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INSECTICIDAL ACTIVITIES OF ESSENTIAL OILS EXTRACTED FROM THREE SPECIES OF *POACEAE* ON *ANOPHELES GAMBIAE SPP*, MAJOR VECTOR OF MALARIA

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Abstract: In this paper, the insecticidal activities on *Anopheles gambiae spp* of the essential oils (EO) extracted from the dry leaves of some species collected in Benin were studied. The essential oil yields are 2.8, 1.7 and 1.4% respectively for *Cymbopogon schoanenthus* (L.) Spreng (*CS*), *Cymbopogon citratus* Stapf. (*CC*) and *Cymbopogon giganteus* (Hochst.) Chiov (*CG*). The GC/MS analysis showed that the EO of *CS* had a larger proportion in oxygenated monoterpenes (86.3%) whereas those of the sheets of *CC* and *CG* are relatively close proportions (85.5% and 82.7% respectively) with. The piperitone (68.5%), δ-2-carene (11.5%), and α-eudesmol (4.6%) are the major components of the EO of *CS* while *trans para*-mentha-1(7),8-dien-2-ol (31.9%), *trans para*-mentha-2,8-dien-1-ol (19.6%), *cis para*-mentha-2,8-dien-1-ol (7.2%), *trans* piperitol (6.3%) and limonene (6.3%) prevailed in the EO of *CG*. The EO of *CC* revealed a rich composition in geranial (41.3%), neral (33%), myrcene (10.4%), and geraniol (6.6%). The biological tests have shown that these three EO induced 100% mortality of *Anopheles gambiae* to 1.1, 586.58 and 1549 $\mu g \cdot cm^{-2}$ respectively for *CC, CS* and *CG*. These effects are also illustrated by weak lethal concentration for 50% anopheles population (*CC*: 0.306; *CS*: 152.453 and *CG*: 568.327 $\mu g \cdot cm^{-2}$) in the same order of reactivity. The EO of *CC* appeared most active on two stocks (sensitive and resistant) of *Anopheles gambiae*.

Key words: *essential oil, Poaceae, insecticide, Anopheles gambiae*

INTRODUCTION

Malaria remains a true problem of public health. In the world, more than two billion people are exposed to this endemic disease [1, 2]. It accounts for 30 even 50% of the hospital admissions and approximately 50% of the external consultations in the developing countries. Malaria is a parasitosis due to *Plasmodium falciparum*, transmitted by the female mosquito-bite. In Benin, the statistics bring back that it represents the first cause of the medical consultations. Indeed, the children under five years of age situation's corresponds to more than 44% of the cases of patients diagnosed in the public health centers and 21.9 % of death in hospitalization [3].
To control the vectors of this pathology, many strategies focused on the use of synthesis insecticides were recommended [3]. But latest years, a certain resistance of new generations of mosquitoes to these insecticides is noticed [4 – 6]. Moreover, the means of chemical fight (Therapeutic Combinations based on Artemisinin) more effective are too expensive for the populations of the most exposed countries [7]. These significant facts cause the urgent search for new alternatives of fight against this endemic disease. Currently, the tendency is with the use of vegetable's extracts, far from toxic, biodegradable, cheaper and with unveiled effectiveness. They are essential oils of aromatic plants containing a noticeable amount of bioactive compounds properties against the vectors of diseases, in particular the malaria mosquitoes (*Anopheles gambiae*) [8, 9].
This study was designed to accompany the prospect for research, starting aromatic vegetable species from Benin, molecules to control the populations of *Anopheles gambiae*. This study thus carried the chemical composition and insecticidal activities of essential oils extracted from the leaves of *Cymbopogon citratus*, *Cymbopogon giganteus* and *Cymbopogon schoanenthus*.

MATERIAL AND METHODS

Plant materials

The dried leafs of *Cymbopogon citratus*, *Cymbopogon giganteus* and *Cymbopogon schoanenthus* were purchased in three different localities from Benin before the flowering stage, and identified at National Herbarium of Abomey-Calavi University, Benin. Seeds of these plants were subjected to hydro distillation for 4 hours in a Clevenger-type apparatus. The essential oil obtained in batches was dried over anhydrous sodium sulfate and, after filtration, stored under refrigeration until tested and analyzed. The yields were reported at the same time with place and date of harvest in Table 1.

Chromatographic analysis of essential oils

GC/MS

The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 7890, coupled to a Hewlett-Packard MS model 5875, equipped with a DB5 MS column (30

m × 0.25 mm; 0.25 µm), programming from 50 °C (5 min) to 300 °C at 5 °C/min, 5 min hold. Helium is carrier gas (1.0 mL·min^{-1}); injection in split mode (1:30); injector and detector temperature, 250 and 280 °C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180 °C; mass spectra data were acquired in the scan mode in m/z range 33 – 450.

Table 1. *Essential oil yield of the studied vegetable species*

Sample of sheets	***Cymbopogon citratus***	***Cymbopogon giganteus***	***Cymbopogon schoanenthus***
Place and date of harvest	Akogbato 05/10/09	Koudo 23/05/09	Boukoumbé 22/05/09
Yield (%)	1.7	1.4	2.8

GC/FID

The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 6890, equiped with a DB5 MS column (30 m × 0.25 mm; 0.25 µm), programming from 50 °C (5 min) to 300 °C at 5 °C/min, 5 min hold. Hydrogen is carrier gas (1.0 mL·min^{-1}); injection in split mode (1:60); injector and detector temperature, 280 and 300 °C respectively. The essential oil is diluted in hexane: 1/30.

The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference substances [10, 11].

Animal material

Anopheles gambiae spp developing either on Kisumu in Kenya (sensitive) or on Ladji in Benin (resistant) were collected from stores and mass-reared to adult under laboratory conditions (temperature: 27 ± 2 °C, relative humidity: 70 – 80% and 12 h: 12 h L: D) and using the method of WHOPES (World Health Organization Pesticides Scheme) to avoid any previous contamination [12].

Biological tests

Three biological tests adapted from the standard protocols of the World Health Organization (WHO) were used: the test of sensitivity, the test in cone and the test in tunnel [13, 14].

Evaluation of the insecticidal activity of essential oils by the test of sensitivity or WHO tube test

To carry out this test, a kit of WHO tubes was used [13]. It is composed of two plastic tubes (height: 125 mm; diameter: 44 mm). The first (tube of exposure), of which the interior was papered of an impregnated essential oil paper with an amount given of range 0 to 400 µg·cm^{-2}. As for the second (tube of observation), has his wall papered of a plain paper. With one of the ends of the observation's tube, a slide provided with a hole (diameter: 20 mm) fixed to which a vacuum cleaner is adapted to facilitate the

introduction of the mosquitoes. To allow a synchronized observation, we blew 25 females of *Anopheles gambiae* (old from 3 to 5 days) inside each tube of observation. By the means of the slide, the females were puffed up in the tubes of exposure, and then they are put in observation. At the end of one hour of exposure, mosquitoes are replaced in the tubes of observation where they are maintained with the juice sweetened to 10% during 24 hours. After this period, we noted the dead number of female anopheles [12]. Four tests are then carried out for each essential oil amount tested. With the exit of the test, the data obtained made it possible to determine the lethal concentrations for 50, 95 and 100% (respectively LC_{50}, LC_{95} and LC_{100}) of the subjugated population of anopheles to essential oil tested.

Effectiveness evaluation of the essential oils by the test in cone

The WHO cone tests consists of introducing unfed five-day-old mosquitoes into a Plexiglas cone attached to the insecticide-treated material. Fifteen mosquitoes were placed in each cone, and four cones were used for each type of material. The contact time was 30 minutes. After exposure, the mosquitoes were placed in small cups, provided with sugar solution and maintained at 27 ± 2 °C with a relative humidity of 80 ± 10% for 24 hours to assess delayed mortality. This test allows an effectiveness evaluation of essential oil on the mosquitoes, complementary to the test of sensitivity. It is made by impregnation with amount corresponding to LC_{100} given starting from the test of sensitivity on mosquito net (25 cm × 25 cm) [14].

The effectiveness evaluation by the test in tunnel of essential oils

The test in tunnel have be done, according WHOPES protocol [14], by releasing 100 non-blood fed female anopheline mosquitoes, aged 5 – 8 days, at 18:00 h in a two third of a tunnel (square section 25 cm × 25 cm × 60 cm) made of glass. At each end of the tunnel, a 25 cm square cage is fitted (extension) and covered with polyester netting. At one third of the length, a disposable cardboard frame is placed with the treated netting sample. The surface (20 cm × 20 cm) of netting to mosquitoes is made with nine holes each 1 cm in diameter. In the shorter section of the tunnel, guinea-pig (as bait) is placed, unable to move. Females are free to fly in the tunnel but have to make contact with the piece of netting and locate the holes in it before passing through to reach the bait. The following morning, at 09:00 h, the mosquitoes are removed and counted separately from each section of the. During tests, cages are maintained at 27 ± 2°C and 80 ± 10% relative humidity under subdued light. Several tunnels are used simultaneously, one tunnel with untreated netting always being used as a negative control. The repulsive effect of insecticides is evaluated by comparing the numbers of mosquitoes gorged with blood in the tunnels with test with those gorged in the pilot tunnel.

Statistical analysis

The recorded raw data underwent a probit regression by using SPSS 16.0 [15]. The values of LC_{50}, LC_{95} and of LC_{100} as well as the line of equation $Y = a + bX$, with a confidence interval of 95%, were established.

RESULTS AND DISCUSSIONS

Chemical study of essential oils

The extraction yields ranged between 1.4 and 2.8% (Table 1). The dry leaves of *C. schoanenthus* are richer in essential oil (2.8%) than those of *C. citratus* (1.7%) and *C. giganteus* (1.4%). These essential oil yields, of each vegetable species considered, are higher than the values obtained during the former investigations [16, 17] due to the periods of harvest of these vegetable species, the nature of the ground and the climatic conditions [18].

Table 2. *Chemical compositions of essential oils of* ***Cymbopogon citratus****,* ***Cymbopogon giganteus*** *and of* ***Cymbopogon schoanenthus***

Chemical compounds	IK	*Cymbopogon citratus*	*Cymbopogon giganteus*	*Cymbopogon schoanenthus*
myrcene	991	10.4	-	-
limonene	1031	-	6.3	-
neral	1245	33	-	-
geranial	1276	41.3	-	-
geraniol	1256	6.6	-	-
trans-para-mentha-1(7),8-dien-2-ol	1194	-	31.9	-
trans-para-mentha-2,8-dien-1-ol	1125	-	19.6	-
cis-para-mentha-2,8-dien-1-ol	1140	-	7.2	-
cis-para-mentha-1(7),8-dien-2-ol	1237	-	7.4	-
trans-piperitol	1205	-	6.3	-
piperitone	1265	-	-	68.4
δ-2-carene	1000	-	-	11.5
α-eudesmol	1662	-	-	4.6
elemol	1552	-	-	3.9
Monoterpenes hydrogens		**11.1**	**7**	**2.6**
Monoterpenes oxygenates		**85.5**	**86.3**	**82.7**
Sesquiterpenes hydrogens		**-**	**-**	**1.2**
Sesquiterpenes oxygenates		**0.2**	**0.9**	**10.4**

The results of Table 2 show that our essential oils consisted mainly of oxygenated monoterpenes (> 80%). Among the oxygenated monoterpenes, geranial (41.3%), neral (33%), and geraniol (6.6%) followed by the myrcene (10.4%) were the major components of the *Cymbopogon citratus* essential oil. The geranial (or citral A) and the neral (citral B), two geometrical isomers, alone constitute 74% of the total mass of this essential oil. Tchoumbougnang *et al*. [16] revealed, in 2009, the majority compounds (geraniol: 15.6%, geranial: 39.3%, neral: 21.9% and myrcene: 14.0%) identical to those found out during current work. This composition of the EO is substantially different from that reported from Brazil plants [19] where geranial (40.8%), neral (36.3%) and beta-myrcene (13.2%**)** constituted the major portion of the EO.

***Cymbopogon giganteus* and *Cymbopogon schoanenthus* essential oils presented monoterpenes as major chemical constituents**

For *Cymbopogon giganteus* essential oil, the major constituents found were: *trans-para*-mentha-1(7),8-dièn-2-ol (31.9%), *trans-para*-mentha-2,8-dièn-1-ol (19.6%), *cis-para*-mentha-2,8-dièn-1-ol (7.2%) and a *trans* pipéritol (6.3%). Its composition out of limonene (6.3%) approaches that (7.7%) found by Alitonou *et al.* [20]. On the contrary, Nyamador *et al.* in 2010 [21] showed that the leaves of *Cymbopogon giganteus* collected in Togo mainly made up of limonene (23%) followed of *para*-mentha-2,8-dien-1-ol duplicated between the *trans* forms (5, 63%) and the *cis* forms (14.3%).
For *Cymbopogon schoanenthus* essential oil, piperitone (68.4%), δ-2-caren (11.5%) and α-eudesmol (4.6%) were the dominant components. Ayedoun *et al.* in 1997 identified the piperitone (60.0%) as principal compound accompanied by elemol (8.4%) of an essential oil extracted from the leaves in Bassila (Benin) [22]. Moreover, Kétoh *et al.* reported [23], that essential oil extracted from the leaves from Togo contained a proportion in piperitone (68.0%) near to the sheets collected in Akogbato (Benin), though δ-2-caren (16.48%) proportion was higher.

Insecticidal activity evaluated on sensitive Anopheles gambiae by the test of sensitivity and the test in cone

The toxicity of EO against *An. gambiae* was evaluated by the test of sensitivity. The statistical data (LC_{50}, LC_{95}, LC_{100}), significance level (Sign) and regression equation were recorded as presented in Table 3. 50% mortality (LC_{50} values) was 0.309 $\mu g \cdot cm^{-2}$, 152.453 $\mu g \cdot cm^{-2}$ and 568.327 $\mu g \cdot cm^{-2}$ respectively for *Cymbopogon citratus*, *Cymbopogon schoanenthus*, and *Cymbopogon giganteus*. We observed that *An. gambiae* was more susceptible to *C. citratus* whose presented the weakest lethal concentrations (LC_{95} = 0.847 $\mu g \cdot cm^{-2}$ and LC_{100} = 1.1 $\mu g \cdot cm^{-2}$). Reactivity of the volatile extract from *C. schoanenthus* is illustrated by the representative slope of the straight regression line (0.005 ± 0.001). Indeed, Kétoh *et al.* already had revealed the insecticidal activities of EO of *C. schoanenthus* against *Callosobruchus maculatus* [22]. They attributed its noxious action to piperitone.

Table 3. *Lethal concentrations of essential oils of three species of* ***Poaceae*** *on* ***Anopheles gambiae*** *sensitive, obtained starting from the test of sensitivity*

	Cymbopogon citratus	***Cymbopogon giganteus***	***Cymbopogon schoanenthus***
LC_{50} [$\mu g \cdot cm^{-2}$]	0.309	568.327	152.453
LC_{95} [$\mu g \cdot cm^{-2}$]	0.847	1261	459.404
LC_{100} [$\mu g \cdot cm^{-2}$]	1.1	1549	586.58
Straight regression line	$Y = (-0.930 \pm 0.120) + (3.040 \pm 0.286)\,X$	$Y = (-1.349 \pm 0.119) + (0.002 \pm 0.001)\,X$	$Y = (-0.817 \pm 0.098) + (0.005 \pm 0.001)\,X$
Significance level	0.000	0.246	0.000

The high rate of significance level (0.246), obtained for the regression of *Anopheles gambiae* mortalities, following the exposure of *C. giganteus* does not throw out the

biocide activity of this EO. It had the lowest reactivity against this stock of mosquitoes (LC_{50} = 568.327 and LC_{100} = 1549 higher).

Table 4. *Number of dead imagoes of* ***Anopheles gambiae*** *after spreading of essential oils of three* ***Poaceae*** *starting from the test in cone*

	Sources of essential oils		
	Cymbopogon citratus	***Cymbopogon giganteus***	***Cymbopogon schoanenthus***
Number of dead imagoes [%]	38	22.7	23.4

These reports are reinforced by the results of the test in cone. We observe, starting from Table 4, that the EO of *C. citratus* remained more active, by showing a higher death rate (38%). However, the results of this test reveal the essential oils of *C. giganteus* and of *C. schoanenthus* have the same reactivity (respective rates of 22.7% and 24.3%). This difference noted can be due to the fact that there is a significant correlation (rate of significance level equal to 0.000) between the amount of EO of *C. schoanenthus* and the mortality of *Anopheles gambiae*. The linear shape of the curve (Figure 1) justifies that the death rate of the anopheles grows when the amount of essential oil increases.

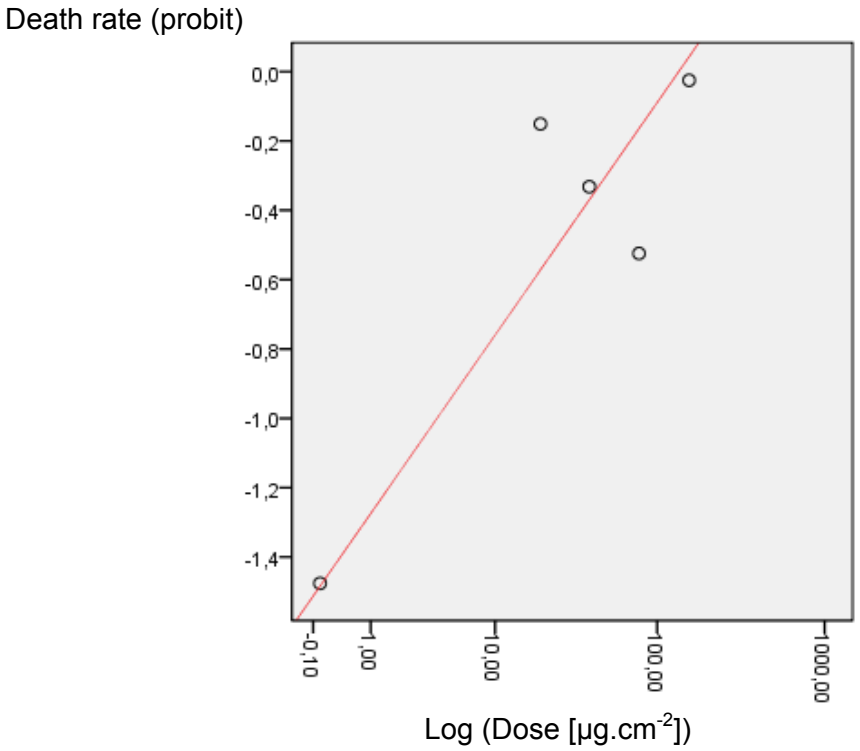

Figure 1. *Variation of the death rate of* ***Anopheles gambiae*** *according to the essential oil amount of* ***Cymbopogon schoanenthus*** *applied*

Bassole *et al.* in 2003 had already shown biological properties of essential oils extracted from dry leaves collected in Burkina-Faso on *Aedes aegypti* and *Anopheles gambiae* mosquitoes [24]. They reported that the biological activity of these essential oils could

be allotted to their chemical composition out of thymol, para-cymene and acetate of thymyle. Tiwary *et al.,* in 2007 had besides shown that the biological activity of the essential oil extracted the sheets of *Zanthoxylum armatum* on three species of mosquitoes would be allotted to the synergistic or antagonistic effect of the whole of their chemical compound [25]. This fact could thus explain the differences in reactivity noted on the level of various essential oils of our studies.
Regard the results previously described, the essential oil of *Cymbopogon citratus* was then selected for the tests on resistant *Anopheles gambiae* taken to Ladji. The results of the tests are consigned in Table 5.

Table 5. *Death rate for* ***Anopheles gambiae*** *in the presence of essential oils of* ***Cymbopogon citrates***

	Sensitive *Anopheles gambiae*		**Resistant *Anopheles gambiae***	
	Concentration [µg.cm^{-2}]		**Concentration [µg.cm^{-2}]**	
	00	22.6	00	22.6
Death rate [%]	00	100	00	04

The results of Table 5 show the weak activity of the diagnostic amount (double of the amount having induced 100% of mortality on sensitive stock of anopheles) of *C. citratus* on *Anopheles gambiae* of Ladji. This amount of essential oil induced only 4% of mortality in this stock of mosquitoes whereas it had caused 100% of mortality in the sensitive population of anopheles. These results are connected with those obtained by Wang *et al.* in 2006 then Djenontin *et al.* in 2009 [26, 27]. They also noted a strong resistance of the stock of Ladji to synthesis insecticides.

CONCLUSION

We conclude that the essential oils extracts from leaves of *Cymbopogon citratus*, *Cymbopogon giganteus* and *Cymbopogon schoanenthus* can be used to control vectors of the malaria. They possess remarkable insecticidal deterrent effects against *Anopheles gambiae* mosquitoes. Because of the prohibitory cost, toxicity and degrading action on the environment of synthesis insecticides, these plants might be used as a source of natural biocides. At the time of this study, we carried out the essential oil extracted to dry leaves collected in Benin. It was observed that their chemical compositions were rich in oxygenated monoterpenes. The insecticidal potential against sensible *Anopheles gambiae* from Kisumu (Kenya), evaluated by protocols inspired of WHOPES methods, were revealed. The essential oil extracted the dry sheets of *Cymbopogon citratus* being revealed most active; we tested the diagnostic amount on resistant *Anopheles gambiae* from Ladji (Cotonou). This amount (2.2 µg·cm^{-2}) induced 100% of mortality on the sensitive stock of anopheles but slightly remains active on the resistant stock.

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