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Influence of urbanization on the sensitivity of female adults of the *Anopheles gambiae* complex to essential oils of some plants from the Littoral region of Cameroon

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Abstract
This study determines the influence of urbanization on vector susceptibility to essential oils of some plants from Cameroon. Essential oils were produced from pericarp of ripe fruits *Citrus* and fresh leaves of *Cymbopogon citratus*, using a Clevenger-type apparatus. Analysis was made by gas chromatography or gas chromatography coupled with mass spectrometry. Sensitivity tests based on the WHO standard protocol were carried out on Ndogbong (urban area) strains of *An. coluzzii* and Logbessou (poorly urbanized area) strains of *An. gambiae*. Essential oils from *C. grandis*, *C. limon* and *C. sinensis* were rich in hydrocarbon monoterpenes (91.2%, 91.43% and 94.92% respectively) with limonene as major compound. The oil from *C. citratus* was rich in oxygenated monoterpenes (75.8%), mostly geranial and neral. Sensitivity tests showed that these essential oils have remarkable adulticide properties vis-à-vis the Logbessou strain of *An. gambiae*. They induced 100% mortality at 50 ppm (*C. citratus*) and 100 ppm (*C. sinensis* and *C. grandis*). However for equal doses, the Ndogbong strain of *An. coluzzii* proved less sensitive; only essential oil from *Cymbopogon citratus* resulted in total mortality of this mosquito strain. Urban environment probably reduces the sensitivity of Anopheles mosquitoes to essential oils with low toxicity. However, the essential oil from *Cymbopogon citratus* kept its toxicity vis-à-vis mosquito strains of both areas (50ppm) and should thus be considered in the design of national control programs against vectors.

Keywords: *An. gambiae*, *An. coluzzi*, *C. grandis*, *C. limon*, *C. sinensis*, *C. citratus*, essential oils, urbanization sensitivity

1. Introduction

Urbanization is a major process with large-scale development project that results in environmental changes. In Africa, urbanization process is recent as well as brutal and uncontrolled. Cameroon is not spared by this process of urbanization [1]. However, population growth, poor sanitation added to poverty in cities, are causing a rapid degradation of urban environment with subsequent proliferation of vectors of diseases such as dengue, yellow fever, filariasis and malaria [2]. Malaria is the first killing endemic disease with over a million of victims per year worldwide [3]. The World Health Organization (WHO) estimated at 198 millions the number of malaria cases and 584,000 deaths in 2013 [4]. In Cameroon, this parasitic disease remains a major public health concern; it is the leading cause of morbidity and mortality in children, infants and pregnant women [5]. Malaria is transmitted through bites of infected female mosquitoes of the genus Anopheles [6]. For years, the international community through the WHO, has been investing much resources for the control of mosquito borne diseases. Notwithstanding the encouraging results achieved in recent decades, the situation remains preoccupying. This may owe to approximate implementation of recommended preventive and curative methods, as well as the up rise of resistant strains of the malaria parasite and vector to drugs and synthetic insecticides respectively. Several studies on vector susceptibility to synthetic insecticides have been conducted in Cameroon. Most vectors showed resistance to dieldrin, DDT, permethrin, deltamethrin and lambda-cyhalothrin [7-11]. Faced with this resistance phenomenon that represents a major obstacle to the prevention and treatment of diseases, the use of plant extracts with proven insecticidal effect is highly recommended. The use of plants for their insecticidal properties is long known in the African tradition [12]. These plants are thus a potential source of new insecticides which is important to further explore. However, variations in the sensitivity of vectors to essential oils based on the eco-climatic facies have been reported in Cameroon [13]. This might result from the selection pressure exerted by environmental factors on mosquitoes, which lead to the selection of genes allowing for better adaptation to the constraints of their living environment. To date, many studies aimed at assessing the impact of environmental factors on the susceptibility of mosquitoes to insecticides have been conducted [14-16]. In current scientific literature however, few studies based on biological insecticides are available. We can mention among other, the works of Akono and his collaborators in Cameroon [13]. Yet such work is needed in order to develop effective vector control strategies including the use of biocides that cause less damage to the environment. This study aims at evaluating the influence of urbanization on the sensitivity of *Anopheles gambiae* and *Anopheles coluzzii* to essential oils of some plants from Cameroon.

2. Materials and Methods

2.1 Collection, identification and breeding of Anopheles larvae

The larvae of *An. gambiae* s.l. were collected in Logbessou and Ndogbong, two neighborhoods of the city of Douala, presenting ecological differences.

Logbessou (4° 05'N and 09° 46'E) is a poorly anthropized suburban area. Apart from some villas under construction, the area is mostly dominated by gallery forests and many cultivated lands. The environment is less polluted and promotes the proliferation of Anopheles larvae which mainly develop intire prints of sand trucks supply current construction sites.

Ndogbong (04° 03'N and 09° 44'E) is a urban and highly anthropized area including among other public universities and numerous industrial companies whose toxic emissions significantly contribute to pollute the environment and may promote the resistance of mosquito populations to insecticides recommended by WHO. Furthermore, the absence of sewage system and the poor household waste collection system promote the proliferation of *Culex pipiens* and *Anopheles coluzzii*, found as species that best adapt in poorly urbanized cities of Africa [17].

From November 2014 to August 2015, the larvae were collected from temporary sunny breeding sites of small size (5 to 20 cm in diameter) then reared at the insectarium. The adults obtained were morphologically identified to species [18-22].

2.2. Essential oils and sensitivity tests

2.2.1 Essential oils

Citrus grandis Osbeck, *Citrus limon* L, *Citrus sinensis* L and *Cymbopogon citratus* Stapf were chosen because of their traditional use as insect repellent in villages of the degraded forest of south Cameroon, presenting with high malaria endemicity. Plant material was harvested in February and August 2014 in an orchard of the Littoral region of Cameroon. They had not received any treatment with chemical insecticides at the time of harvest.

Extraction of essential oils was obtained by hydro distillation of plant material using a Clevenger-type apparatus for 3 to 4 hours. Residual traces of water were then removed by filtration through anhydrous sodium sulfate. The essence obtained was weighed, then put in dark glass bottles to be stored in the refrigerator at a temperature of about 4 °C. Analysis of the chemical composition was made by Gas Chromatography (GC) and Gas-Phase chromatography coupled with Mass Spectrometry (GC / MS) [13].

2.2.2 Sensitivity tests

The methodology was inspired from the WHO protocol [4]. Preliminary experiments were carried out to select the concentrations to be used. These concentrations were obtained by diluting the stock solution (half diluted) indifferent quantities of acetone solution. Using a mouth aspirator, 20 to 25 previously identified female mosquitoes were introduced into an observation tube lined with un-impregnated paper. After one hour of observation, mosquitoes were transferred to an observation tube lined with Whatman n°1 paper (12x15 cm^2), impregnated with diluted essential oil. During the observation, knocked mosquitoes were counted at regular time intervals of 5 minutes. At the end of the exposure period, the mosquitoes were transferred back to the observation tube. A swab with 10% sugar solution was placed on top the observation tube and mortality was determined after 24 hours of observation. For each concentration of essential oil, 4 lots of 20 to 25 mosquitoes were prepared. A control batch of 20 to 25 mosquitoes was exposed to acetone only. For species showing resistance to essential oils, a batch was pre-exposed to Piperonyl butoxide (PBO) then, tested as described above, in order to determine the nature of the resistance. The test was considered valid for a mortality rate less than 5% in the control group; if the mortality rate exceeds 20%, the test was not considered valid and was redone. On the reverse, if comprised between 5% and 20%, the overall mortality of mosquitoes exposed to the essential oil was corrected according to Abbott's formula [23] defined as follows:

$$\text{Corrected } \%M = \frac{\%\text{ test mortality} - \%\text{ control mortality} \times 100}{100 - \%\text{ control mortality}}$$

2.3 Molecular identification of members of the Gambiae complex

DNAs were extracted from the legs and wings of specimens of *An. gambiae* s.l. that died after exposure to essential oils. The molecular identification of the members of this complex was made using the technique described by Fanello [24].

2.4 Statistical Analyses

Statview version 5.0 (SAS Institute, Inc, USA) and XLSTAT version 2003 packages were used for statistical analysis. Kruskal Wallis H test was used to compare mean number of knocked down mosquitoes. The simplified Henry table that transforms percentages of knocked down mosquito into probit enabled the determination of the necessary time for obtaining 50% (tkd_{50}) and 95% (tkd_{95}) adult female mosquitoes 'knock down'. Significance level was set at p-value <0.05.

3. Results

3.1 Essential oils

The essential oils extracted from the pericarp of fruits of Citrus species were light yellow, with the extraction yields ranging from 0.18% (w/w) for *C. grandis* to 0.8% (w/w) for *C. sinensis.* The extraction yield of *C. citratus* leaves was 0.3% (w/w) (table 1).

Table 1: Data on essential oils extraction from studied plants.

Plant				Harvest		Essential oil		Yield (%)
Family	Species	Organ	Mass (g)	Location	Date	Colour	Mass (g)	
Rutaceae	*Citrus grandis*	Pericarps	4800	Mbanga	12/08/2014	Light yellow	8.41	0.18
Rutaceae	*Citrus limon*	Pericarps	3300	Mbanga	14/08/2014	Light yellow	6.55	0.20
Rutaceae	*Citrus sinensis*	Pericarps	1700	Mbanga	27/02/2014	Light yellow	12.77	0.80
Poaceae	*Cymbopogon citratus*	Leaves	1650	Ndogpassi	15/08/2014	Light yellow	4.84	0.30

The chemical composition of oils varied from one plant to other. It was dominated by limonene in oils of *C. sinensis* (92.87%), *C. grandis* (90.7%) and *C. limon* (84.71%) and by geranial (31.17%), neral (32.78%) and myrcene (19.23%) in *C. citratus* oil (Table 2).

Table 2. Chemical composition of essential oils from pericarps of *Citrus limon, Citrus grandis, Citrus sinensis* and leaves of *Cymbopogon citratus*

Compound	Mass percentages (%)			
	C. limon	***C. grandis***	***C. sinensis***	***C. citratus***
Aromatic compound	0.07	0.83	0.22	0.16
Methylnaphthalene I Eugenol	0.07	0.83	0.22	- 0.16
Monoterpenes	92.67	97.51	95.65	96.47
Hydrocarbon monoterpenes	90.93	92.73	93.84	19.79
α-thuyene	0.12	-	-	-
α-Pinene	0.30	0.26	0.34	-
Camphene	-	-	-	-
Δ³Carene	0.17	-	0.15	-
Sabinene	4.23	-	-	-
β-Pinene	0.03	1.02	0.24	-
Myrcene	-	0.52	0.15	19.23
β-Phéllandrene	0.08	-	0.03	-
Limonene	84.71	90.7	92.87	0.02
(E)-β-Ocimene	1.06	-	-	-
(Z)-β-Ocimene	-	-	-	0.19
α-terpinene	0.23	-	0.04	-
p-Cymene	-	-	-	-
γ- Terpinene	-	0.23	0.02	0.35
Oxygenated monoterpenes	1.74	4.78	1.81	76.68
1.8 Cineole	-	-	-	0.45
E-Sabinene hydrate	0.12	0.15	0.01	-
Linalool I oxyde	0.03	0.53	0.12	-
Linalool II oxyde	-	1.10	-	-
Linalool	0.02	0.51	0.04	1.02
Isocitral	-	-	-	0.27
(E)-Chrysanthenal	-	-	0.2	-
Citronellal	-	-	-	0.23
(Z)-Chrysanthenol	-	-	-	1.02
(E)-Chrysanthenol	-	-	1.05	1.45
Limonene I oxyde	0.06	0.17	-	-
Terpinen-4-ol	-	-	-	-
α-Terpineol	-	0.18	-	-
Myrtènal	0.34	-	-	-
α-Pinene oxyde	0.07	1.08	-	-
γ-Terpineol	0.37	0.42	-	-
Carvone	0.27	-	-	-
Carveol	0.11	0.31	0.07	-
Neral	0.08	-	-	32.78
Nerol	0.12	0.02	-	-
Lynalyleacetate	0.06	0.04	-	-
Geraniol	0.04	0.27	0.25	7.23
Geranial	-	-	-	31.17
Neryl acetate	-	-	-	1.06
Bornyl acetate	0.05	-	0.07	-
Sesquiterpenes	0.79	0.66	0.05	0.24
Hydrocarbon sesquiterpenes	0.73	0.66	0.05	0.15
Bicycloelemene	-	-	-	-

β-Elemene	0.23	0.02	-	-
Santalene	-	-	-	-
β-Caryophyllene	0.14	0.32	-	0.12
(E-E)α-Bergamotene	0.08	-	-	0.03
Bicyclogermacrene	0.05	-	-	-
Germacreme D	-	0.15	-	-
β-Bisabolene	-	-	0.05	-
γ-cadinene	0.23	0.17	-	
Oxygenated sesquiterpenes	0.06	0.00	0.00	0.09
Globulol	0.06	-	-	-
Eugenol	-	-	-	0.09
Linear compound	2.07	0.35	1.26	1.41
Octanol	-	-	-	-
Octanal	2.07	-	1.07	-
Decenol	-	0.03	0.12	-
Decanal	-	-	0.07	-
Nonanal	-	0.32	-	--
Undecan-2-one	-	-	-	0.21
Tridecan-2-one	-	-	-	0.15
6-Methyl-hept-5-en-2-one	-	-	--	1.05

3.2 Molecular identification of mosquitoes

Molecular identification of mosquitoes that died under the influence of essential oils showed that the species captured in Logbessou was *An. gambiae* while that captured in Ndogbong was *An. coluzzii*.

3.3 Susceptibility testing

3.3.1 Knockdown time

Values of tkd_{50} and tkd_{95} are summarized in tables 3 and 4. For *An. gambiae* (Logbessou strain), the lowest tkd_{50} and tkd_{95} were obtained with essential oil of *C. grandis* and are respectively 0.01 and 0.42 minutes at 200 ppm. The highest tkd_{50} and tkd_{95} were obtained with essential oil of *C. limon* and are respectively of 14.14 and 338.43 minutes at 50 ppm. For the species *An. coluzzii* (Ndogbong strain), the lowest tkd_{50} and tkd_{95} were obtained with essential oil of *C. grandis*, 0.14 min and 1.49 min respectively at 200 ppm. The highest tkd_{50} and tkd_{95} were obtained with the essential oil of *C. limon*, 151.46 and 3961.79 minutes respectively, at 50 ppm.

Table 3: Knock-down time of adult female *Anopheles gambiae* exposed to various doses of essential oils for 60 minutes

				Knock-down time (min)	
Mosquito	**Essential oils**	**Concentrations (ppm)**	**N**	**Tkd_{50}**	**Tkd_{95}**
An. gambiae (Logbessou)	*Citrus grandis*	200	80	0.01	0.42
		100	80	0.97	4.89
		50	80	3.64	10.54
	Citrus limon	200	80	1.99	7.36
		100	80	6.48	15.88
		50	80	14.14	338.43
	Citrus sinensis	200	80	0.03	0.67
		100	80	0.92	4.84
		50	80	1.86	7.35
	Cymbopogon citratus	200	80	0.043	0.84
		100	80	0.82	4.49
		50	80	1.48	6.41

Table 4: Knock-down time (Tkd) of adult female *An. coluzzii* exposed to various doses of essential oils for 60 minutes

		Concentrations		Knock-down time (min)	
Mosquito	**Essential oils**	**(ppm)**	**N**	**Tkd_{50}**	**Tkd_{95}**
An. coluzzii (Ndogbong)	*Citrus grandis*	200	80	0.14	1.49
		100	80	0.98	4.93
		50	80	6.32	12.88
	Citrus limon	200	80	0.79	4.36
		100	80	16.44	158.45
		50	80	151.46	3961.79
	Citrus sinensis	200	80	0.57	3.65

		100	80	0.90	4.70
		50	80	50.35	212.30
		200	80	0.14	1.59
	Cymbopogon citratus	100	80	1.55	6.25
		50	80	6.94	15.97

N= Group size; ppm =part per million

3.3.2 Mortality rate

Table 5: Mortality rate of female *An. gambiae* (Logbessou) after 24h of observation

	Concentration		**Mortality rate after 24h of observation**		
Essential Oils	**(ppm)**	**N**	**Test group (%)**	**Control group (%)**	**Status**
	200	80	100	0	Sensitive
Citrus grandis	100	80	100	0	Sensitive
	50	80	93	0	Probable resistance
	200	80	90	0	Resistant
Citrus limon	100	80	83	0	Resistant
	50	80	15	0	Resistant
	200	80	100	0	Sensitive
Citrus sinensis	100	80	100	0	Sensitive
	50	80	98	0	Sensitive
	200	80	100	0	Sensitive
Cymbopogon citratus	100	80	100	0	Sensitive
	50	80	100	0	Sensitive

N= Group size

Table 6: Mortality rate of female *Anopheles coluzzii* (Ndogbong) after 24h of observation

	Concentration		**Mortality rate after 24h of observation**		
Essential Oils	**(ppm)**	**N**	**Test group (%)**	**Control group (%)**	**Status**
Citrus grandis	200	80	98	0	Sensitive
	100	80	96	0	Probable resistance
	50	80	23	0	Resistant
Citrus limon	200	80	90	0	Resistant
	100	80	29	0	Resistant
	50	80	1	0	Resistant
Citrus sinensis	200	80	100	0	Sensitive
	100	80	95	0	Probable resistance
	50	80	65	0	Resistant
	200	80	100	0	Sensitive
Cymbopogon citratus	100	80	100	0	Sensitive
	50	80	100	0	Sensitive

N= Group size

Table 7: Mortality rate of female *Anopheles coluzzii* (Ndogbong) pre-exposed to piperonyl butoxide (PBO), after 24h of observation

	Concentration		**Mortality rate after 24h of observation**		
Essential oil	**(ppm)**	**N**	**Test group (%)**	**Control group (%)**	**Status**
	200	80	100	0	Sensitive
Citrus grandis+ 4% PBO	100	80	100	0	Sensitive
	50	80	100	0	Sensitive
	200	80	100	0	Sensitive
Citrus limon+ 4% PBO	100	80	98	0	Sensitive
	50	80	45	0	Resistant
	200	80	100	0	Sensitive
Citrus sinensis+ 4% PBO	100	80	100	0	Sensitive
	50	80	100	0	Sensitive
	200	80	100	0	Sensitive
Cymbopogon citratus+ 4% PBO	100	80	100	0	Sensitive
	50	80	100	0	Sensitive

N=Group size ppm =part per million

Table 8 Comparison of mortality rates of *Anopheles gambiae* and *Anopheles coluzzii* exposed to the *Citrus grandis* and Citrus *sinensis* essential oils at 50 ppm. *Kruskal Wallis test H (p<0.05).*

	Source of the essential oil	
Mosquito species	*Citrus grandis*	*Citrus sinensis*
Anopheles gambiae	23.25 ± 6.3	24.5 ± 5.1
Anopheles coluzzii	5.75 ± 2.9	16.25 ± 6.5
P-value	0.0004	0.02

Mortality rates of *An. gambiae* and *An. coluzzii* female adults exposed to essential oils are summarized in tables 5 and 6 respectively. Apart from the essential oil of *C. grandis* for which we suspected resistant at 50 ppm, females *An. gambiae* proved sensitive to all concentrations of *C. citratus*, *C. sinensis* and *C. grandis*. They also proved totally resistant to all concentrations of *C. limon*. On the other hand, females *An. coluzzii* proved resistant to all concentrations of *C. limon* and totally susceptible to *C. citratus* oil. The Kruskal Wallis H test showed that at 50 ppm concentration, *An. gambiae* (Logbessou strain) is significantly more sensitive to essential oils of *C. sinensis* and *C. grandis* than *An. coluzzii* (Ndogbong strain) (table 7). However, pre-exposure of *An. coluzzii* to a synergist, the Piperonyl butoxide (PBO) increased the sensitivity of this mosquito species to essential oils (table 8).

4. Discussion

The extraction yield of the leaves of *C. citratus* is 0.3% (w/w). This value is lower than that recorded with samples collected in Brazil (1.3%) and Cameroon (0.6%) [25, 26]. The essential oil obtained in the present study was characterized by a high content of myrcene (19.23%), neral (32.78%) and geranial (31.17%). These results are in accordance with that recorded in the previous work from Benin, Burkina Faso, Brazil and Portugal, showing that the chemical composition of this oil is homogeneous, myrcene, neral and geranial being the main compounds regardless of the geographic origin of the plant [26-28].

The extraction yields of essential oils from pericarp of ripe fruit *C. grandis*, *C. sinensis* and *C. limon* were 0.18%; 0.2%; 0.8% (w/w) respectively. A slight difference in yields was noted with respect to that recorded in previous work [29]; however, the pericarp of *C. sinensis* has always shown a higher content of essential oil than pericarps of other *Citrus* species. Furthermore, the present work shows that these oils are all characterized by a high content of hydrocarbon monoterpenes, mainly limonene. These results corroborate that found in Cameroon Vietnam and Tunisia respectively [30-32]. Limonene therefore appears as the characteristic compound of pericarp of all *Citrus* species.

Molecular identification of mosquitoes that died under the influence of essential oils showed that the species captured in Logbessou was *An. gambiae* while that captured in Ndogbong was *An. coluzzii*. These results show that *An. coluzzii* fits better in polluted areas of the city of Douala compared with *An. gambiae* that prefers least polluted suburbs [13, 17].

It appears from values of tkd_{50} and tkd_{95} that, for each concentration of essential oils of *C. grandis* and *Cymbopogon citratus*, the tkd_{50} and tkd_{95} proved higher for *An. coluzzii* than *An. gambiae*. The same observation was made for all the essential oils at 50 ppm, suggesting a higher sensitivity of *An. gambiae* female adults to essential oils, compared with *An. coluzzii* (figure 1).

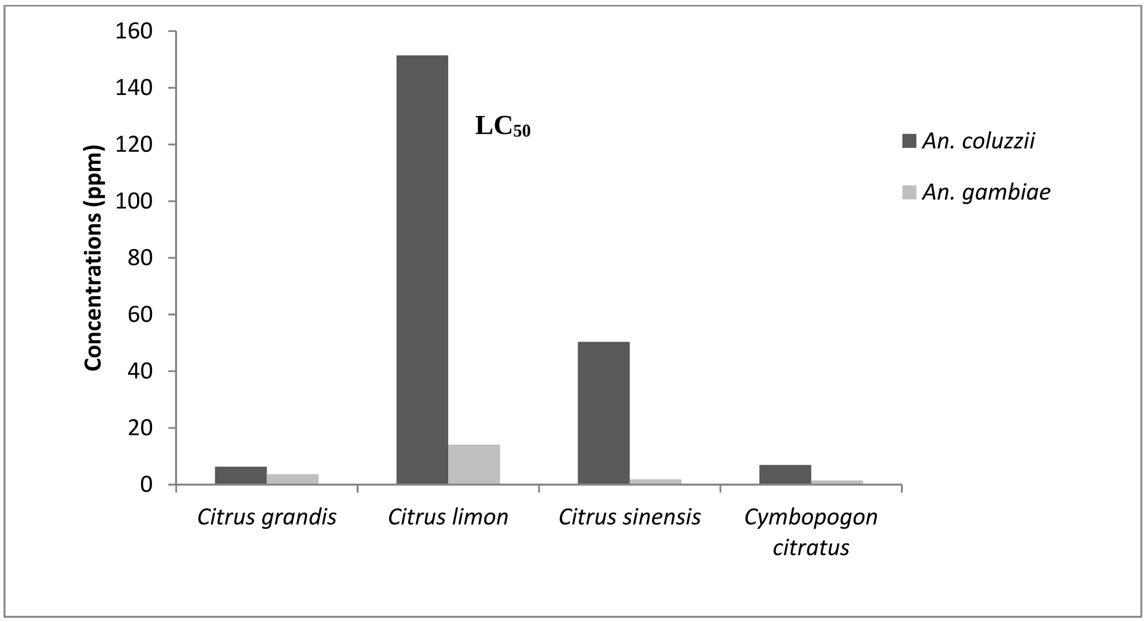
Mortality rates of *An. gambiae* and *An. coluzzii* showed that the toxicity of a volatile oil depends on the concentrations, but more on its chemical composition [33].

The essential biological properties of the oil of *C. citratus* have been described by many authors. These authors showed that this volatile oil has excellent antiplasmodial and larvicidal properties respectively against *Plasmodium falciparum* in vitro and *An. funestus* [26]. Long before, others had related the larvicidal activity of this essential oil vis-à-vis *Aedes aegypti* to its major constituents (neral and geranial) [34]. Similar studies to evaluate the toxicity of the oil of *C. citratus* on *Sitophilus oryzae*, a beetle pest of stored foodstuffs related its toxicity to citral [35]. Moreover, Machado and his collaborators have shown that citral is an anti-leishmaniasis agent [36]. Given the above, it would be reasonable to think that the sensitivity of the female *An. gambiae* and *An. coluzzii* to the oil of *C. citratus* is due to its high content in neral and geranial.

The insecticidal and insect repellent activities of the essential oils of pericarp of fruits of various *Citrus* species have been described. However, it remains difficult to say with accuracy which is the most toxic species. Scientific literature shows that when three samples are tested simultaneously, the oil of *C. grandis* pericarp proves to be the most toxic in some areas and the oil of *C. limon* in other areas [13, 32]. The main point on which the literature agrees is the origin of this toxicity. In fact, the chemical composition of oils from pericarp of these *Citrus* species is dominated by limonene. This compound is considered by many authors as the characteristic compound of all Citrus [13, 30, 31, 32, 37]. Pure limonene has shown excellent insecticidal activity on developmental stages of some mosquito species [38-41]. Toxicity differences observed with samples of different *Citrus* species in our work may be due to minority compounds with potential insecticidal property. These minor compounds would act in synergy with limonene to boost the toxic effect of the essential oil. Some studies have shown the toxic activity performed by these minor compounds vis-à-vis certain pathogens. For instance, Ojimelukwe and Alder [42] and Lucia [43] have assessed the insecticidal activity of α-pinene vis-à-vis *Tribolium confusum* and β-pinene on *Aedes aegypti* respectively. Furthermore, Simeon Buochberg [44] has observed that the toxic potential of phenolic compounds was increased when associated with terpinene. The absence of these minor compounds insecticide potential in some of our oils is the cause of the differences in toxicity of citrus oils vis-à-vis the anopheles.

The Kruskal Wallis H test showed that at 50 ppm concentration, *An. gambiae* (Logbessou strain) is significantly more sensitive to essential oils of *C. sinensis* and *C. grandis* than *An. coluzzii* (Ndogbong strain). The resistance of *An. coluzzii* captured in Ndogbong could be explained by the important pollution of this area. In fact, the Ndogbong neighborhood hosts several industrial companies whose toxic wastes are not always properly managed. Moreover, household waste collection and treatment system is poor. In some places, gutters are clogged preventing smooth flow of water during the rainy season and promoting the establishment of stinking water. Recent studies have shown a strong link between the toxic waste from industrial companies whose chemical composition is close to pyrethroids and resistance of malaria vectors to conventional insecticides. Antonio [45] who worked in Ndogpassi, a poorly urbanized and polluted area of the city of Douala, found that vectors that had previously been exposed to these toxic emissions developed resistance to pyrethroid insecticides. The authors attributed this resistance to the West African kdr allele (L1014F) and the East African kdr allele (L1014S). Mosquitoes presenting both types of mutations have previously been found in Cameroon and Gabon [8, 46]. Given the frequency of occurrence of these resistance alleles in some malaria endemic areas, they might be involved in the *An. coluzzii* (Ndogbong strain) resistance to essential oils. This study shows that the resistance of *An. coluzzii* to essential oils can also be attributed to the action of detoxification enzymes. Pre-exposure of *An. coluzzii* to Piperonyl butoxide increased the sensitivity of this mosquito species to essential oils. This result proves that in addition to genetic resistance, there is a metabolic resistance through P450s oxidase in this strain of *An. coluzzii*. The resistance of *An. coluzzii* through detoxification enzymes has previously been reported in Cameroon [9]. Recent investigations have also reported metabolic type resistance in *An. coluzzii* in Youpwe, on the coastal area of Douala (Etang, Personal communication). From the above, it should be inferred that the resistance of *An. coluzzii* to essential oils may result either from kdr mutations, from the action of the P450s oxidase enzyme or from both actions (multidrug resistance) as showed by Nguessan [47].

The sensitivity of the Logbessou strain *An. gambiae* of *C. sinensis* and *C. grandis* to essential oils may be explained by the more natural environment of this neighborhood. Logbessou is a barely anthropized suburb in which some forest islets are still visible with some few residential houses.



LC_{50}= Lethal concentration 50

Fig 1: Comparison of sensitivities of *An. gambiae* and *An. coluzzii* to essential oils at 50ppm, based on their lethal concentrations (LC_{50})

4. Conclusion

This study shows that uncontrolled urbanization might reduce the sensitivity of Anopheles mosquitoes to essential oils with low toxicity such as that from *C. grandis*, *C. sinensis* and *C. limon*. However, the essential oil from *Cymbopogon citratus* kept its toxicity vis-à-vis mosquito strains of the study sites. Vector resistance to biocides should therefore be taken into account for the development of effective vectors control strategies in polluted areas and should thus be considered in the design of national control programs against vectors.

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