Larvicidal Activity of Two Cymbopogon Species Leaf Extracts and Essential Oils against *Anopheles gambiae* Gilles (Diptera: Culicidae)

Aurore Christelle Sengue Batti[1], Lame Younoussa[2*], Elias Nchiwan Nukenine[3], Mallam Kary Oumarou[3] and Abraham Fomena[1]

[1]*Department of Animal Biology and Physiology, Faculty of Science, P.O.Box 812, University of Yaounde 1, Cameroon.*
[2]*Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, P.O.Box 13033, Yaoundé, Cameroon.*
[3]*Department of Biological Sciences, Faculty of Science, University of Ngaoundere, P.O.Box 454, Ngaoundere, Cameroon.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors ENN, AF, ACSB and LY designed the study. Author LY performed the statistical analysis. Authors ACSB, MKO and YL wrote the protocol and author LY wrote the first draft of the manuscript. Authors ENN, AF and LY managed the analyses of the study. Authors ACSB, MKO and YL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present investigation aimed to assess the toxic effect of hexane, acetone and methanol extracts of *Cymbopogon citratus* and *Cymbopogon giganteus* against 3rd and 4th instar of *Anopheles gambiae* larvae under laboratory conditions.
Place and Duration of Study: Plant products were extracted from November 2017 to February 2018 in the Chemistry laboratory, while the larvicidal tests were conducted from April to June 2018

Corresponding author: E-mail: younoussalame@yahoo.com, lamey2004@gmail.com;

in the laboratory of Applied Zoology of the Faculty of Science, University of Ngaoundere, Cameroon.

Methodology: Twenty five (25) 3rd and 4th instars of *An. gambiae* were subjected to methanol, acetone and methanol plant extracts of the two plants at doses of 1000, 500, 250 and 125 mg/L each while their essential oils were tested at concentrations of 200, 100, 50 and 25 mg/L. Dichlovos tested at the recommended dose of 1000 mg/L was performed as positive control while 1mL of tween-80 in 99 mL of natural breeding site water was used as negative control. Larval mortality was recorded after 24 h post treatment for plant extracts and after 1, 6, 12, 18 and 24 h post-exposure for plant essential oils.

Results: At the highest concentration of 1000 mg/L, *C. citratus* extract caused 100% mortality of mosquito larvae while 84, 81 and 88% mortality of larvae were recorded with hexane, acetone and methanol leaf extract of *C. giganteus*, respectively. *C. citratus* (LC_{50}=58.32 mg/L) and *C. giganteus* (LC_{50}=372.36 mg/L) hexane extracts were more potent than acetone and methanol extracts of the plants. *C. citratus* extracts were the most effective against larvae compared to *C. giganteus* extracts. Essential oil of *C. citratus* (LC_{50}=27.98 mg/L after 1h) was also the most toxic on mosquito larvae compared to *C. giganteus* (LC_{50}=180.07 mg/L after 1h) essential oil.

Conclusion: *C. citratus* plant and specially its hexane extract and essential oil could be taken into consideration as a new source of botanical insecticide and may be used in the mosquito control programs for *An. gambiae* larvae elimination in their breeding sites.

1. INTRODUCTION

Mosquito-borne diseases constitute nowadays, major human health problems and are responsible of the significant morbidity, mortality and economic burden to population of tropical and subtropical countries [1]. In sub-Saharan African zones, malaria is the most important disease and still remains a major source of illness and death. It is caused by the *Plasmodium* parasites transmitted to the human being through *Anopheles* species mosquito bites [2]. An estimated 219 million cases and 435,000 related deaths from malaria were reported in 2017 by WHO [3]. In Cameroon, a mortality rate of 116/1,000 for malaria cases surpasses those of the African region (104/1,000) as well as neighboring countries such as the Central African Republic [4]. In health facilities of the country, 48% of total hospital admissions were due to suspicion of severe malaria, and 19% of nationwide deaths were assigned to that disease [5].

To control and eliminate malaria in sub-Saharan countries, different strategies developed against malaria parasite and mosquito vectors are facing problems, including a lack of sustainable and predictable funding, conflicts in malaria endemic zones, irregularity of climate conditions and mosquito resistance to insecticides commonly in uses [6]. Moreover, synthetic insecticides in uses for their larvicidal, adulticidal or repellent effects were revealed to be toxic for human and animal and possess deleterious impact on non-target insects [7]. Thus, the search for alternative, simple, effective and sustainable methods of mosquito control is promptly needed. Botanicals derived from plant products are relatively less toxic, environmentally safer, more biodegradable, and more target specific and may constitute alternatives to those hazardous synthetic residual insecticides for mosquito control [8]. Several plants from diverse families have been proven to have toxic properties against the immature stages of mosquitoes and the major phytochemical components responsible for the toxic effect on these insects include Saponins, flavonoids, tannins, alkaloids, glycosides, steroids and terpenoids [9]. However, miscellaneous research papers have reported the efficacy of plant derived products against *Anopheles gambiae* larvae. The most recent includes the leaves of *Hyptis suaveolens*, *Chenopodium ambrosoides*, and *Lippia adoensis* methanol extracts and their essential oils against larvae of *An. gambiae* [10]; a significant larvicidal efficacy of clove and cinnamon essential oils against laboratory *Anopheles gambiae* (*sensu stricto*) and wild *An. arabiensis* larvae [11]; and a potent larvicidal activity of dichloromethane *H. suaveolens* extract against 4th instar larvae of *An. gambiae* [12]. Vivekanandhan et al. [13] reported recently also, a significant toxicity of hexane, petroleum benzene, chloroform, ethylacetate, and acetone extracts of *Acanthospermum hispidum* against *An. stephensi* larvae. Essential oil of *E. globulus*

showed also a strong larvicidal activity against *An. stephensi* larvae [14,15].

Commonly known as lemon grass, *Cymbopogon citratus* (DC.) Stapf belonging to the family of Poaceae family is a perennial tropical grass plant with long and thin leaves largely widespread in tropical and subtropical regions of Asia, South America, and Africa [16]. The plant is mostly cultivated for their essential oil which possesses antibacterial, antifungal, analgesic, and mosquito repellent properties [17,18,19,20]. Previous studies reported interesting uses of *C. citratus* as a natural pesticide and insecticide. From Gabon, Obame-Engonga et al. [21] reported a toxic effect of *C. giganteus* essential oils against *An. gambiae* eggs and larvae. The essential oil of *C. citratus* showed a significant oviposition-deterrent and ovicidal activity towards three mosquito vectors, *Aedes aegypti, Anopheles dirus* and *Culex quinquefasciatus* [22]. The insecticidal activity of *C. citratus* was reported against the common housefly, *Musca domestica* [23], and exerted toxic effect against *Tribolium castaneum* [24].

The plant species *Cymbopogon giganteus* Chiov. (Poaceae) is a perennial grass largely widespread in African tropical and savannah regions. In folk medicine, the plant is used to treat skin disorders and malaria [25]. The plant also possesses anti-inflammatory, antimicrobial, anti-trypanosomal, cytotoxicity and anti-plasmodial properties [26,27,28]. The essential oil of *C. giganteus* was reported to cause low insecticidal activity on *An. gambiae* eggs and larvae but that activity was significantly optimized when combined to *Eucalyptus citriodora* [21]. The present study aimed to investigate the larvicidal activity of hexane, acetone and methanol as well as essential oils of *C. citratus* and *C. giganteus* against the larvae of *An. gambiae* under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Plant Material

2.1.1 Plant collection and processing

The leaves of *Cymbopogan citratus* and *Cymbopogon giganteus* were collected early in the morning (6:00 to 8:00 am) at Dang (latitude 7°24.949'N, longitude 13°32.870'E) Ngaoundere in the Adamaoua region of Cameroon in July

2017. Plants were identified by professor Mapogmetsem Pierre Marie a botanist of the Faculty of Science, University of Ngaoundere and then were confirmed at the National Herbarium of Cameroon at Yaounde through the comparison to the voucher specimen no 18628/SRF/Cam in comparison with the plant material sample of Dang Daniel 202 for *C. citratus* and no 8373/SFR/Cam compared with Letouzey 6532 voucher sample for *C. giganteus*. The plant leaves collected were shade dried during three weeks (26±2°C; 78±2% relative humidity), then crushed in a wood mortar and passed through 1mm mesh size sieve. Each plant powder obtained was preserved in the glass bottles then kept in a refrigerator at 4°C until used for extraction.

2.1.2 Plant extraction process

The cold maceration method performed of Perry et al. [29] was used for plants extraction. Indeed, 500 g of each plant leaf powder was macerated in 2.5 L of hexane for 3 days in the glass jar (5 L) and agitated twice a day. Then, the maceration was filtrated through Whatman No.1 filter paper to obtain hexane filtrate and residue. The residue was dried and then macerated in 2.5L of acetone and processed as described previously to obtain acetone filtrate and residue. At last, the dry residue was macerated in the 2.5L of methanol solvent as described previously to obtain methanol filtrate and residue. Each filtrate was concentrated separately using rotary evaporator (BÜCHI R-124) to obtain hexane, acetone and methanol extracts. The dry plant extracts were stored at -4°C until its use for phytochemical screening and bioassays. The extraction yield of each extract was determined using the following formula:

$$\text{Extraction yield (\%)}$$
$$= \frac{\text{Weight of the extract obtained (g)}}{\text{Weight of the plant powder used (g)}} \times 100$$

2.1.3 Extraction of plant essential oils

Essential oils were extracted directly from fresh leaves of plants by hydro-distillation method using Clevenger apparatus for 3 hours. The oil was separated from water using separating funnel and traces of water in the oil was removed using anhydrous sodium sulphate and then stored in airtight containers rolled with aluminum foil to avoid light until needed for bioassay. The extraction yield of each essential oil obtained was calculated using the following formula:

$$\text{Oil yield (\%)} = \frac{\text{Weight of essential oil obtained}}{\text{Weight of plant fresh leaves used}} \times 100$$

2.2 The Strain of *Anopheles gambiae*

The larvae of *An. gambiae* were collected from the lake water in July 2018 around the campus of the University of Ngaoundere. Larvae of *Anopheles* species were detected and collected according to their horizontal position on the water surface and *An. gambiae* larvae were identified in the laboratory following the identification keys performed by Gillies & Coetzee [30] and Gillies & De Meillon [31]. The larvae of *An. gambiae* therefore identified were reared according to Das et al. [32] protocol in the laboratory (27±2°C; 76±4% Relative Humidity). The first generation was used for the larvicidal assay.

2.3 Larvicidal Bioassay

The standard larvicidal assessment method described by WHO [33] was used to evaluate the efficacy of *C. citratus* and *C. giganteus* extracts and essential oils against *An. gambiae* larvae in the laboratory. The hexane, acetone and methanol extracts and essential oils were dissolved in 0.5 mL of Tween-80 and different concentrations of 1000, 500, 250 and 125 mg/L for plant extracts and 200, 100, 50 and 25 mg/L of plant essential oils were prepared in the volume of 100 mL with tap water in the 250 mL plastic cups. Twenty five fourth instar larvae were transferred into the each test solutions prepared. Four (4) replicates were maintained for each concentration and mortality of mosquito larvae was recorded 1, 6, 12, 18 and 24 h post-exposure for essential oils and after 24 h for plant extracts. Dead larvae were detected when appendages did not move when probed with needle. Data were adjusted for control mortality using Abbott's formula [34], if mortality in the control sets exceeded 5 %.

2.4 Statistical Analyses

The percentage of larval mortality data were subjected to the ANOVA procedure using SPSS 16.0. Tukey test (P=0.05) was applied for mean separation. Lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality of *An. gambiae* larvae 24 h after treatment application were determined using Probit analysis (SPSS 16.0).

3. RESULTS

3.1 Plant Extraction Yields

The yield of the plant extracts and essential oils obtained varied among the plant species according to the solvent used for the extraction (Table 1). Among the extracts of the two plant species, hexane extracts yields were low compared to acetone and methanol extracts. Between the two plant species, essential oil of *C. citratus* yield was superior to the *C. giganteus* extraction yield.

3.2 Larvicidal activity of plant extracts against *An. gambiae* larvae

The hexane, acetone and methanol extracts of *C. citratus* and *C. giganteus* exerted a significant larvicidal activity against the third and fourth instar larvae of *An. gambiae* under laboratory conditions after 24 h post-exposure (Table 2 & 3). The toxic activity of these two plant species significantly augmented with the increasing concentrations. Globally, no larval mortality was recorded in the negative control while 100% mortality of *An. gambiae* larvae was registered in the positive control (Dichlovos 1000 mg/L).

Table 1. Yield of the plant solvent and hydrodistillation extraction

Plant species	Plant products	Weight of plant material used (g)	Yield (%)
C. citratus	Hexane extract	500	9.33
	Acetone extract	500	10.83
	Methanol extract	500	12.92
	Essential oil	200	1.24
C. giganteus	Hexane extract	500	5.28
	Acetone extract	500	7.24
	Methanol extract	500	8.73
	Essential oil	200	0.83

After application of *C. citratus* extracts as presented in Table 2, larval mortality significantly varied from 82to 100% ($F_{(5,18)}$ = 800.33, P < 0.001) for hexane extract, from 28 to 100% ($F_{(5,18)}$ = 877.07, P < 0.001) for acetone extract and from 60 to 100% ($F_{(5,18)}$ = 1481.84, P < 0.001) for methanol extract, respectively tested at the concentrations of 125 and 1000 mg/L. Among the *C. citratus* extracts tested, the hexane extract of the plants with LC_{50} value of 58.32 mg/L was revealed as the most toxic on *An. gambiae* larvae compared to acetone (LC_{50} = 161.83 mg/L) and methanol (LC_{50} = 109.59 mg/L) extracts of the plant.

With *C. giganteus* extracts applied at the lowest dose of 125 mg/L and at the highest concentration of 1000 mg/Lon *An. gambiae* larvae as showed in Table 3, the larval mortality significantly increased respectively from 11% to 80% (at 1000 mg/L) ($F_{(5,18)}$ = 199.06, P < 0.001)

for hexane extract, from 10% to 81% ($F_{(5,18)}$ = 251.65, P < 0.001) for acetone extract and 16% to 88%($F_{(5,18)}$ = 776.33, P < 0.001) for methanol extract. Comparing efficacy of *C. giganteus* plant extracts each other, the hexane extract with LC_{50} value of 372.36 mg/L was revealed as the most effective followed by methanol extract (LC_{50} = 161.83 mg/L) extract and acetone extract (LC_{50} = 161.83 mg/L).

3.3 Larvicidal Activity of Plant Essential Oils against *An. gambiae* Larvae

The essential oil of the plant species caused significant larvicidal activity against *An. gambiae* larvae in the laboratory. The efficacy of the essential oils of *C. citratus* and *C. giganteus* assessed significantly increased with the augmenting concentration and exposure time (Fig 1 A& B).

Table 2. Percentage mortality of *An. gambiae* larvae treated with plant hexane, acetone and methanol extracts and LC_{50} as well as LC_{95} (mg/L) of *C. citratus*, 24 h post-exposure

Extracts	Conc (mg/L)	% mortality	R^2	Slope±SE	LC_{50} (LFL-UFL)	LC_{95} (LFL-UFL)	χ^2
Hexane	0	0.00±0.00c					
	125	82.00±3.46b					
	250	100.0±0.00a	0.31	3.00±0.39	58.32	206.08	24.84*
	500	100.0±0.00a			(32.02-77.90)	(177.35-262.80)	
	1000	100.0±0.00a					
	Dichlovos (1000 mg/L)	100.0±0.00a					
	$F_{(5, 18)}$	800.33***					
Acetone	0	0.00±0.00e					
	125	28.00±2.82d					
	250	87.00±1.00c	0.50	3.84±0.19	161.83	432.91	72.38***
	500	93.00±1.91b			(137.57-184.58)	(357.80-578.84)	
	1000	100.0±0.00a					
	Dichlovos (1000 mg/L)	100.0±0.00a					
	$F_{(5, 18)}$	877.07***					
Methanol	0	0.00±0.00d					
	125	60.00±1.63c					
	250	85.00±1.91b	0.60	3.39±0.24	109.59	334.76	17.70ns
	500	100.0±0.00a			(98.70-119.30)	(303.29-379.12)	
	1000	100.0±0.00a					
	Dichlovos (1000 mg/L)	100.0±0.00a					
	$F_{(5, 18)}$	1481.84***					

Mean of mortality ± standard deviation within a column followed by the same letter did not differ significantly according to Tukey test (P= 0.05); [ns]P>0.05; *P<0.05; ***: p<0.001; LFL: Lower Fiducial Limit; UFL: Upper Fiducial Limit; Number of replicates: 4

Table 3. Percentage mortality of *An. gambiae* larvae treated with plant hexane, acetone and methanol extracts and LC_{50} as well as LC_{95} (mg/L) of *C. giganteus*, 24 h post-exposure

Extracts	Conc (mg/L)	% mortality	R^2	Slope±SE	LC_{50} (LFL-UFL)	LC_{95} (LFL-UFL)	χ^2
Hexane	0	0.00±0.00f					
	125	11.00±1.91e					
	250	40.00±3.65d	0.85	2.32±0.11	372.36	1897.46	40.46***
	500	59.00±4.12c			(327.96-	(1442.42-	
	1000	84.00±3.65b			423.48)	2776.39)	
	Dichlovos (1000 mg/L)	100.0±0.00a					
	$F_{(5, 18)}$	199.06***					
Acetone	0	0.00±0.00f					
	125	10.00±1.15e					
	250	34.00±2.58d	0.90	2.28±0.11	420.97	2205.85	30.48**
	500	54.00±2.58c			(376.75-	(1702.73-	
	1000	81.00±4.72b			472.86)	3118.78)	
	Dichlovos (1000 mg/L)	100.0±0.00a					
	$F_{(5, 18)}$	251.65***					
Methanol	0	0.00±0.00f					
	125	16.00±1.63e					
	250	44.00±1.63d	0.89	2.27±0.11	325.43	1719.10	16.14[ns]
	500	62.00±2.00c			(303.50-	(1470.41-	
	1000	88.00±1.63b			348.62)	2071.45)	
	Dichlovos (1000 mg/L)	100.0±0.00a					
	$F_{(5, 18)}$	776.33***					

*Mean of mortality ± standard deviation within a column followed by the same letter did not differ significantly according to Tukey test (P= 0.05); [ns]P>0.05; **P<0.01; ***: p<0.001; LFL: Lower Fiducial Limit; UFL: Upper Fiducial Limit; Number of replicates: 4*

Tested with essential oil of *C. citratus* at the lowest dose of 25 mg/L, mosquito larval mortality varied significantly ($F_{(4, 15)}$=105.22, p < 0.001) from 46% after 1 h to 100% after 24 h post-exposure was recorded. Essential oil of *C. citratus* (applied at 100 and 200 mg/L) and the commercial larvicide Dichlovos (tested at the recommended dose of 1000 mg/L) exhibited 100% mortality of larvae at all exposed periods. The LC_{50} values of *C. citratus* against *An. gambiae* larvae declined with exposure period and values of 27.98, 20.21 and 10.97 mg/L were recorded respectively after 1, 6 and 12 h post-treatment (Table 3).The values of LC_{95} of *C. citratus* essential oil against *An. gambiae* larvae recorded after 1, 6 and 12 h post-exposure were 76.96, 62.00 and 35.94 mg/L, respectively.

The essential oil of *C. giganteus* tested at the lowest dose of 25 mg/L caused also a moderate significant ($F_{(4, 15)}$=119.55, p < 0.001) larval mortality ranging from 0% after 1 h to 54% after 24 h post exposure. Tested at the highest concentration of 200 mg/L, larval mortality significantly ($F_{(4, 15)}$=82.75, p < 0.001) varied from 48% after 1h to 100%after 24 h post-treatment. The LC_{50} and LC_{95} values of *C. giganteus* against *An. gambiae* larvae decreased with the increasing exposure times (Table 4). The LC_{50} recorded were 180.07, 95.00, 42.13, 24.64 and 22.93 mg/L after 1, 6, 12, 18 and 24 h post-treatment, respectively. LC_{95} values of 1030.56 mg/L (1h), 547.82 mg/L (6h), 162.08 mg/L (12h), 67.93 mg/L (18h) and 49.83 mg/L (24h) of *C. giganteus* essential oil against mosquito larvae assessed were also registered.

Considering the essential oils of the two plant species tested on mosquito larvae, *C. citratus* essential oil (LC_{50} = 27.98 mg/L after 1 h post-exposure) was revealed to be more toxic against *An. gambiae* compared to *C. giganteus* essential oil (LC_{50} = 180.07 mg/L after 1 h post-exposure).
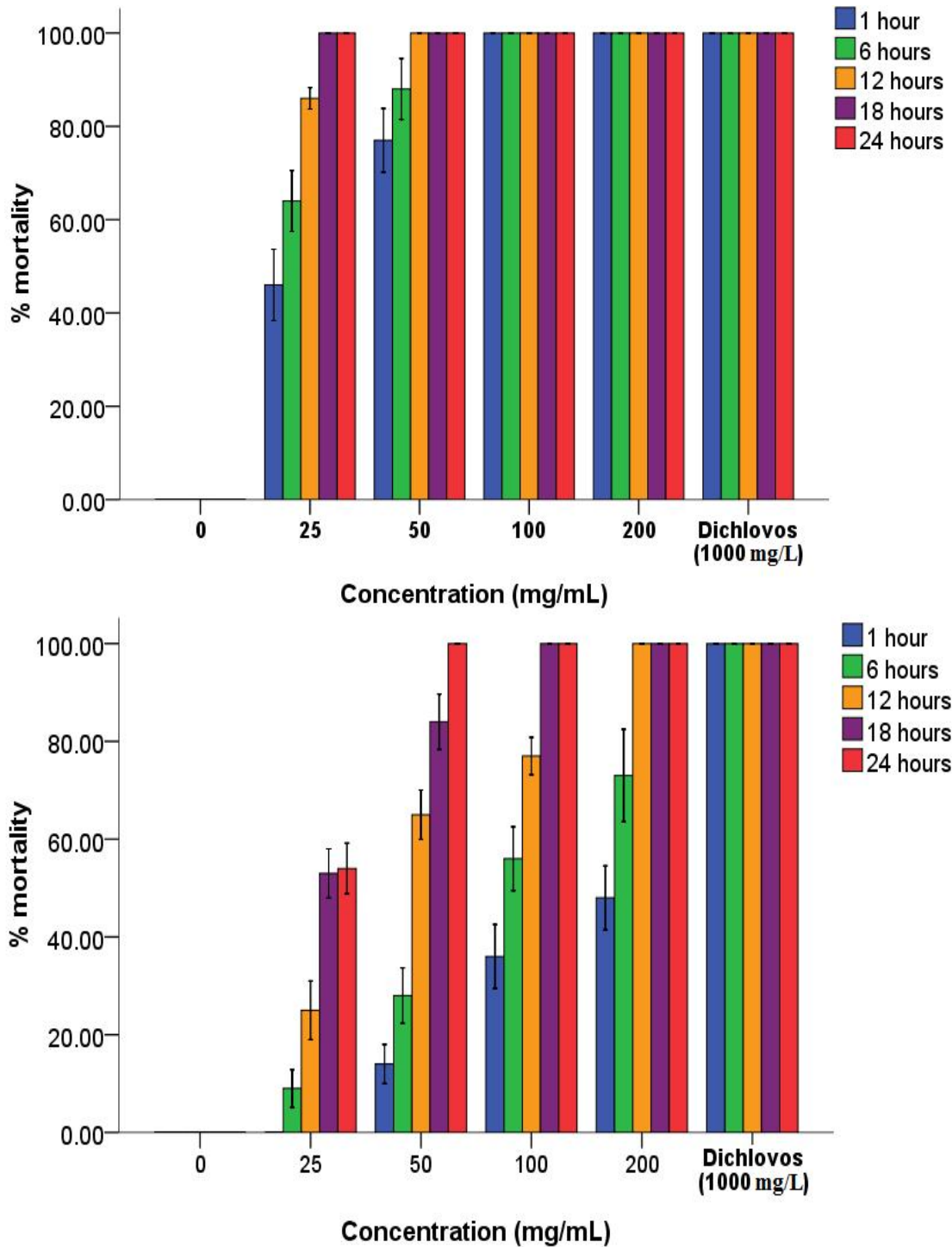
Fig. 1. Percentage mortality of *An. gambiae* larvae treated with *C. citratus* (A) and *C. giganteus* (B) leaf essential oils after 1, 6, 12, 18 and 24 hours post-exposition, PC= Positive control (Dichlovos 49%)

Table 4. LC$_{50}$ and LC$_{90}$ (mg/L) of *C. citratus* and *C. giganteus* leaf essential oils against *An. gambiae* larvae after 1, 6, 12, 18 and 24 hours post-exposure

Plant species	Time (h)	Slope±SE	R^2	LC$_{50}$(95% FL)	LC$_{95}$(95% FL)	χ^2
C. citratus	1	3.74±0.22	0.61	27.98(24.60-31.02)	76.96(66.10-95.58)	36.05**
	6	3.38±0.26	0.54	20.21(16.45-23.27)	62.00(53.50-77.11)	29.07*
	12	3.19±0.48	0.34	10.97(7.08-13.99)	35.94(32.42-41.44)	9.75ns
	18	-	-	-	-	-
	24	-	-	-	-	-
C. giganteus	1	2.17±0.13	0.85	180.07(149.45-233.37)	1030.56(641.06-2203.95)	47.19***
	6	2.16±0.11	0.84	95.00(83.83-108.99)	547.82(402.39-840.89)	34.62***
	12	2.81±0.13	0.79	42.13(6.13-48.11)	162.08(129.77-222.68)	54.63***
	18	3.73±0.24	0.56	24.64(21.97-27.00)	67.93(60.11-80.08)	21.60ns
	24	4.88±0.39	0.34	22.93(20.39-25.00)	49.83(44.16-59.82)	26.07*

nsP>0.05; *P<0.05; **P<0.01 and ***: p<0.001; FL= Fiducial Limit. -: no determined because of no or complete mortality at all concentrations tested

4. DISCUSSION

Results of the present study showed that the extracts and essential oils of the two plant species assessed (*C. citratus* and *C. giganteus*) were toxic when applied on *An. gambiae* larvae. Generally, hexane, acetone and methanol extracts as well as essential oils of the two plant species significantly exerted concentration-dependent toxic effect against *An. gambiae* larvae.

Previous studies worldwide reported the efficacy of *Cymbopogon* species extracts against mosquito species. Indeed, the methanol extract of *C. citratus* exhibited potent larvicidal activity against late third instar larvae of *An. arabiensis* [35]. Methanol and hexane extracts of *C. citratus* caused also a significant larvicidal activity against *An. gambiae* larvae [36]. *C. citratus* significant inhibited growth and caused mortality in later developmental stages of *Aedes aegypti* [37]. The hydrolate *C. citratus* was also revealed as promising larvicidal activity product against *Ae. albopictus* and *Cx. quinquefasciatus* mosquito species [38]. The phytochemical secondary metabolites contained in these plant species might be the responsible of the larval mortality observed. Earlier, Bilal and Haman [39] reported the important role of flavonoids, terpenoids, alkaloids, steroids and phenols, responsible of the biological activities against insects. Plant secondary metabolites in contact with mosquito larva may penetrate through oral or cuticle way to affect midgut epithelium or gastric caecae and the malpighian tubules in the insect larva [40]. These phytochemical compounds might also act at the proton transferring sites, leading to mitochondria dysfunction that can cause cellular and physiological disturbances by inhibiting acetylcholinesterase, the disruption of potassium and sodium ion exchange, and unbalance of mitochondrial respiration [41].

Among the solvent extracts of two plant species assessed, the non-polar solvent hexane extracts was the most toxic followed by methanol extract and then acetone extract. Similar observation was noticed by Kemabonta et al. [42] in which, hexane extract (LC$_{50}$=19.49% v/v) of *Lantana camera* was more effective on *An. gambiae* larvae than ethanol extract (LC$_{50}$=46.98% v/v) of that plant on mosquito larvae. This is also in agreement with the observation of Egunyomi et al. [43] in which the hexane extract of *C. citratus* killed more mosquitoes that came to physical contact of the extract than methanol plant extract indicating that the active compounds of these plant species might be more soluble in hexane solvent. Similar observation was reported with methanol extract of *C. citratus* that repelled more *An. stephensis* and *An. culicifacies* female adults compared to chloroform and water extracts of the plant [44].

From the results of this present investigation, essential oils of the two plant species significantly exhibited dose-dependent larvicidal property against larvae of *An. gambiae*. In the same way, the essential oil of *C. giganteus* showed a low insecticidal activity on *An. gambiae* eggs (LC$_{50}$ of 107.77 µg/mL) and larvae (LC$_{50}$ of 64.4 µg/mL) but combined to *Eucalyptus citriodora* exhibited a significant larvicidal activity (7.33 µg/mL) and ovicidal activity (13.64 µg/mL), respectively against *An. gambiae* [21].

Indeed, plant essential oils constitute a complex mixture of phytochemical compounds including majorly monoterpenes and sesquiterpenes molecules which might act singly or in synergy causing the mortality of mosquito larvae [45]. Eight volatile components of essential oils compounds including (-)-perillyl alcohol, (-)-isopulegol, (+)-limonene epoxide, (+)-limonene, terpinen-4-ol, terpinolene, (-)-carvone epoxide, and (-)-hydroxydihydrocarvone were revealed to be potent against 3rd instar larvae of *An. gambiae s.s.* [43]. Four p-menthadienol isomers as major molecules in *C. giganteus* and major terpenes in *C. citratus* essential oils including geranial (10%-48%) neral (3%-43%), borneol (5%), geraniol (2.6%-40%), geranyl acetate (0.1%-3.0%), linalool (1.2%-3.4%), and nerol (0.8%-4.5%) reported in the previous studies [27, 46,47,48,49] might also be present in the essential oils assessed in this present study and probably might be the responsible of the insect mortality. From Benin, Nonviho et al. [50] and Bossou et al. [51] identified also geranial and neral in *C. citratus*, and E-p-mentha-1(7), Z-carveol, E-p-mentha-2,8-dienol and 8-dien-2-ol in *C. giganteus* essential oils as major constituents, and were found potent against the malarial vector *An. gambiae*. From Cameroon, study conducted by Tchoumbougnang et al. [52] revealed acyclic monoterpenes including geraniol (15.6%), geranial (39.3%), neral (21.9%) and myrcene (14.0%) as the main compounds found in the essential oil of *C. citratus* are such as which caused a remarkable larvicidal activity against *An. Gambiae* larvae.

Globally, the toxic effect of essential oils is higher than those of extracts in this present study and could be attributed to their volatile properties with rapid action in insect. Because of their lipophilic nature, essential oils in the contact of insect organs might interfere immediately with behavioral, physiological and biochemical functions of insects explaining their rapid action compared to plant extracts [53]. Total breakdown of nervous system followed by the death of the insect occurs when essential oil molecules interfere with the neuromodulator GABA-gated chloride or octopamine channels causing therefore their disruption [54,55].

5. CONCLUSION

Extract and essential oils of *C. citratus* and *C. giganteus* significantly exhibited larvicidal activity *An. gambiae* larvae. Hexane extracts of *C. citratus* (LC_{50}=58.32 mg/L) and *C. giganteus*

(LC_{50}=372.36 mg/L) were the most toxic for mosquito larvae compared to acetone and methanol extracts of the plants. Comparing the two plant species, *C. citratus* extracts tended to be more effective on *An. gambiae* larvae than *C. giganteus* extracts. In the same way, essential oil of *C. citratus* (LC_{50}=27.98 mg/L after 1h) was the most potent against mosquito larvae assessed compared to *C. giganteus* (LC_{50}=180.07 mg/L after 1h) leaf essential oil. Thus, hexane extract and essential oil of *C. citratus* may be considered as a best candidate for a new botanical insecticide to control *An. gambiae* larvae in their breeding sites.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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