Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Diversity of aggressive Aedinian fauna and susceptibility of *Aedes albopictus* (Skuse, 1894) to *Ageratum conyzoides* L., 1753 and *Chromolaena odorata* L. Robinson, 1970 (Asteraceae) essential oils in some towns from southern Cameroon

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Abstract

Background: A good knowledge of Aedes diversity and their biting cycle is necessary for better planning of vector control interventions against arboviruses diseases. This study aims to determine the diversity of Aedes and the biting cycle of *Aedes albopictus* in urban and suburban areas of Douala, Kribi, and Ayos to propose a method for vector control based on *Ageratum conyzoides* L., 1753 and Chromolaena odorata L., 1970 (Asteraceae) essential oil.

Methods: Larvae were sampled by dipping method and reared by Desfontaine method. The aggressiveness of Aedes was determined by the outdoors Human Landing Catches method from 6 am to 6 pm. Captured mosquitoes were morphologically identified using identification keys. Essential oils from fresh leaves of *A. conyzoides* and *C. odorata* were extracted by hydrodistillation using Clevenger while the chemical composition was determined by GC-MS. Insecticide tests were carried out according to WHO protocol on matures *Ae. albopictus* larvae.

Results: *Ae. albopictus* (n=3499; 80.5%) was the most abundant species in urban and suburban areas followed by *Ae. unilineatus* (n=392; 9.02%) and *Ae. aegypti* (n=210; 4.83%). The mean biting rate of Aedes was 21.52 bites/person/day (b/p/d). In urban and suburban areas *Ae. albopictus* (18.22 b/p/d) was the most aggressive species followed by *Ae. unilineatus* (2.04 b/p/d) and *Ae. aegypti* (1.09 b/p/d). Precocene I (54.4%) and Androencecalinol (24.69%) were the major compounds of *A. conyzoides* fresh leaves essential oil. Geijerene (20.02%) and trans-Muurola-4(14), 5-diene (19.15%) were the major compounds of *C. odorata* fresh leaves essential oil. *A. conyzoides* fresh leaves essential oil was the most effective with 100% mortality after 10 hours of exposure at 75 ppm concentration.

Conclusion: This study reveals that Aedes was more aggressive in urban areas than in suburban areas. *Aedes albopictus* was the most aggressive species. *A. conyzoides* fresh leaves essential oil should be considered in the implementation of control strategies against Aedes.

Keywords: Aedes diversity; biting cycle; fresh leaves; urban; suburban.

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Citation on this article: Ngo Hondt OE, Akono-Ntonga P, Tsila HG, Nopowo Takap FN, Soh Teukam W, Ngaha R, Offono Enama L, Mbongue RS, Eyisap Ekoko W, Moussango VD, Tedjou Nouboudem A, Kopya E, Ntoumba AA, Monkam Tchamaha F, Talipouo A, Awono Ambeme HP, Lehman LG. Diversity of aggressive Aedinian fauna and susceptibility of Aedes albopictus (Skuse, 1894) to Ageratum conyzoides L., 1753 and Chromolaena odorata L. Robinson, 1970 (Asteraceae) essential oils in some towns from southern Cameroon. Investigational Medicinal Chemistry and Pharmacology (2022) 5(2):65; Doi: https://dx.doi.org/10.31183/imcp.2022.00065

Background

Aedes (Diptera: Culicidae) are mosquitoes with a medical interest. Their females play a significant role in the transmission of many viruses such as dengue, Chikungunya, Zika, and amaril viruses, particularly in Africa, which is one of the most affected continents [1-3]. Aedes albopictus appears to be the most common species in Central African cities. This species was first identified in Africa in 1990 from the Asian continent. It was reported in Central Africa, notably in Cameroon, two decades ago [4]. This species is thought to have contributed to the re-emergence of dengue and chikungunya in Central Africa due to its abundance [5].

The control of arboviruses diseases is a global priority. The strategy adopted by countries in the sub-region includes early detection and management of cases combined with prevention using Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Insecticide Spraying (IRS). The implementation of these interventions has yielded promising results. In Gabon, for example, the prevalence of yellow fever has decreased from 15% in 2010 to 10% in 2016. The trend is similar in other countries such as Equatorial Guinea and Cameroon [6].

However, the increased application of insecticides in the natural environment pollutes the environment and can affect non-target animal populations [7, 8-9]. Moreover, vector strains resistant to these chemicals are increasing in sub-Saharan Africa [10-13]. In this context, the use of insecticides in public health is less and equally less recommended, thus the development of new means of vector control has become a priority in the research programs of many countries. Work is increasingly oriented towards strategies that consider environmental protection, particularly natural molecules derived from essential plant oils [14]. As a result, the plants of the Cameroonian pharmacopoeia constitute an inexhaustible source of bioactive molecules that simply need to be explored. This is the case of certain plants that have been used for thousands years in the South Cameroon forest in prevention and treatment of numerous pathologies in traditional medicine. This is the case of *Ageratum conyzoides* and *Chromolaena odorata*, two plants from the Asteraceae family. Originating respectively from Central and South America, these plants have been successfully introduced in Cameroon due to favorable eco-climatic conditions. Most naturopaths use them in the treatment of peptic ulcers, intestinal inflammation, pancreatitis, rheumatoid arthritis, coughs, and umbilical hernia [15, 16]. Others use them as insect repellents for mosquito control. Regarding the uses above, we proposed to consider a strategy for the control of *Aedes albopictus* based on the essential oils of these plants.

However, to be effective and sustainable, this strategy should necessarily integrate a perfect knowledge of vectors and their biting behavior [17-19].

In Cameroon, few works have been carried out on the diversity of Aedes species aggressive to humans because most entomologists focus their interest on malaria vectors. Apart from the investigations carried out in Douala [20, 21], no other large-scale study is reported in this central African country concerning *Aedes albopictus*. However, due to their geographical position, some towns in the country, such as Ayos and Kribi, are strategic points for international trade, especially in used tires or containers in which Aedes eggs can survive for several weeks in a quiescent state [22]. Furthermore, the abusive use of pesticides in agriculture and the use of insecticide-treated bed nets could select resistance genes within the vector populations. It is in this context that this study proposed to determine the aggressive Aedes diversity as well as the susceptibility of *Aedes albopictus* (Skuse, 1894) to *A. conyzoides* and *C. odorata essential* oils in the urban and suburban areas of some towns in southern Cameroon.

Methods

Study sites

The present study was carried out in the cities of Ayos, Douala, and Kribi (Figure 1). Kribi (2° 57′ N, 9° 55′ E) is a city located 180 km from the city of Douala [23]. Its climate is a coastal variant of the Guinean subequatorial climate. Rainfall is abundant (2825 mm on average/year) and distributed throughout the year. Poor management of wastewater, household waste, and used tires is favorable to the proliferation of mosquitoes. Mosquito sampling took place in suburban (Mpangou) and urban (Mokolo and Ndombe) areas of Kribi city.

Ayos (3° 54′N, 12° 31′E) is located 123 km East of Yaoundé. The climate is equatorial savannah [24]. Average annual temperatures range from 23°C to 30°C. The average annual rainfall is 831.7 mm. Mosquito sampling took place in suburban (Ebabodo) and urban (Jamot and Bamiléké neighborhoods) areas of Ayos town.

Douala (4° 03′ N, 9° 42′ E) is the economic capital of Cameroon. The climate is equatorial, hot, and very humid. The temperature varies between 22°C and 34°C, the relative humidity is 80°C to 90°C [25]. There is anarchic urbanization and poor waste management, which contributes to the proliferation of Aedes. Mosquito sampling took place in suburban (Yassa and PK 21) and urban (Ndogbong) areas of Douala.

In all three cities, vector control is focused on the use of insecticides through indoor spraying, LLINs, and environmental sanitation.

Capture of adult female mosquitoes and morphological identification

Daytime Human Landing Catches made a sampling of adult female mosquitoes possible. This is a direct method of measuring host-vector contact and consists of a volunteer sitting on a chair or stool, capturing mosquitoes that land on their bare legs with hemolysis tubes [26]. For each site, the captures took place outside eight houses, from 6 am to 6 pm and at a rate of two consecutive days per quarter during the study period. For each house, two volunteers were used for the captures for a total of 4 men per day. All volunteers were subject to prophylaxis against malaria and yellow fever.

Captured female mosquitoes were morphologically identified using the specialized keys of Edwards (1941), Jupp (1996), Rueda (2004), Huang (2004), Harrison et al, (2016), and Coetzee (2020).

Plant collection and extraction of essential oils

The plant material (fresh leaves of *A. conyzoides* and *C. odorata*) was collected in March and April 2019 in Douala. The plants collected were identified by the botanists of the Plant Biology laboratory of the Faculty of Sciences of the University of Douala, and not subjected to any chemical insecticide treatment that could influence the chemical composition of the volatile essences. The essential oils were extracted by hydrodistillation using a Clevenger

[27]. The duration of the distillation was 3 to 4 hours. Traces of water were removed by filtration with anhydrous sodium. The essence obtained was put in dark glass bottles, weighed, and stored at 4°C.

Sampling and processing of Aedes larvae

Aedes larvae were sampled by the 'dipping' method [28]. Larvae was collected every two days in urban and suburban areas of Douala, Ayos, and Kribi in all artificial and natural sites likely to host Aedes larvae. These larvae were transported and reared under laboratory conditions until they emerged as adults.

Larvicidal tests

These tests were performed on mature larvae (stages 3 and 4) of *Aedes albopictus*. They consisted in evaluating the mortality of *Ae. albopictus* larvae in the presence of diluted solutions of essential oils following a methodology inspired by the World Health Organization protocol [29]. Indeed, 20 larvae were collected and placed in 8 cm diameter bowls each containing 99 ml of spring water to which 1ml of diluted test solution was added. The screening test was run out to select a range of concentrations for the actual tests. For this purpose, stock solutions of essential oils of each sample were prepared in 90° ethanol. From these, dilutions were made to give final concentrations of 25 ppm, 75 ppm, 100 ppm, 600 ppm, 700 ppm, and 800 ppm. Four replicates were made for each dilution. Two control bowls were also set up under the same conditions as the test bowls. The negative control contained only ethanol (in the same proportions as for the tests, i.e. 1%) with no trace of essential oil. Dead larvae were counted every 5 minutes for 1 hour, then every hour for 10 hours, and finally after 24 hours of exposure to the volatile extracts solubilized in water.

Determination of the chemical composition of essential oils

Analysis by gas chromatography

The chemical analysis of the essential oils by Gas Chromatography was carried out using a Hewlett Packard electronic pressure chromatograph (HP 6850 series), equipped with an HP-5 capillary column (60 m x 0.25 µm), with a film thickness of 0.25 Jlm and an FID detector regulated at 260°C, fed with a H2/air mixture and a split-splitless injector regulated at 275°C. The injection mode was split (leakage ratio: 1/50, flow rate 66 ml/min). The carrier gas used was nitrogen with a flow rate of 1.7 ml/min. The column temperature was programmed from 50 to 240 °C at 5 °C/min.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was performed on a Hewlett-Packard gas chromatograph (HP 6890 series) coupled with a mass spectrometer (HP 5973 series). Fragmentation was carried out by electronic impact under a field of 70 Ev. The column used was a HP-5MS capillary column (30m x 0.25 mm), and the film thickness was 0.25 Jlm. The temperature of the column was programmed from 70 to 240 °C at 5 °C/min. The carrier gas was helium with a fixed flow rate of 1.5 ml/min. The injection mode was split (leakage ratio: 1/70, flow rate 112 ml/min). The apparatus was connected to a computer system managing a library of NIST 98 mass spectra. The identification of the constituents was carried out based on their Kovats indices (KI) and gas chromatography-mass spectrometry (GC-MS).

Statistical analysis of the data

Statview version 5.0 (SAS Institute, Inc., USA) and XLSTAT version 2003 were used to compare the means of larval mortality using the Kruskal Wallis H test ($p<0.05$). Henry's simplified table, which transforms mortality percentages into probits, was used to determine the concentrations necessary to obtain 50% (LC_{50}) and 95% (LC_{95}) of dead larvae. Excel 2016 software was used to calculate the mean mortality rates and standard deviations of dead larvae as a function of exposure time.

Results

Diversity of the aggressive Culicinae fauna

The diversity of the aggressive Culicidae fauna is recorded in Table 1. *Ae. albopictus* (n=3499; 80.5%), occupied the bulk of the aggressive fauna followed by *Ae. unilineatus* (n=392; 9.02%) and *Ae. aegypti* (n=210; 4.83%). In Ayos, 2 377 female mosquitoes of 12 species were captured. The abundance of aggressive fauna in the urban area (n=2150; 90.45%) was significantly higher than in the suburban area (n=227; 9.55%) ($p <0.0001$). In Douala, 945 female mosquitoes of seven species were captured. The abundance of aggressive fauna in the urban area (n=738; 78.1%) was significantly higher than in the suburban area (n=207; 21.1%) ($p = 0.0005$). In Kribi, 1 023 female mosquitoes of six species were captured. The abundance of aggressive fauna in the urban area (n=758; 74.1%) was significantly higher than in the suburban area (n=265; 25.9%) ($p <0.0001$).

Biting cycles of Aedes albopictus

The biting cycles of *Aedes albopictus* in urban and suburban areas of our study sites are represented in Figure 2. From this figure, it appears that this cycle differs from one site to another and could be subdivided into 2, 3, 4, or 5 phases. However, the peaks of activity, in general, were observed between 6 am - 8 am and 4 pm - 6 pm in urban areas, and 8 am-10 am and 4 pm - 6 pm in suburban areas.

Aedes bite rates

A total of 4 132 female Aedes were captured by 192 volunteers during the study period. The overall average Aedes aggression rate was 21.52 bites/person/day (b/p/d). *Ae. albopictus* was the most aggressive species (18.22 b/p/d) followed by Ae. unilineatus (2.04 b/p/d) and *Ae. aegypti* (1.09 b/p/d). *Ae. albopictus* and *Ae. aegypti* were the only species whose biting rate in urban areas was significantly higher than in suburban areas with P=0.01 for *Ae. aegypti* and P=0.0213 for *Ae. albopictus*. In urban areas, the overall mean biting rate was 37.09 b/p/d. Ayos (65.13 b/p/d) had the highest aggression rate followed by Kribi (23.69 b/p/d) and Douala (22.46 b/p/d). *Ae. albopictus* was the most aggressive species in all localities. In suburban areas, however, the overall average rate of aggressiveness was 5.95 b/p/d. Kribi (8 b/p/d) had the highest aggression rate followed by Douala (6.28 b/p/d) and Ayos (3.56 b/p/d). *Ae. albopictus* was the most aggressive species in all locations. In general, the biting rates of *Ae. albopictus* and *Ae. aegypti* were higher in urban areas than in suburban areas with p=0.0213 and p=0.01 respectively for *Ae. albopictus* and *Ae. aegypti* (Table 2).

Chemical composition of A. conyzoides and C. odorata fresh leaves essential oils.

The chemical composition of *A. conyzoides* and *C. odorata* fresh leaves essential oils are recorded in Table 3 and Table 4. The essential oil of *A. conyzoides* fresh leaves had a high monoterpene content (79.3%). Precocene I (54.4%), Andro encecalinol (24.69%) and trans-α-bergamotene (15.5%) were the major compounds in this essential oil. The essential oil of C. odorata fresh leaves had a high monoterpene content (55.42%). Geijerene (20.02%), trans-Muurola-4(14), 5-diene (19.15%), Pregeijerene (13.83%), Dictamnol (10.46%) and α-pinene (10.05%) were the major compounds in this essential oil.

Biological activities of essential oils

Mortality rates of *Ae. albopictus* larvae varied according to the concentrations, botanical origin of the extracted essential oils and the origin of larvae (Table 5). The essential oils of both plants were effective on the mature larvae's stages of *Ae. albopictus* but at different levels of toxicity. The essential oil from *A. conyzoides* fresh leaves was the most active. It caused total mortality of mature *Ae. albopictus* strain larvae at 75 ppm concentration after 24 h of exposure. For the same concentration and exposure duration, C. odorata fresh leaves essential oil induced partial mortality of the larvae (Figure 3). The high activity of *A. conyzoides* fresh leaves essential oil is justified by the low LC_{50} values (19.39 ppm; 34.72 ppm; 26.89 ppm) recorded with this oil compared to those recorded with *C. odorata* oil (657.12 ppm; 705.1 ppm; 568.72 ppm), respectively in Ayos, Douala and Kribi.

Discussion

The objective of this study was to determine some patterns of arboviruses persistence in the cities of Douala, Kribi and Ayos through the study of Aedes diversity and their biting behavior. Some solutions have been considered through evaluation of the larvicidal activity of *A. conyzoides* and *C. odorata* essential oils on *Ae. albopictus* strains from Douala, Kribi and Ayos.

The present study shows an impressive diversity of mosquitoes in our study sites. This high diversity is the result of the presence of a wide variety of mosquito breeding sites in the study localities, which differed from one to another in terms of typology (temporary or permanent), nature (artificial or natural) and the physicochemical properties of water. Our results showed that, 98.3% of breeding sites identified were artificial. Similar observations have been made in several cities in Africa and Asia [30-32]. In the urban areas, except in Ayos town where brick molds were most represented, used tires and containers mainly represented artificial breeding sites. The rapid urbanization in Ayos could explain the high presence of brick molds in this locality. This has led to the emergence of brick factories where molds are stored and constitute preferential sites for Aedes larvae during the rainy season. This observation is in line with those made in the Maldives [33] and in Cameroon [34]. The latter's work showed that in urban areas, brick molds are the most attractive oviposition sites and a favorable factor of *Ae. albopictus* and *Ae. aegypti* proliferation. The same applies to use tires found in large numbers in the urban sites of Douala and Kribi. The presence of such sites is a permanent risk of *Ae. albopictus* proliferation [35].

Suburban sites, with a natural environment, present artificial and some natural breeding sites such as cocoa pods and bamboo holes. Ayos is an important cocoa production area in the Central Region. After the cocoa season, broken and bean-deprived cocoa pods are usually attractive breeding sites for Aedes larvae.

Our results show that *Ae. albopictus* larvae are more abundant in breeding site than *Ae. aegypti* larvae in suburban and urban areas of Kribi, Ayos and Douala. *Ae. Aegypti* is found in smaller proportions. Similar observations have been made in several cities in Africa and Asia, indicating a decline or even a progressive decrease of *Ae. aegypti* populations in the areas indicated [36]. Some authors believe that *Ae. Aegypti* is a species complex with the predominant form in sub-Saharan Africa being the dark sylvatic form (*Ae. aegypti formosus*) which generally develops in natural habitats and feeds preferentially on forest hosts [37, 38]. If this information is correct, we would be tempted to link the probable decline of this species to the deep environmental changes undergoing in Douala, Ayos and Kribi. *Ae. albopictus* is originated from Southeast Asia forests [39] and introduced into Africa in the 1990s [40]. It was collected for the first time in Central Africa, notably in Cameroon in 2000 [41]. Many studies suspect the introduction of this species as one of the main causes of the displacement and decline of *Ae. aegypti* populations in Cameroon, in Africa and in many parts of the world [31]. Indeed, *Ae. albopictus* is a species with great ecological plasticity that allows it to adapt to a wide variety of habitats and biotopes while feeding on a wide range of hosts [42].

In all study localities, *Ae. albopictus* was the most aggressive species towards humans. This high aggressiveness is not only due to the strong competitive adaptation of this species but also to the strong anthropophagic tendency shown by females of this species compared to other Aedes. Indeed, some studies have shown that *Ae. albopictus* females are less zoophagous than those of *Ae. aegypti*, and preferentially feed on human blood [43, 44-45]. This trophic attitude is one of the arguments that could explain the high aggressiveness of *Ae. albopictus* in the study localities [46-47].

Our work shows that the biting behavior of *Ae albopictus* is essentially diurnal with peaks in the evening (between 4 pm and 6 pm). This aggressiveness seems to be related to the inhabitant's behavior. This trophic behavior has been observed in numerous studies in Africa and Europe [38-48] and in Asia [49]. Unfortunately, studies on the understanding of this phenomenon explaining the rather diurnal aggressive activity of Aedes are not yet included in the existing scientific literature. However, this aspect of research appears to be of primary importance in improving control strategies against these vectors. This aspect of the research will be the subject of our future investigations.

Our results show that *C. odorata* fresh leaves (0.92%) have higher essential oil content than those of *A. conyzoides* (0.067%). These yields contrast with those obtained in Nigeria, the West Cameroon region, Togo, and Benin [50-51, 52-53, 54-55]. This variation would be related to the plant organ from which the oil is extracted, the nature of the soil, the time of collection, and the time needed for extraction [56-57]. Several other factors can influence plant performance, such as the developmental phase of the plant, the pollination cycle, seasonal variations, and the pathophysiological condition of the plant [58-59].

Chemical composition analysis shows that *A. conyzoides* fresh leaves essential oil is predominated by hydrogenated compounds (74.22%) with Precocene I (54.4%) as the majority compound. These results are different from those obtained in Ivory Coast, Europe, and China [60-62], which present β-Caryophyllene and Ageratochromene (Precocene II) as the majority compounds.

However, our results are in agreement with those observed in Ivory Coast, Burkina Faso, and China samples [63-66]. The latter found Precocene I to be the majority compound. The analysis of the chemical composition of *C. odorata* fresh leaves essential oil shows that Geijerene is the major component (20.02%). These results are close to those obtained in India [67] where geijerene (26.34%) was the majority compound. Pregeijerene and geijerene represented 33.85% of our sample; this result is in line with that of the sample from Cameroon [68]. However, these results are different from those recorded with the samples from Togo and West Cameroon region [52, 54]. These differences in chemical composition for the same plant species could be explained by variations in tissue differentiation (secretory cells and excretory cavities, etc.) necessary for the formation of essential oils and the ontogenetic phase of the plant [69]. This composition could also be influenced by the climatic conditions as well as the chemical composition of the soil where the plant was collected.

The results of the anti-larval tests show that *Ageratum conyzoides* fresh leaves essential oil has a strong larvicidal activity against mature *Ae. albopictus* larvae. This result corroborates that obtained in China [62]. This result showed a high sensitivity of *Ae. albopictus* stages 4 and 3 larvae to the essential oil of fresh leaves of *A. conyzoides*, and demonstrated that this high sensitivity is due to the combined action of precocene II and precocene I. In view of the above, we would be tempted to associate this sensitivity of the larvae with the presence of these molecules in our sample of *A. conyzoides* oil. Moreover, *Chromolaena odorata* fresh leaves essential oil showed a low larvicidal activity towards the mature larvae of *Ae. albopictus* with an LC_{50} between 560 and 710 ppm. This low activity could be due to the extraction process of the active principles from *C. odorata* fresh leaves. Indeed, the extraction technique and solvent influence the chemical composition of the plant as well as its degree of toxicity [70, 71]. Previous studies on *An. gambiae* and *Ae. vittatus* larvae [71, 72] have shown that *C. odorata* extracts are likely to induce 100% larval mortality after 24 hours of exposure from 100 ppm (n-hexane extract), 160 ppm (methanol extract), and up to 600 ppm (ethyl acetate extract). These observations are close to the results obtained in Kribi where 100% larval mortality was observed after 24 hours of exposure at 800 ppm.

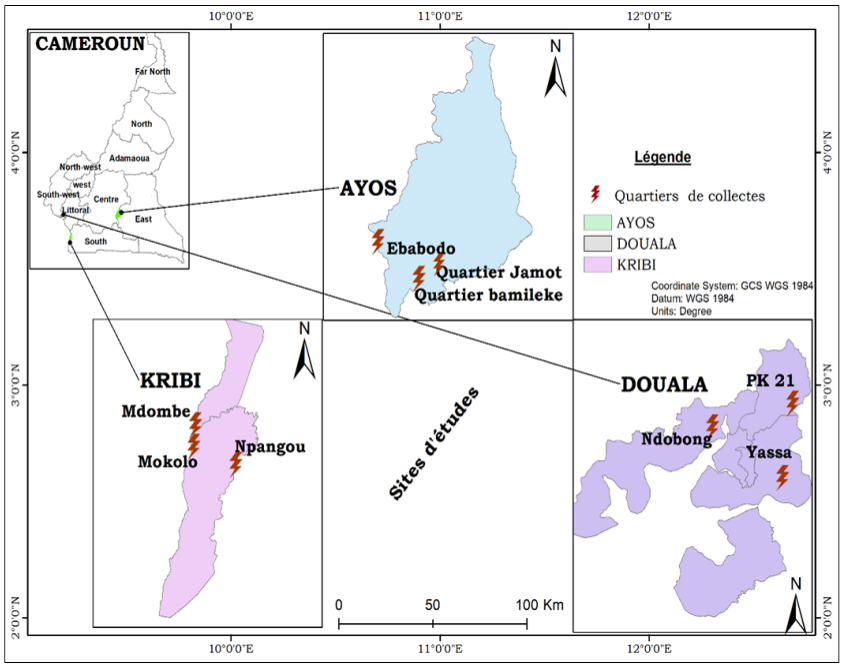

Figure 1. Study sites

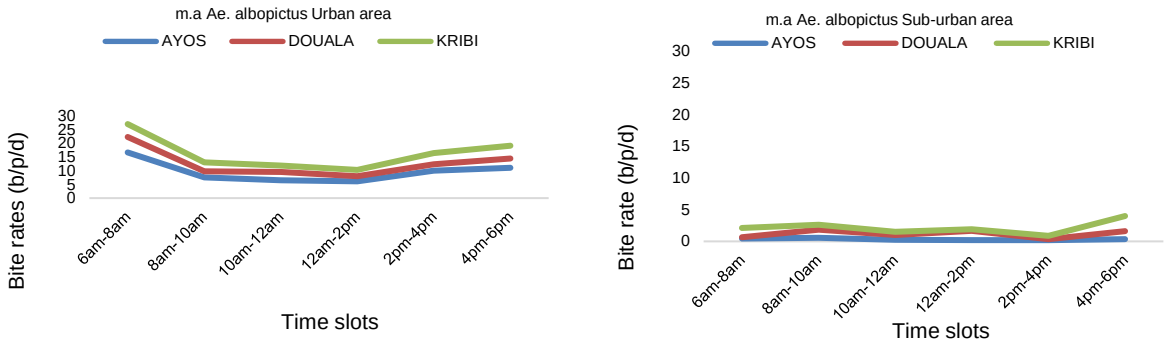

Figure 2. Biting cycles of *Aedes albopictus* in urban and sub-urban areas

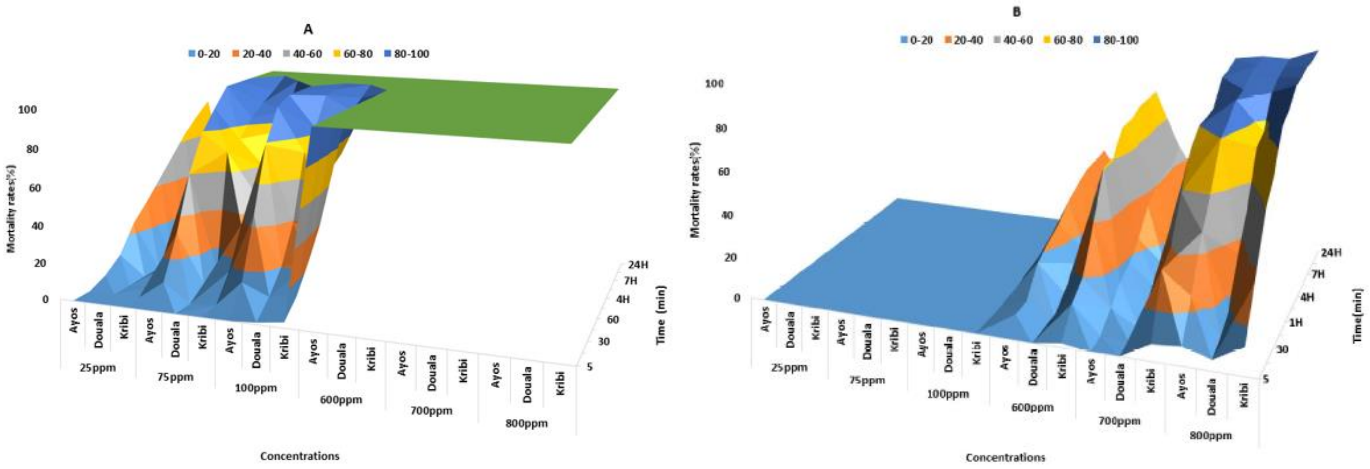

Figure 3. Variations in mortality rates (%) of *Aedes albopictus* larvae exposed to the essential oil of fresh leaves of *Ageratum conyzoides* (A) and *Chromolaena odorata* (B) in the study sites

Table 1. Diversity of aggressive Culicidae fauna in the study sites

Species	Urban area			Sub-urban area				
	Ayos	Douala	Kribi	Ayos	Douala	Kribi	P	Total
An. ziemani	3 (0. 14%)	0	0	9 (3. 96%)	0	0	0.5504	12 (0. 28%)
An. coustani	2 (0. 09%)	0	0	0	0	0	0.3559	2 (0. 04%)
An. gambiae s.l.	1 (0. 05%)	1 (0. 14%)	0	0	0	5 (1. 88%)	0.5976	7 (0. 16%)
An. paludis	6 (0. 27%)	0	0	11 (4. 84%)	0	0	0.4575	17 (0. 39%)
Ae. albopictus	1803 (83. 9%)	593 (80. 35%)	685 (90. 37%)	64 (28. 19%)	164 (79. 2%)	190 (71. 7%)	0.0167	3499 (80. 5%)
Ae. aegypti	126 (5. 86%)	19 (2. 57%)	34 (4. 48%)	4 (1. 76%)	0	27 (10. 19%)	0.0100	210 (43. 83%)
Ae. unilineatus	149 (6. 93%)	107 (14.5%)	37 (4. 88%)	27 (11. 89%)	34 (16. 42%)	38 (14. 34%)	0.2003	392 (9. 02%)
Ae. neomelaniconium	2 (0. 09%)	0	0	16 (7. 04%)	0	0	0.2163	18 (0. 41%)
Ae. simpsoni	4 (0. 18%)	0	2 (0. 26%)	3 (1. 32%)	3 (1. 45%)	1 (0. 4%)	0.9081	13 (0. 3%)
Cx. quinquefaciatus	2 (0. 09%)	11 (1. 49%)	0	14 (6. 16%)	0	4 (1. 5%)	0.7602	31 (0. 71%)
Ma. africana	23 (1. 07%)	0	0	44 (19. 38%)	0	0	0.3546	67 (1. 54%)
Er. chrysogaster	29 (1. 34%)	7 (0. 95%)	0	35 (15. 41%)	6 (2. 9%)	0	0.4927	77 (1. 77%)
Total	2150 (100%)	738 (100%)	758 (100%)	227 (100%)	207 (100%)	265 (100%)	0.0272	4345 (100%)

Table 2. Bite rates (b/p/d) of *Aedes* species caught in the study sites

Species	Urban area			Sub-urban area				
	Ayos	**Douala**	**Kribi**	**Ayos**	**Douala**	**Kribi**	**P**	**Total**
Ae. albopictus	56.34	18.53	21.41	2.00	5.13	5.94	0.0213	18.22
Ae. aegypti	3.94	0.59	1.06	0.13	0.00	0.84	0.0100	1.09
Ae. unilineatus	4.66	0.31	1.16	0.84	1.06	1.19	0.2003	2.04
Ae. neomelaniconium	0.06	0.00	0.00	0.50	0.00	0.00	0.2163	0.09
Ae. simpsoni	0.13	0.00	0.06	0.09	0.09	0.03	0.9081	0.07
Total	65.13	22.46	23.69	3.56	6.28	8	1.356	21.52

Table 3. Chemical composition of the essential oil of fresh leaves of *Ageratum conyzoides*

Identified compounds	**Percentage (%)**
Monoterpenes	79.3
Hydrocarbon monoterpenes	54.61
alpha pinene	0.03
alpha phellandrene	0.05
Precocene I	54.4
Camphene	0.13
Oxygenated monoterpenes	24.69
Androencecalinol	24.69
Sesquiterpenes	20.7
Hydrocarbon sesquiterpenes	19.61
beta cubebene	0.57
(Z)-beta-Fanesene	0.13
trans-α-bergamotene	15.5
trans-Muurola-4(15).5-diene	1.49
beta Bisabolene	0.89
trans-cadina-1.4-diene	1.03
Oxygenated sesquiterpenes	1.09
(E)-Nerolidol	0.17
Eudesma-4(15).7-diene-1-beta-ol	0.39
6- acethyl-2.2-dimethyl chroman	0.22
Caryophyllene oxide	0.21
Phytone	0.04
6.10.14-Trimethylpentadecane-2-one	0.06

Table 4. Chemical composition of the essential oil of fresh leaves of *Chromolaena odorata*

Identified compounds	**Percentage (%)**
Monoterpenes	55.42
Hydrocarbon monoterpenes	54.65
alpha pinene	10.05
Sabinene	0.85
β-pinene	4.35
Myrcene	1.16
p-cymene	0.19
Limonene	0.49
(E)-β-ocimene	2.98
γ-terpinene	0.14
Geijerene	20.02
Pregeijerene	13.83
n-Tridecane	0.59
Oxygenated monoterpenes	0.77
1.8-cineole	0.51
Linalool	0.1
Terpinene-4-ol	0.09
alpha Terpineneol	0.07
Sesquiterpenes	44.57

Hydrocarbon sesquiterpenes	30.29
clavukerine A	0.13
isogeijerene C	0.1
δ-elemene	0.24
β-elemene	0.86
β-copaene	0.3
alpha Huelene	2.09
trans-Muurola-4(14).5-diene	19.15
germacrene-A	2.56
Zonarene	3.04
α-cadinene	1.82
Oxygenated sesquiterpenes	14.28
8-epi-dictamnol	1.53
Dictamnol	10.46
caryophyllene oxide	0.13
Viridiflorol	0.18
humulene-1.2-epoxyde	0.13
epiα-cadinol	0.32
α-cadinol	1.18
Phytol	0.35
TOTAL	99.99

Table 5. Mean mortality of mature *Aedes albopictus* larvae after 10 h exposure to different concentrations of essential oils of the studied plant species in the study sites

study sites		**Concentrations (ppm)**								
	Essential oils	**25**	**75**	**100**	**600**	**700**	**800**	**Controle**	**H**	**P**
Ayos	*Ageratum*	16 ±	20 ± 1	20 ± 0	20 ± 0	20 ± 0	20 ± 0	0 ± 0	26.89	0.0002
	Chromolaena	0 ± 0	0 ± 0	0 ± 0	10 ± 3	10 ± 2	19 ± 1	0 ± 0	26.30	0.0002
Douala	*Ageratum*	1 ± 2	20 ± 1	20 ± 0	20 ± 0	20 ± 0	20 ± 0	0 ± 0	26.61	0.0002
	Chromolaena	0 ± 0	0 ± 0	0 ± 0	4 ± 1	6 ± 1	18 ± 2	0 ± 0	26.55	0.0002
Kribi	*Ageratum*	7 ± 1	20 ± 0	20 ± 0	20 ± 0	20 ± 0	20 ± 0	0 ± 0	26.93	0.0001
	Chromolaena	0 ± 0	0 ± 0	0 ± 0	16 ± 14	18 ± 1	20 ± 1	0 ± 0	26.75	0.0002

Conclusion

This survey shows a high diversity of Aedes in Ayos, Douala, and Kribi because of a variety of breeding sites for this genus. Aedes species bite mainly between sunrise and sunset. *Ae. Albopictus* was the most aggressive species in urban and suburban areas. Biting rates were higher in urban areas compared to suburban areas. *Ageratum conyzoides* fresh leaves essential oil may be one of the substances capable of effectively controlling these arboviruses vectors.

Abbreviations

GC-MS: Gas Chromatography-Mass Spectrometry
An.: *Anopheles*
Ae.: *Aedes*
LC_{50}: Lethal Concentration 50
LC_{95}: Lethal Concentration 95
ppm: parts per million

Authors' Contribution

OENH, PAN, and HPAA designed the study. OENH, RN, LOE, WST, WEE, FNNT, EK, AAN, ATN, AT and RSM carried out the field activities. OENH, PAN, and FMT drafted the manuscript and analyzed the data. VDM, HGT and HPAA critically revised the manuscript. OENH, PAN, and HPAA conceived and designed the study. LGL, HPAA and PAN revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Acknowledgments

The authors of this manuscript thank the local administration and populations of the different study sites. We are grateful for their contribution to the realization of this work.

Conflict of interest

The authors declare no conflict of interest

Article history:

Received: 05 August 2022
Received in revised form: 24 August 2022
Accepted: 25 August 2022
Available online: 25 August 2022

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