipes reported by Dossou *et al.* (2025). The results of our study revealed that some essential oils evaluated were effective against wild An. gambiae to some degree in the human arm-in-cage assay. Three essential oils and two combinations evaluated have a protection time exceeding the 120-minute efficacy threshold. Moreover, *Chromolaena odorata* oil and the combination 2 (*Chromolaena odorata* + *Ocimum gratissimum*) have higher repellent efficacy than the commercial chemical repellent used as a positive control. The essential oils alone were sufficient to repel mosquitoes for up to 3.5 hours at a time. However, combining them to improve repelling duration is evident in this study. Indeed, all the essential oil combinations showed greater protection time than the combined oils in isolation. The combination of *Chromolaena odorata* and *Ocimum gratissimum* essential oils was highly effective as repellent with a protection time of 4 hours. Based on that, we assume that when two single oils were mixed, the ratio of active ingredients changed, and this change may increase the effective protection time against mosquito bites.

Previous research suggested the additive effects among constituents of plant essential oils and mixtures of oils as well as the search of new additives that could make longer the time of protection. This could be a good alternative to replace the chemical repellents (Nerio *et al.*, 2010). The repellent performance of essential oils depends on their chemical constituents (Auysawasdi *et al.*, 2016). The best documented are mainly monoterpenes, sesquiterpenes, alcohols and phenols that is neurotoxic to mosquitoes (Huff and Pitts, 2019; Saad *et al.*, 2019; Tian *et al.*, 2020). Further studies will be required to determine the exact constituents of the natural resources.

The volatility and oxidation essential oils remain challenging of their use as mosquito repellent. Additives such as vanillin, coconut oil and olive oil are recommended for stabilizing the essential oil to last the protection time (Auysawasdi *et al.*, 2016; Sritabutra and Soonwera, 2013).

In this study, no volunteers complained of irritation after applying essential oils. However, a serious study of the irritability of these essential oils could be envisaged. Essential oil-based repellents are considered safer and may have a lower probability of resistance development in vector mosquitoes and can be applied to skin without affecting non-target organisms or the environment (Bottrell and Schoenly, 2018).

This study highlighted the effectiveness of essential oil combinations and identified the most effective one (*Chromolaena odorata* + *Ocimum gratissimum*). This combination could be candidate for the formulation of safe and effective mosquito repellent. National malaria control programs should capitalize on and promote these essential oil-based recipes as individual vector control measures.

Conclusion

This study demonstrated the potential of essential oils derived from *Syzygium aromaticum*, *Citrus aurantiifolia*, *Chromolaena odorata*, *Ocimum gratissimum*, *Hyptis suaveolens* and *Cymbopogon citratus* for use as mosquito repellents against *An. gambiae*.

Chromolaena odorata oil was highly effective against wild malaria vector *An. gambiae*. When combined with *Ocimum gratissimum* oil, the combination exhibited additive protection time. Study of operational feasibility and dermal irritability is essential before these essential oil combinations can be safely deployed as alternative mosquito repellents for individual use.

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