International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2020; 7(1): 16-24

Received: 02-11-2019
Accepted: 06-12-2019

Tia vama Etienne
Péléforo Gon Coulibaly University (UPGC) Côte d'Ivoire

Doannio JM Christian
Péléforo Gon Coulibaly University (UPGC) Côte d'Ivoire

Adima amissa Augustion
Institut Polytechnique Houphouët Boigny (INP-HB) Yamoussoukro, Côte d'Ivoire

Corresponding Author:
Tia vama Etienne
Péléforo Gon Coulibaly University (UPGC) Côte d'Ivoire

Repellent effect of some essential oil from Ivorian ethnomedicinal plant against malaria vector, *Anopheles gambiae* (Giles, 1902)

Tia vama Etienne, Doannio JM Christian and Adima amissa Augustion

Abstract
To address the resistance developed by mosquitoes to pyrethroids in sub-Saharan Africa in general and by *Anopheles gambiae* in Côte d'Ivoire in particular, it has become urgent to seek insecticides with novel modes of action. Plant extracts such as essential oils, which generally contain a high proportion of bioactive molecules with insecticidal properties, could be a promising avenue. One alternative to control is to find natural repellents to complement the use of impregnated mosquito nets as recommended by WHO to reduce contact between humans and the malaria vectors. This study was undertaken to identify the active compounds of some essential oils and to study their repellent effect against adults of *An. gambiae* under laboratory conditions. Their effects were compared to that of DEET used as a positive control. The most promising extracts were those from *Cymbopogon citratus*, *Cymbopogon nardus* and *Lippia multiflora*. The repellent effect varies from one EO to another and the behavioural response of adults was significantly influenced by the concentration of plant extracts. The most abundant compounds were acyclic or monocyclic mono terpenes. The use of plant repellent essential oils for the formulation of an anti-mosquito product for the control of the malaria vector as an alternative control method can thus be considered.

Keywords: Essential oils, repellent activity, *Anopheles gambiae*, DEET, Mosquito, Côte d'Ivoire

Introduction

In most countries south of the Sahara, malaria remains a major health problem. More than 3 billion people are at risk and 300 million malaria outbreaks are recorded each year [1].

In Côte d'Ivoire, malaria remains one of the most important diseases. This disease is the first reason for consultation with 57% of morbidity. Malaria accounts for 62% of hospitalization cases among children under five (5) years of age and 36% among pregnant women [2]. Parasites of the genus *Plasmodium*, particularly *Plasmodium falciparum* transmitted to humans by the female *Anopheles gambiae*, are responsible for malaria [3]. *An. gambiae* is present throughout the country and transmits malaria from the southern forest to the savannah regions in the north of the country.

For malaria control, the use of pyrethroid-treated mosquito nets and indoor spraying have been proposed as vector control measures. These control strategies have contributed to improved public health in many countries [4]. Although these tools are currently the best means of protection, impregnated nets are still inaccessible and expensive for rural areas.

As a result, many people in our regions still do not tolerate the use of insecticide-treated mosquito nets today. In addition, pyrethroids are currently the only class of insecticides used to control the malaria vector in Africa.

Inevitably, the intensive use of this insecticide in recent years has caused pyrethroid resistance in the main malaria vector [4]. This is a real problem for treated nets. So, malaria, which is still relevant today, continues to challenge the control tools put in place by man through the expansion of the vector's resistance. Faced with this situation, it is clear that in order to combat resistance, new control strategies must be developed, including the search for bioactive molecules of plant origin with several compounds possessing different modes of action.

The search for natural repellents that are effective and free of side effects will there fore provide an added benefit in terms of personal protection against mosquito bites. This is all the more so as the desire to return to natural means and the ever-increasing interest of biologists in using methods that are less polluting for man and his environment are topical. Plants, for their part, offer an excellent source of natural biologically active substances that deserve to be promoted.

Today, the prospects for the discovery of bioactive molecules of plant origin remain more promising than ever. Plant extracts such as essential oils generally contain a high proportion of active molecules with insecticidal properties as well as the synergistic effects induced by the chemical components of these essential oils are reported in the literature [5-7]. Indeed, many monoterpenes, contained in the essential oils of aromatic plants, have insect-repellent properties against certain mosquitoes, in particular *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say) [8]. However, research on the repellent potential of plant essential oils against malaria vectors, *An. gambiae*, has remained rare in recent years. While WHO recommends the use of repellents in addition to impregnated mosquito nets to reduce contact between humans and the vector [3].

It is in this dynamic of research that this study is oriented, which aims to evaluate the repellent properties of essential oils from local plants against *An. gambiae* and then to identify their chemical constituents.

Materials and methods

Plant material and extraction of essential oils

Native plant species from Côte d'Ivoire, including *Lippia multiflora* (Verbenaceae), *Cymbopogon citratus, Cymbopogon nardus* (Poaceae) and *Ocimum gratissimum* (Labiatae), were selected for this study. These plants have been selected because of their traditional use in rural areas as insect repellents. The leaves of *C. citratus, C. nardus* and *O. gratissimum* were collected in March 2014 from the Abidjan region (N 5°20' 11″; W 4°1'36″) from formally identified and localized plants. Flowers of *L. multiflora* were collected at the same time in Korhogo (N 9°27' 28″; W 5°37' 46″). The various plant materials were dried at the laboratory's ambient temperature (28±2 °C) for one week before extraction. The essential oils were obtained by steam distillation with a Clevenger device for 4 hours. The principle of steam entrainment used in this study is similar to that described in the previous article [9]. After drying on anhydrous magnesium sulphate, the essential oils were stored at 4°C in a dark place until their analysis.

Mosquito strain

Repellent tests were carried out on *An. gambiae* females precisely on the reference strain "Kisumu". *An. gambiae* colonies were bred in the insectarium of the LIN-IRD in Montpellier (France). The strain was maintained in the breeding chamber under conditions of temperature of 27±2°C and 80% relative humidity (RH) with a photoperiod of 16h : 8h. From the larvae of *An. gambiae* fed with fish feed (Teratim), adults were obtained which were then collected in cages (25x25x25 cm) and fed with 10% sweet juice. Fasting females used for bioassays were 4 to 5 days old.

Chromatographic analyses

• GC-FID analysis

Chromatographic analyses of the essential oils tested were performed on a Varian gas chromatograph (model CP-3380) equipped with a HP 5 J&W Agilent non-polar fused silica capillary column (5% phenyl 95% methyl polysiloxane: 30 m x 0.25 mm i.d. 0.25 µm; Agilent Santa Clara, CA) and a flame ionization detector (FID) set at a temperature of 250°C. The injector temperature is 220 °C and the oven temperature was programmed from 60° to 220 °C at a rate of 3 °C/min, then maintained at 220 °C for 20 minutes. The carrier gas used was nitrogen (N2) with a flow rate of 0.8 ml/min. 1 µL of an essential oil solution (10% essential oil in ethyl ether) was manually injected in split mode (leakage ratio: 1/100). A mixture of alkane solution (C9-C22) was injected to calculate the retention indices (IR) for each compound according to the formula :

$$IR = [Tr(x) - Tr(n)]/[Tr(n+1) - Tr(n)] \times 100 + 100n$$

The relative percentage of the different components were obtained by electronically integrating the signals obtained by GC-FID (assuming that all components are characterized by the same response coefficient to the detector).

• GC-MS analysis

GC-MS analyses were performed on a Hewlett-Packard gas chromatograph (GC 5890, Series II) equipped with a HP-5 fused silica capillary column (5% phenyl 95% methyl polysiloxane:
30 m x 0.25 mm i.d. 0.25 µm). The mass detector was a quadrupole type (model 5972). The temperature of the column is programmed from 70 to 200 °C at a rate of 10°C/min. The injector and transfer temperatures were 220°C and 250°C respectively. The carrier gas was Helium, the flow rate of which was fixed at 0.06 ml/min. 1 µL of an essential oil solution (10% essential oil in ethyl ether) was injected in split mode (leakage ratio : 1/100). The ionization energy was equal to 70 eV; electron multiplier 1400 eV; scanning area [35-300] (m/z)]; scanning rate 2.96 scan/s. The identification of the components was carried out on the basis of their retention index calculated by comparing retention times with those of a series of alkanes (C9-C22) and by comparing their mass spectra with those of the NBS 75 K/ NIST 98 libraries in the literature [10].

Preparation of essential oils for the repellent test

Four (4) essential oils from the above-mentioned plants were used in this trial. For each essential oil, two concentrations 0.1 and 1% (v/v) were obtained in a dilution in the complex solvent composed by 10% Genapol x 80, 10% water and 80% ethanol to mimic the final formulation and fix the essential oil as long as possible. These two concentrations were selected after preliminary testing and based on published data [11].

To compare our plant extracts with some chemicals repellents, DEET was tested with the same concentrations using equal solvents and formulations against the same mosquito strain.

Repellent test procedure

The tests were conducted in the Laboratory for the Control of Pests (LIN) of the IRD of Montpellier (France) within laboratory rooms maintained at 27 ± 2 °C and relative humidity 60±10% (RH). The repellent effect of essential oils was evaluated using a device (HITSS : *High-Throughput Screening System*) originally developed by Grieco and al [12] against *Aedes aegypti* and adapted in our study for use against *An. gambiae*. The HITSS device used for repellent tests consists of two (2) metal chambers (10.2 cm in diameter and 0.6 cm thick) closed with a plug at one end each. The chambers were joined by a funnel-shaped section with a flight valve that allows mosquitoes to move through the device. One of the metal chambers was marked as a treated compartment (treatment chamber) and the other was designated as an untreated chamber. Wattman paper (10 x 30 cm) impregnated with 3.5 mL of the product or simple solvent and dried at laboratory temperature for 15 minutes was wrapped around the grid on the inner cylinder and then introduced into the treatment chamber. The grid prevents mosquitoes from coming into direct contact with the impregnated paper. The inner cylinder of the untreated chamber was covered with printing paper (10 x 30 cm) without any product or solvent.

For each test in the devices thus constituted, ten (10) female mosquitoes (*An. gambiae*) were introduced into the treatment chamber using a mouth vacuum cleaner. After a period of acclimatization (1 min.) of the mosquitoes, the flight valves were then opened for 10 minutes to allow them to move from the treated chamber to the untreated chamber. At the end of the tests, the valves were closed and the number of mosquitoes in each chamber of the device was counted after having put them under CO_2. A product was repellent when the most insects take refuge in the untreated chamber of the HITSS and the test was validated when the percentage of escape in the control was below 10%. For each product, each concentration was repeated six (6) times and between replicates, the assembly of the device was dismantled and cleaned with ethanol/acetone solution (1:1) to remove any trace of odour. The device was cleaned at the end of the tests each evening with TFD4 detergent (FranklabS.A, France) and only one product was tested per day to reduce the risk of contamination and interactions between the volatile constituents of the essential oils studied.

Fig 1: Device used to evaluate the repellent effect of essential oils: treatment chamber **A** and untreated chamber **B**.

Statistical analysis

The various tests were repeated six times. The results obtained were expressed as an SD average and subjected to an analysis of variance (ANOVA). The cumulative repellent rates according to the essential oil doses were subjected to a one-dimensional analysis of variance. Where there were differences between treatments, the averages were separated by the Student test (SPSS software 7 :1) at the 5% significance level.

Results

Chemical composition

The essential oils were extracted by steam distillation and analyzed by gas chromatography coupled with mass spectrometry (GC-FID and GC/SM). The results of the chemical analyses performed by GC-FID and GC-MS are presented in Table 1, where the compounds are listed in order of elution on a DB5 column. The different essential oils have completely different chemical compositions, even if they are exclusively terpenic. The essential oil of *Ocimum gratissimum* is a mixed complex of compounds, of which 38 compounds have been identified constituting 99.7% of the total quantity of the oil. The fractions of oxygenated and hydrocarbon monoterpenes such as thymol (27.0%) and γ-terpinene (26.0%), p-cymene (11.0%), α-thujene (7.0%) respectively, constitute the majority of the essential oil composition.

In *Cymbopogon citratus* essential oil, 25 compounds have been identified. They constitute 98.8% of the chemical composition of the essential oil. The main chemical compositions that constitute the main fraction of this essential oil are acyclic monoterpenes; mainly myrcene (29.9%), geranial (28.0%) and neral (22.0%). *Cymbopogon nardus* essential oil contains several volatile compounds of which 27 have been identified, corresponding to 95.0% of the total composition of the oil. The main compounds are mainly geraniol (51.6%), citronellol (17.0%) and citronellal (5.0%). Thirty-two (32) compounds have been identified in *Lippia multiflora* essential oil corresponding to 97.3% of the total composition of the extract. The fraction of oxygenated monoterpenes such as citral (34.8%; geranial and neral) and hydrocarbon monoterpenes such as p-cymene (20.0%) and α-phelandrene (10.5%) constitute the majority of the composition of this essential oil.

Repellent effect

The repellent activity of the essential oils of four (4) aromatic plants and DEET has been studied on the behaviour of adult females of *An. gambiae* under treatment conditions.

The repellent rates of mosquitoes exposed to essential oils and DEET and compared to the control are presented in Table 2. Analysis of the table shows that there is a significant difference between the rate of mosquitoes displaced in treatment and control for all products tested. The rate of mosquitoes displaced in treatment and control differs significantly from one plant to another and these effects are positively dependent on concentration.

Table 2: Comparison of the repellent effect observed in treaments and controls

Products	Concentration % (mg/l)	P-value	Rate displaced mosquitoes	
			Processing	Witnesses
O. gratissimum	0,1	0,002	2,00	0,33
	1	0,000	3,50	0,33
C. citratus	0,1	0,000	4,17	0,7
	1	0,000	7,7	0,7
C. nardus	0,1	0,000	3,17	0,17
	1	0,000	7,7	0,17
L. multiflora	0,1	0.021	1,33	0,50
	1	0,000	9,7	0,50
DEET	0,1	<0,144	0,33	0,000
	1	0,044	1,17	0,17

Three of the four (4) essential oils showed a high rate of mosquitoes displaced at all concentrations tested compared to the control. They are *C. citratus*, *C. nardus* and *L. multiflora*.

Compared to the control, there is no significant difference between the rate of displaced mosquitoes for the essential oil *O. gratissimum* and the synthetic chemical, DEET.

The repellent effects of plant extracts and DEET evaluated at different concentrations (0.1% and 1%) are illustrated in Table 3. The products tested have a varied repellent potential, particularly at both concentrations. Again, repellent rates were significantly influenced by concentration. The repellent activity was the refore positively in fluenced by the in crease in concentration.

Table 3: Repellent rate of *An. gambiae* exposed to different doses of essential oil and DEET

Products	Average number of displaced mosquitoes	
	0,1%	1%
O. gratissimum	1,17±0, 32^{bc}	1,92±0, 52^{bc}
C. citratus	2,42±0, 57^{a}	4,17±1,08^{ab}
C. nardus	1,67±0, 54^{ab}	3,92±1,16^{ab}
L. multiflora	0,92±0, 19^{bcd}	5,08±1,41^{a}
DEET	0,17±0, 11^{d}	0,67±0,26^{c}

The essential oil of *O. gratissimum* has a very low repellent effect even at the highest concentration. On the other hand, three essential oils have a significant repellent effect at all concentrations. These are the essential oils of *C. citratus*, *C. nardus* and *L. multiflora*. Conversely, at any concentration, DEET did not appear to be effective in repelling mosquitoes.
Among the products tested, essential oils would seem to be the most active or repellent. The graphical representation of the variables (Figure 2), along axes F1 and F2, makes it possible to distinguish two groups of aromatic compounds. We have oxygenated monoterpenes (OM) which are positively correlated with both concentrations (0.1% and 1%) of essential oils unlike hydrocarbon monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes.

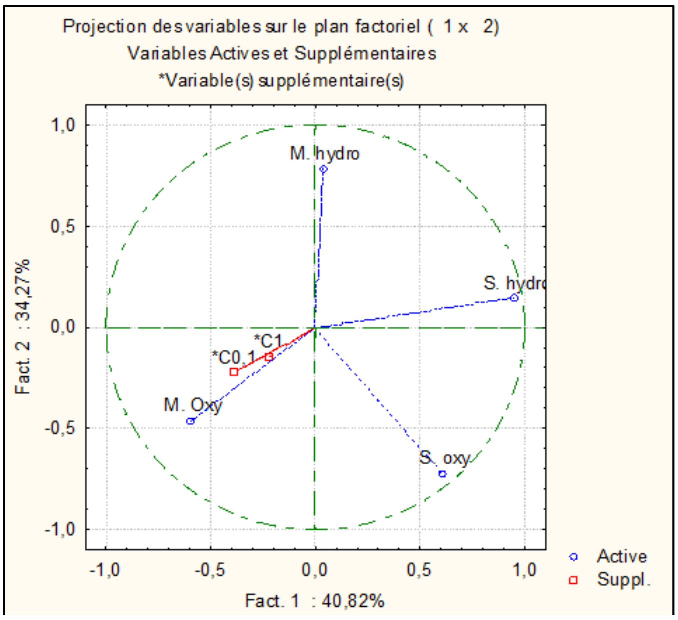

Fig 1: Correlation between the concentration of essential oils and the content of monoterpene compounds

This result indicates that each increase in MO level in the EO and its concentration leads to an increase in the repellency effect. The representation of observations indicates that the essential oils of *C. nardus*, *C. citradus* and *L. multiflora* rich in oxygenated monoterpenes have a high repellent potential at 0.1% and above, unlike the essential oils of *O. gratissimum*, rich in hydrocarbon compounds.

Discussion

The analysis of the chemical composition of the four (4) essential oils revealed that the composition of the essential oil varies according to the chemotype which generally reflects the majority compound. The essential oil extracted from the leaves of *O. gratissimum* consists essentially of monoterpenes, i. e. 91%, of which 61.8% are hydrocarbon and 29.2% are oxygenated. Oxygenated monoterpenes such as thymol (27.0%) and hydrocarbons such as γ- terpinene (26.0%), p-cymene (11.0%), α-thujene (7.0%), myrcene (5.0%) constitute the majority
of the essential oil composition. These results corroborate those obtained from the work of Tchoumbougnang and al [13], Akantetou and al [14] and Ouedraogo and al [15]. Indeed, in the chemotypes studied by these authors, a high composition of hydrocarbon elements was detected in *O. gratissimum* oils. On the other hand, Cameroon's is relatively rich in oxygenated monoterpenes [16, 17].
Thymol, γ-terpinene and *p*-cymene were the main molecules of the essential oil of *O. gratissimum*. These chemical constituents and their proportions are relatively not in the same orders of magnitude as those of Kpodekon and al. [18] in Benin. These authors found more thymol (43.4%), p-cymene (12.3%) and less than γ-terpinene (12.1%). The remarkable presence of thymol in the sample studied is generally singular with essential oils of *O. gratissimum* [16, 17, 19]. Koffi and al [20] reach the same conclusion with an oil from a locality in southern Côte d'Ivoire. In their analyses, more thymol (43.3%) and *p*-cymene (16.8%) were found, but γ-terpinene is absent or in trace form. These differences could be explained by the physiological state of the plant at harvest, as well as the time at which the leaves are harvested and the drying time [21-23]. These factors probably explain the fact that thymol and to a lesser extent γ-terpinene vary in thymol-type oils from the locality in southern Côte d'Ivoire.
The essential oil of *C. citratus* is marked by the presence of acyclic monoterpenes such as myrcene (29.9%), geranial (28.0%) and neral (22.0%). The presence of these two main constituents, neral and geranial, which are isomers is singular with essential oils of *C. citratus* but the chemical constituents are not in the same orders of magnitude as that of Noudjou, [17] and Akono and *et al.* [24] in Cameroon. These two compounds, together with geraniol, show the importance of oxygenated monoterpenes, which together represent 61.2%. The importance of monoterpenes is reported in other essential oils of *C. citratus* [24-26]. Despite these similarities between the essential oils of *C.citratus of* various authors, some differences can be noted, in particular, myrcene, generally inproportions of 10.4 to 12% [24] is the main compound (29.9%) in theanalyzed oil. Kanko and al [27] reported the presence of myrcene in an essential oil from Côted'Ivoire. In their analysis, the myrcene represents only 12%.
The compositional variations observed in the essential oil of *C. citratus*, namely the increase in myrcene content and the decrease in geraniol, can be justified by the geographical origin and physiological state of the plant.
The essential oil of *C. nardus* contains a little more oxygenated monoterpenes (76.9%) with less than 6% hydrocarbon monoterpenes. Oxygenated monoterpenes such as geraniol (51.6%), citronellol (17.0%) and citronellal (5.0%) are the most abundant. This composition is quite different from that of a Togo essential oil in which we find citronellal (30.58%), geraniol (23.93%), elementol (12.04%), geranyl acetate

(8.68%) and citronellol (7.65%) [28]. In the oil analysed, elemol represents only 6.0%, and citronellal, which is the minority component (5.0%), represents the majority component in the compositions reported by Nyamador [28]. It can therefore be assumed, in this case, that the geographical origin of the plant has a considerable influence on the composition of the essential oil.

The essential oil of flowers of *L. multiflora* contains relatively equal proportions of oxygenated monoterpenes (41.3%) and hydrocarbon monoterpenes (45.6%). The richness in hydrocarbon monoterpenes is in line with Bayala's [29] work according to which *p-cymene* is the major compound with 25.27%. In the oil analysed, geranial, neral and linalol are the main oxygenated compounds. This essential oil thus presents a composition close to the references of literature. Various studies carried out on the chemical composition of the essential oil in the sub-region have revealed the presence of several chemotypes, including *L. multiflora* Mold. citral chemotype; *L. multiflora* Mold. chemotype 1.8-cineole/ néral/ géranial; *L. multiflora* Mold. chemotype 1.8 cinéole; *L. multiflora* Mold. chemotype Linalool; *L. multiflora* Mold. chemotype – terpinéol [27, 30] and *L. multiflora* Mold. chemotype nérolidol [6]. Several chemotypes have been described for the species originating from Côte d'Ivoire. Indeed, Kanko obtained twelve (12) groups of majority compounds in sixteen (16) regions of the country [30]. It should be noted, however, that it has a very different composition from the Nigerian [31] and Beninese [32] species.

The variations observed in the chemical composition of each of these oils could be related to the ecology, the period during which the plants were harvested in the year and especially the soil structure on which the plants grew.

Under our experimental conditions, the essential oils of the four local aromatic plants and DEET tested have variable effects on the behaviour of adults of *An. gambiae* (Table 2). Indeed, the rate of mosquitoes displaced in treatment and control differs significantly from one product to another ($p<0.05$).

However, these repellent effects are positively dependent on concentration (0.1 and 1%) (Table 3). Thus, depending on the concentration of the products tested, we distinguish three product groups. The first group is made up of repellent essential oils, i.e. they have a repellent action from the lowest concentration. The second group consists of essential oils that induce repulsion only at the highest concentration and the last group, consisting of less repellent product regardless of the increase in concentration. Among the products tested, it is the essential oils of *C. citratus*, *C. nardus* and *L. multiflora* which have a significant repellent effect from the low concentration. These observations confirm their insecticidal [6, 33] and acaricidal [34] properties. Essential oils belonging to plants of the citronella genus (Poaceae) are commonly used as ingredients in commercial plant-based insect repellent preparations, mainly *C. nardus* [35].

The repellent effect of essential oils of *C. citratus* and *C. nardus* against mosquitoes has been reported in Thailand [8, 36]. According to these authors, lotions based on these essential oils have shown excellent protection against *A. aegyti* (L) and *C. quinquefasciatus* (Say). [37] showed that the essential oils of *C. citratus* and *C. nardus* used individually have a significant repellent effect on *A. arabiensis*. These authors also indicated that the combination of formulations based on these two (2) essential oils in proportions of 10 or 20% gave better protection than individual effects.

Citronellol and geraniol are two substances with proven repellency. They are found in particular in the foliage of lemongrass (*Cymbopogon citratus*) and some *Cymbopogon* such as *C. nardus*.

The insecticidal activity by fumigation of these essential oils on other species of the genus *Callosobruchus* has been reported. According to Nyamador [28], the essential oil of *C. nardus* reduces the number of eggs laid by females of *C. maculatus* and *C. subinnotatus* with a reduction rate of more than 80% for *C. maculatus* and more than 90% for *C. subinnotatus* compared to the control.

The results obtained with the essential oil of *L. multiflora* show that it had a remarkable repellent effect on *An. gambiae* depending on the oil concentration. The repulsion rate at 0.1%, for example, is 0.92±0.2, 5.08 ± 1.4 for the 1% concentration. Literature data indicate that the effectiveness of a repellent depends on several factors, including the concentration of the product used. Yao [38], for example, observed a link between the protection time and the concentration of the essential oil used. It indicates that the essential oil of *L. multiflora* at a dilution of 10% provided a longer protection time (33.3 min) on *An. gambiae* compared to the 5% (14.6 min) and 1% (4.3 min) dilutions.

The essential oil of *O. gratissimum* has a moderate repellent effect on *An. gambiae* and its effect is theoretically identical regardless of the concentration tested. Our results of the repellent effect of *O. gratissimum* essential oil against *An. gambiae* do not match those reported by Oparaocha and al. [39]. These authors have shown that a formulation of the essential oil of *O. gratissimum*, with an olive oil at a concentration of 30%, produces a repulsion of more than 96% against *A. gambiae*. Similar results were obtained by Kweka and Prabhu [40] with an *O. gratissimum* oil-based formulation against *C. quinquefasciatus*. These observations show that the essential oil-based formulation of *O. gratissimum* with olive oil improves the repellency of this oil.

Comparing the repellent potential of the essential oils tested with DEET against *A. gambiae*, it appears that the latter was not effective in repelling mosquitoes at both concentrations.

According to Ladj-Minost [41], instead of having a repellent effect perse, DEET would act on the receptors of attractive compounds by competing with them, which is why this molecule is an agonist. Attractive information would not be routed to the central olfactory system. It would act by masking attractive "odor neutralizer" odors that would confuse insects by inactivating just enough receptors to disrupt the mosquito, or even make it insensitive to odors it should find attractive.

According to Peterson and Coats [42], DEET is a synthetic repellent that is stable in the environment but is less volatile compared to essential oils. It remains the most widely used mosquito repellent. However, toxic effects have been reported with the use of DEET, including encephalopathy in children, urticaria syndrome, anaphylaxis, hypotension and decreased heart rate.

Our results suggest that these essential oils can be considered a potential repellent that could reduce mosquito attacks. The repulsion observed against *An. gambiae* can be attributed to high concentrations of monoterpenes. The essential oils tested were characterized by their richness in oxygenated monoterpenes such as thymol, citral, geraniol, citronellol, citronellal and linalol. Several studies report the insecticidal potential of these terpene compounds [24, 34, 43]. Indeed, several authors had concluded that the biological effects might be either the result of a synergism of all the molecules or could reflect only those of the main molecules. In that sense, for biological purposes, it could be more informative to study the entire oil rather than some of its components because the concept of synergism seems to be important [24, 44]. Tak *et al.* [45] demontrated that essential oils often have stronger insecticidal activity than any of their individual constituents.

Table 1: Chemical composition

Compounds	**IR exp**	**IR lit**	***O. gratissimum***	***C. citratus***	***C. nardus***	***L. multiflora***	**Identification**
Hydrocarbon Monoterpen			**61,8 %**	**35,4 %**	**5,9 %**	**45,6 %**	
α-Thujene	932	924	7,0			0,3	GC, MS, IR
α- Pinene	940	932	2,0			1,0	GC, MS, IR
Camphene	956	946	0,1				GC, MS, IR
Sabinen	979	969	1,0			0,3	GC, MS, IR
β- Pinène	985	974	1,0			0,1	GC, MS, IR
3-Octanone	989	979	0,1				GC, MS, IR
6-methyl-5-Hepten-2-one	990	981		4,4	0,3	1,0	GC, MS, IR
Myrcene	995	988	5,0	29,9		1,0	GC, MS, IR
6-methyl-5-Hepten-2-ol	996	989			0,5		GC, MS, IR
α- Phellandrene	1010	1002	0,5			10,5	GC, MS, IR
δ-3-Carene	1016	1008	0,1				GC, MS, IR
α- Terpinene	1022	1014	4,0				GC, MS, IR
p-Cymene	1030	1020	11,0	0,1	0,1	20,0	GC, MS, IR
Limonene	1034	1024	1,0	0,1	5,0	5,0	GC, MS, IR
β- Phellandrene	1031	1025				5,0	GC, MS, IR
1,8-Cineol	1035	1026	0,1	0,5		0,8	GC, MS, IR
(Z)-β-Ocimen	1039	1032	0,5	0,3			GC, MS, IR
(E)-β-Ocimen	1050	1044	0,3	0,1		0,6	GC, MS, IR
γ-terpinen	1065	1054	26,0				GC, MS, IR
cis-hydrate de sabinen		1065	0,1				GC, MS
beta-Cymenen	1084	1082	2,0				GC, MS, IR
Terpinolene	1089	1086					GC, MS, IR
Oxygenated Monoterpen			29,2 %	61,2 %	76,9 %	41,3 %	
Linalol	1102	1095	0,1	1,0	0,8	3,7	GC, MS, IR
Perillen	1102	1102		0,3			GC, MS, IR
1,3,8-p-Menthatrien	1116	1108	0,1				GC, MS, IR
cis-p-Menth-2-1-ol	1123	1118				0,2	GC, MS, IR
Camphre	1153	1141					GC, MS, IR
Isopulegol	1151	1145			0,3		GC, MS, IR
Citronellal	1156	1148		0,5	5,0		GC, MS, IR
(Z)-Isocitral	1162	1160		0,4		0,3	GC, MS, IR
Borneol	1168	1165	0,1				GC, MS, IR
epoxide Rosefuran	1177	1173		0,8	0,2	0,2	GC, MS, IR
Terpinèn-4-ol	1182	1174	1,0				GC, MS, IR
(E)-Isocitral	1180	1177		0,6		0,6	GC, MS, IR
p-Cymen-8-ol	1188	1179	0,1				GC, MS, IR
cis-Pinocarvéol	1203	1182				0,4	GC, MS, IR
□- Terpineol	1192	1186	0,1				GC, MS, IR
Citronellol	1231	1223		0,4	17,0	0,1	GC, MS, IR
Thymol, methyl ether	1235	1232	0,2				GC, MS, IR
Neral	1242	1235		22,0	0,1	15,0	GC, MS, IR
Geraniol	1255	1249	0,2	6,3	51,6	1,0	GC, MS, IR
Geranial	1273	1264		28,0	0,1	19,8	GC, MS, IR
Citronellyl formate	1280	1271		0,1			GC, MS, IR
Thymol	1294	1289	27,0	0,8	0,6		GC, MS, IR
Geranyl formate	1303	1292			0,1		GC, MS, IR
Itronellyl acetate	1352	1350			0,4		GC, MS, IR
Eugenol	1361	1356			0,7		GC, MS, IR
Cavacrol	1301	1298	0,3				GC, MS,IR
Hydrocarbon Sesquiterpen			8,7%	2,2%	3,2%	9,1%	
α- Copaene	1381	1374	0,3				GC, MS,IR
Géranyl acetate	1382	1379		1,4	0,6		GC, MS, IR
β- Cubeben		1387	0,1				GC, MS
β- Elemen		1389	0,1		1,0		GC, MS
β- Caryophyllene	1428	1417	3,0	0,2		2,4	GC, MS, IR
trans-α-Bergamoten	1436	1432		0,2			GC, MS, IR

β- Gurjunen	1441						GC, MS, IR
α- Humulen	1461	1452	0,4			4,5	GC, MS, IR
(E)-β-Farnésene	1458	1454		0,1		1,0	GC, MS, IR
Allo-aromadendrene		1158				0,1	GC, MS, IR
Precocene I	1464	1461		0,1			GC, MS, IR
Germacren D	1488	1484	0,3		0,7	0,7	GC, MS, IR
β- Selinen	1495	1489	3,0				GC, MS, IR
Tridecanon	1490	1495		0,2			GC, MS, IR
α- Selinen	1502	1498	1,0				GC, MS, IR
Bicyclogermacren	1505	1500					GC, MS, IR
β- Bisabolen	1505	1505				0,3	GC, MS, IR
γ-Cadinen	1522	1513			0,4		GC, MS, IR
7-epi-selinen	1527	1520	0,4				GC, MS, IR
δ-Cadinen		1522	0,1		0,5	0,1	GC, MS
Oxygenated Sesquiterpen			0%	0%	9,0%	1,3 %	
Elemol	1558	1548			6,0		GC, MS, IR
(E)-Nerolidol	1559	1561				0,5	GC, MS, IR
Germacren D-4-ol	1587	1574			2,0		GC, MS, IR
Spathulenol	1584	1577					GC, MS, IR
Oxyde de caryophylene	1590	1582				0,7	GC, MS, IR
epoxide II Humulen		1608				0,1	GC, MS
γ-Eudesmol		1630			0,1		GC, MS
epi-α-Muurolol		1640			0,1		GC, MS
β-Eudesmol		1649			0,1		GC, MS
α-Cadinol	1662	1652			0,7		GC, MS, IR
Sclaren	2027						GC, MS, IR
Abiétatrien	2063						GC, MS, IR
	Total		99, 7%	98, 8%	95, 0%	97, 3%	

Conclusion

In short, our work shows that some of the essential oils extracted from local plants are interesting repellents against *An. gambiae*, major malaria vector, the most common species found in many parts of the country. These results suggest that plant extracts have a potential repellent effect of varying magnitude between the products tested and the concentrations. For us, in carrying out this work, the objective was less the comparison of the repellent action of essential oils with synthetic repellents, but rather to find a repellent of natural origin sufficiently effective at low concentrations. This is done with a view to substitution, considering the limited effectiveness of synthetic repellents, and the disadvantages associated with their use.

Most of the se essential oils are very volatile, which contributes to their short life as insect repellents. However, this problem can be solved by using hairsprays or careful formulation to improve their longevity. Further research on improving the formulation technique is needed to increase the repellent effect of the essential oil in order to reduce the high volatility of the active ingredients.

Acknowledgement

We are grateful IRD-Montpellier for financial support in the form of a Grant in Aid for Scientific Research The authors would also like to thank the "glyco and nano vector for therapeutic screening" team at the Max Mousseron Biomolecules Institute (IBMM) of the University of Montpellier 1 for the chemical characterization of essential oils.

Conflicts of interest

We have no conflict of interest to declare.

References

1. World Health Organisation. World malaria report 2011. WHO Global Malaria Programme. Geneva : WHO, 2011, 248. ISBN : 978 92 4 156440 3.
2. Chadi Fakih. Malaria in Côte d'Ivoire : current situation and control strategies. Doctoral thesis, U.F.R. des Sciences Pharmaceutiques, Université Bordeaux (France), 2014, 143.
3. World Health Organization, Implementation of the global malaria control strategy. Report of a WHO Task Force on the implementation of the Global Plan of Action for Malaria Control. Geneva, (WHO, Technical Report Series, No. 839), 1993.
4. Ranson HN, Guessan R, Lines J, Moiroux N, Nkuni Z *et al*. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 2011; 27:91-98.
5. Nagarajan Y, Venkatraman A, Mohamed YSA, Radhakrishnan M, Arumugam C, Muniyasamy MP. Chemical properties of essential oil from *Rhizophora mucronata* mangrove leaf against malarial mosquito *Anopheles stephensi* and flarial mosquito *Culex quinquefasciatus*. Asian Pacific Journal of Tropical Disease. 2015; 5(Suppl 1):S67-S72.
6. Tia VE, Adima AA, Niamké SL, Gnago AJ, Thibaud M, Lozano P *et al*. Chemical composition and insecticidal activity of essential oil of two aromatic plants from Ivory Coast against *Bemisia tabaci* G. (Hemiptera: Aleyrodidae). Natural Product Communications. 2011; 6 (8):1183-1188.
7. Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitology Research. 2010; 107:1455-1461.

8. Mayura Soonwera, Siriporn Phasomkusolsil. Mosquito repellent from Thai essential oils against dengue fever mosquito (*Aedes aegypti* (L.)) and filarial mosquito vector (*Culex quinquefasciatus* (Say)). African Journal of Microbiology Research. 2014; 8(17):1819-1824.
9. Tia EV, Lozano P, Menut C, Lozano YF, Martin Niamké TS, Adima AA. Potentiality of essential oils in biological control of the white fly *Bemisia tabaci* Genn. Phytotherapy. 2013; 11:31-38.
10. Adam's RP. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry. 4th Edition. *Allured Publishing Corporation*, (Ed.). Carol Stream, Il 60188-2787. USA, 2007, 803.
11. Achee NL, Sardelis M, Dusfour I, Chauchan K, Grieco J. Characterization of spatial repellent, contact irritant, and toxicant chemical actions of standard vector control compounds. Journal of American Mosquito Control Association. 2009 ; 25:156-167.
12. Grieco JP, Achee NL, Andre RG, Roberts DR. A novel high-throughput screening system to evaluate the behavioral response of adult mosquitoes to chemicals. Journal of American Mosquito Control Association. 2005 ; 21:404-411.
13. Tchoumbougnang F, Dongmo PMJ, Sameza ML, Mbanjo EGN, Fotso GBT, Zollo PHA *et al*. Larvicidal activity against *Anopheles gambiae* Giles and chemical composition of essential oils from four plants cultivated in Cameroon. Biotechnology, Agronomy, Society and Environment. 2009; 13:77-84.
14. Akantetou PK, Koba K, Nenonene AY, Poutouli WP, Raynaud C, Sanda K. Evaluation of the insecticidal potential of *Ocimum canum* Sims essential oil on *Aphis gossypii* Glover (Homoptera : Aphididae) in Togo, International Journal of Biological and Chemical Sciences. 2011; 5(4):1491-1500.
15. Ouédraogo I, Sawadogo A, Nebie RCH, Dakouo D. Evaluation of the toxicity of the essential oils of *Cymbopogon nardus* L. and *Ocimum gratissimum* L. against *Sitophilus zeamais* Motsch and *Rhyzopertha dominica* F, the main maize pests in storage in Burkina Faso. International Journal of Biological and Chemical Sciences. 2016; 10(2):695-705, 2016.
16. Nguemtchouin MGM. Formulation of insecticides in powder form by adsorption of essential oils of *Xylopia aethiopica* and *Ocimum gratissimum* on modified Cameroonian clays. Doctoral thesis, Ecole nationale supérieure de chimie, Montpellier, 2012, 293.
17. Noudjou-Wandji Félicité E. Use of the essential oil for the protection of grains against devastating insects in Northern Cameroon (thèse de doctorat in French). Gembloux, Belgium Gembloux Agricultural University, 2007, 201.
18. Marc Tchokponhoue Kpodekon, Kadoeito Cyrille Boko, Jacques Georges Mainil, Souaibou Farougou, Philippe Sessou, Boniface Yehouenou *et al*. Composition chimique et test d'efficacité in vitro des huiles essentielles extraites de feuilles fraîches du basilic commun (*Ocimum basilicum*) et du basilic tropical (*Ocimum gratissimum*) sur *Salmonella enterica* sérotype Oakland et Salmonella enterica sérotype Legon. Journal de la Société Ouest-Africaine de Chimie, 2013; 035:41- 48
19. Gueye S, Diop MT, SecK D, Sembene M. Biochemical fractions activity of *Annona senegalensis* Pers. extract leaves to protect groundnut against the seed-beetle *Caryedon serratusol*. (Coleoptera, Chrysomelidea, Bruchinae). International Journal of Plant, Animal and Environmental Sciences. 2011; 1:122-130.
20. Koffi AM, Tonzibo ZF, Delort L, Ruiz N, Caldefie-Chézet L, Chalchat JC. Correlation between the chemical composition and antifungal activity of essential oils predominantly thymol on *Candida albicans* and *Aspergillus fumigatus*. Phytotherapy. 2013; 11:134-139.
21. Madeira SVF, Rabelo M, Soaresa PMG, Souza EP, Meirelesa AVP, Montenegro C *et al*. Temporal variation of chemical composition and relaxant action of the essential oil of *Ocimum gratissimum* L. (Labiatae) on guinea-pig ileum. Phytomedicine. 2005; 12:506-509.
22. Silva de VGM, Craveiro AA, Matos AJF, Machado LIM, Alencar WJ. Chemical variation during daytime of constituents of the essential oil of *Ocimum gratissimum* leaves. Fitoterapia. 1999; 70:32-34.
23. Gupta SC. Variation in herbage yield, oil yield and major component of various Ocimum species/varieties (chemotypes) harvested at different stages of maturity. Journal of Essential Oil Research. 1996; 8:275-279.
24. Akono Ntonga P, Baldovini N, Mouray E, Mambu L, Belong P, Grellier P. Activity of *Ocimum basilicum*, *Ocimum canum*, and *Cymbopogon citratus* essential oils against *Plasmodium falciparum* and mature-stage larvae of *Anopheles funestus* S.S. Parasite. 2014; 21:33.
25. Sidibé L, Chalchat JC, Garry RP, Lacombe L, Harama M. Aromatic plants of Mali (IV): chemical composotion of essential oils of Cymbopogon citratus (DC) Stapf and C. giganteus (Hochst.) Chiov. Journal of Essential Oil Research. 2001a; 13:110-112.
26. Cimanga K, Apers S, Bruyne De T, Miert VS, Hermans N, Totté J *et al*. Chemical composition and antifungal activity of essential oil of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of Essential Oil Research, 2002a; 14:382-387.
27. Kanko C, El-Hadj Sawaliho B, Kone S, Koukoua G, N'Guessan YT. Study of the physico-chemical properties of the essential oils of *Lippia multiflora*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Cymbopogon giganteus*. C. R. Chemistry. 2004; 7:1039-1042.
28. Nyamador Seth Wolali. Influence of treatments based on essential oils on the reproductive capacities of *Callosobruchus subinnotatus* pic. and *Callosobruchus maculatus* f. (Coleoptera: bruchidae): mechanism of action of the essential oil of *cymbopogon giganteus* chiov. Doctoral thesis, Faculty of Science, University of Lome-Togo, 2009, 176.
29. Bayala B. Study of the antioxidant, anti-inflammatory, antiproliferative and antimigratory properties of essential oils of some medicinal plants from Burkina Faso on prostate cancer cell lines and glioblastoma. Doctoral thesis, Blaise Pascal University. 2014, 141.
30. Kanko C, Koukoua G, N'Guessan YT, Lota ML, Tomi F, Casanova J. Composition and intraspecific variability of the leaf oil of *Lippia multiflora* Mold. from the Ivory Coast. Journal of Essential Oil Research. 1999; 11:153-158.
31. Owolabi MS, Ogundajo A, Lajidé L, Oladimeji MO, Setzer WN, Palazzo MC. Chemical composition and antibacterial activity of essential oil of *Lippia multiflora* Moldenke from Nigeria. Records of Natural Product, 2009; 3(4):170-177.

32. Avlessi F, Alitonou G, Sohounhloue, Menut, Bessière JM. Aromatic plant of tropical West Africa. Part XIV. Chemical and biological investigation of *Lippia multiflora* Mold. essential oil from Benin. Journal of Essential Oil Research, 2005; 17(4):405-407.
33. Kimutai A, Ngeiywa M, Mulaa M, Njagi PGN, Ingonga J, Nyamwamu LB *et al*. Repellent effects of the essential oils of *Cymbopogon citratus* and *Tagetes minuta* on the sandfly, *Phlebotomus duboscqi*. BMC Res Notes. 2017; 10:98.
34. Mateus AC, Caio MOM, Marcio GS, Fernando TG, Marcio CAP, Erick D. Arcaricidal activity of essential oils from *Eucalyptus citriodora* and *Cymbopogon nardus* on larvae of *Amblyomma cajennense* (Acari: Ixodidae) and *Anocentor nitens* (Acari: Ixodidae). Parasitology Research. 2010; 10:987-992.
35. Deletre E, Martin T, Campagne P, Bourguet D, Cadin A, Menut C *et al*. Repellent, irritant and toxic effects of 20 plants extracts on adults of the malaria vector Anopheles gambiae mosquito. PLoS ONE. 2013; 8:e82103.
36. Soonwera M, Phasomkusolsil S. Effcacy of Thai herbal essential oils as green repellent against mosquito vectors. Acta Tropica. 2015; 142:127-130.
37. Solomon B, Gebre-Mariam T, Asres, K. Mosquito repellent actions of the Essential Oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Eucalyptus citriodora*: Evaluation and Formulation Studies. Journal of Essential Oil-Bearing Plants. 2012; 15:766-773.
38. Yao Charles Alexis. Evaluation of the repellent effect of *Lippia multiflora* Mold essential oil. (verbenacees), with regard to three species of mosquito vectors and pests in Côte d'Ivoire. PhD thesis, UFR des Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët Boigny ex-Université de Cocody-Abidjan, 2000, 102.
39. Oparacha ET, Iwu I, Ahanakuc JE. Preliminary study on mosquito repellent and mosquitocidal activities of *O. gratissimum* (L.) growth in eastern Nigeria. Journal of Vector Borne Diseases. 2010; 47:45-50.
40. Kweka EJ, Prabhu K. Parasites and Vectors, *Asian* Pacific Journal of Tropical Biomedicine. 2012; 5:189
41. Ladj-Minost Audrey. Long-lasting arthropod repellents for the veterinary industry: pharmacotechnical study, *in situ* development and efficacy. Thesis Doctoral School of Chemistry, Claude Bernard Lyon University 1 France, 2012, 312.
42. Peterson C, Coats J. Insect repellents : past, present and future. Pesticide outlook, 2001.
43. Lames Y, Elias NN, Simon PYD, Charles OE. Repellent activity of the creams formulated from *Annona senegalensis* and *Boswellia dalzielii* leaf fractions and essential oils against *Anopheles gambiae* (Diptera: Culicidae). Asian Pacific Journal of Tropical Diseases, 2016; 6(12):973-978.
44. Freitas FP, Freitas SP, Lemos GC, Vieira IJ, Gravina GA, Lemos FJ. Comparative larvicidal activity of essential oils from three medicinal plants against *Aedes aegypti* L. Chemistry & Biodiversity, 2010; 7:2801-2807.
45. Tak JH, Jovel E, Isman MB. Contact, fumigant, and cytotoxic activities of thyme and lemongrass essential oils against larvae and an ovarian cell line of the cabbage looper, *Trichoplusia* ni. Journal of Pest Science. 2016; 89:183-193