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Chemical composition and Insecticidal activity of essential oils of *Cinnamomum zeylanicum*, *Citrus grandis*, *Citrus medica* and *Citrus sinensis* leaves from Cameroon on *Anopheles gambiae* Giles, 1902

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Abstract
Mosquito resistance to conventional insecticides is a barrier to malaria prevention in endemic countries. This study proposes an alternative method for vector control, based on the use of essential oils from some plants from Cameroon. Essential oils were obtained by hydro-distillation of *Cinnamomum zeylanicum*, *Citrus grandis*, *Citrus medica* and *Citrus sinensis* leaves, using a Clevenger type apparatus. The determination of the chemical composition was made by Gas Chromatography (GC) and coupling of Gas Chromatography with Mass Spectrometry (GC/MS). Biological tests were performed on stage IV larvae and pupae of *Anopheles gambiae* s.l. according to WHO standard protocol. Essential oils from *Ci. zeylanicum, C. grandis*, *C. sinensis* and *C. medica* leaves are rich in ß-cubebene (78.10%), cis-decahydro-naphthalene (16,09%), Z-ß-ocimene (33.03%) and ß-pinene (20.69%) respectively. At 400 ppm, the oils of Citrus induce larval mortality (≥ 95%) and pupal mortality (≥ 94%) after 10 hours of exposure. At equal concentration, *Ci. zeylanicum* oil induced pupal and larval mortality of 30% and 100% respectively. The LC_{50} and LC_{95} values showed that *Ci. zeylanicum* (Larvae: LC_{50} = 98.95 and LC_{95} = 159.80; Nymphs: LC_{50} = 489.2 and LC_{95} = 1820.78) is a good larvicide and *C. sinensis* (larvae: LC_{50} = 136.98; LC_{95} = 342.54; Nymphs: LC_{50} = 78.41; LC_{95} = 126.11) is a good nymphocide. The essential oils of these plants should be strongly recommended for the development of natural biocides.

Keywords: Essential oils, *Cinnamomum zeylanicum*, *Citrus grandis, Citrus medica*, *Citrus sinensis*, larvicide, nymphocide, *Anopheles gambiae* s.l.

1. Introduction
Malaria is one of the leading health problems in the world [40]. The infection is mainly transmitted to humans by infective bite of a female mosquito of the genus Anopheles. Between 300 and 500 million clinical cases occur each year worldwide, mostly in sub-Saharan Africa where it results in about 1 to 2 million deaths per year, including one child every 30 seconds [41]. Malaria consumes on average 25% of household budgets; it is therefore an obstacle to the development of tropical Africa.

In recent decades, malaria endemic countries have deployed multiple strategies for fighting against the disease. Early diagnosis and prompt management of cases, chemoprophylaxis with sulfadoxine-pyrimethamine in pregnancy and integrated vector control including distribution of mosquito nets, indoor residual spraying and biolarvicides are the main strategies they use. In addition, free care for children under 5 years old and mass distribution of LLIN to households are implemented in Cameroon. These measures have resulted in the decline of mosquito density and malaria prevalence in some ecological facies, but they face some limitations. In fact, adding to the emergence and spread of drug resistant strains of Plasmodium as well as vector resistance to insecticides [9], side effects of insecticides have been recorded in humans, non-target animals and the environment [10,18]. Faced with these multiple challenges, the use of biocides is an interesting alternative. Previous studies have shown that many aromatic plants have interesting insecticidal properties and could thus play a

leading role in the development of biocides [2, 16, 29, 38]. The insecticidal properties of aromatic plants may mostly derive from secondary metabolites (essential oils) whose initial role is to protect the plants from potential pests. Thus, the biocides obtained from essential oil have more intense insecticidal properties [6]. *An. gambiae* s.l. is a mosquito species whose larvae develop preferentially in the small breeding sites that are easy to identify and manage. In this context, vector control should primarily target the mosquito larvae with the use of biolarvicides since due to their highly volatile nature, essential oils have little impact on adult forms. In Taiwan, control strategy using biolarvicides was planned in the fight against malaria vectors [28]. By cons, despite the impressive plant biodiversity in Cameroon, no such larva control strategy has ever been conducted.

With a view to implement larvae control strategy in Cameroon, this study was aimed at collecting, extracting and determining the chemical composition of essential oils from fresh leaves of *Cinnamomum zeylanicum*, *Citrus grandis*, *Citrus medica* and *Citrus sinensis*, as well as assessing their larvicidal and nymphocidal activities on *Anopheles gambiae* s.l., the main malaria vector in Cameroon.

2. Materials and methods

2.1 Collection, identification and breeding of Anopheles larvae

The larvae of *An. gambiae* s.l. were collected in Yassa (3°58'59"N- 9°49'0"E), a suburb located in an industrial area on the eastern exit of the city of Douala. From July to September 2015, the larvae were collected from temporary sunny breeding sites of small size (5 to 20 cm in diameter), devoid of vegetation and with low organic matter dissolved. They were later morphologically identified to species at the insectarium, using specialized taxonomic identification keys [14]. Larval identification being often rough, 500 larvae were randomly selected from those collected in the field and reared to adult stage then identified using the key of Gillies and De Meillon [17]. Anopheles larvae were reared in trays (30 x 20 x 10cm) containing water collected from breeding sites. The larval density was 100 larvae per 100 cl of water. These larvae were fed with Tetrababy fish food, a powder with high contains in proteins and minerals [13]. After 3 days of culture for the larvae to get used to their new living conditions, they were separated according to their development stages and underwent insecticides tests.

2.2 Harvesting of plants and extraction of essential oils

Plants were chosen for their traditional use as repellents in many sub-Saharan villages in Africa. These plants had never received chemical treatment. They were harvested in different forest areas of Cameroon (Obala, Yabassi and Kekem) in May and July 2015, and were identified in the Laboratory of Plant Biology by Pr. Dibong Siegfried. The extraction of essential oils was made by hydro-distillation using a Clevenger type apparatus for 5 h. The essential oils obtained were filtered through anhydrous sodium sulfate to remove residual traces of water, then conditioned in small dark bottles and stored in a fridge at 4° C for further analysis.

2.3. Analysis of the chemical composition of essential oils

2.3.1 CPG analysis

Gas chromatography of essential oils was performed on a Varian CP-3380 Gas Chromatograph equipped with a flame ionization detector (FID) and a non-polar column characterized by a 5% polyméthylsilicone (DB 5) stationary phase. The essential oil to be analyzed was diluted with pentane to 10% (v/v). Then, 0.5 µl was introduced in the column head to be vaporized at the SPLIT type injector. The operating conditions were as follows: temperature of the injector and detector 200 °C; oven temperature ranging from 50 °C to 200 °C with a gradient of 5 °C·min^{-1}; nitrogen was used as carrier gas with a 1ml·min^{-1} flow; a Star chromatography work station (STARWS) software was used for recording. Quantitative analysis was performed by integration of the measurements of surfaces peak and results were given as relative percentages

2.3.2 Coupling of GC and MS

A 5970 type Hewlett-Packard GC/MS coupling apparatus, equipped with a capillary column of fused silica with a bonded DB1 type stationary phase was used. The mass detector was quadrupole type; the ionization energy used was 70eV. The operating conditions were as follows: temperature program ranging from 70 °C to 200 °C with a gradient of 10 °C·min^{-1}; the injection temperature was 220 °C; flow rate of carrier gas (helium) was 0.6ml·min^{-1}; the injection volume was 0.1µL of essential oil diluted to 10% with hexane.

2.3.3 Identification of essential oils components

Identification of components of essential oils was made on the basis of their retention indices and their mass spectra as compared with literature data [1, 22].

2.4 Larvicidal or nymphocidal tests

The tests were designed to evaluate the insecticidal activity of various concentrations of diluted essential oils extracted vis-à-vis the mature stage larvae (stage 4) and pupae of the *An. gambiae* s. l. The methodology was inspired by the WHO protocol [39]. The mature larvae and pupae were divided in batches of 25, in bowls of 5 cm in diameter, each containing 99.9 mL of spring water. Preliminary experiments were used to select a range of concentrations for the actual tests. For this purpose, stock solutions of essential oils of each sample were prepared by diluting 10000µl of essential oil in 10 000 µl of 70% ethanol, resulting in ½ diluted essential oil solutions. With these, several other dilutions were made from the stock solution and varying amounts of 70% ethanol solution. The following concentrations were obtained: 400 ppm, 200 ppm, 100 ppm and 50 ppm. 0.1 ml of solution of each of the concentration was then introduced in a bowl containing 99.9 ml of spring water and larvae or nymphs. Four replicates were performed for each of the concentrations. The negative control contained 99.9 ml of water source and 0.1ml of 70% ethanol in addition to the biological material. Counting of dead larvae and nymphs was done every 30 minutes for 10 hours. Larvae and pupae were considered dead when they did not respond to various stimuli.

2.5 Data analyses

Analyses were performed using the Staview version 5.0 package (SAS Institute, Inc, USA). The Kruskal Wallis H test was used to evaluate the effect of time and concentration of essential oils on the mortality of nymphs and larvae .The significance level was set at a probability value lower than 0.05.

3. Results and discussion

Essential oils from fresh leaves of *Cinnamomum zeylanicum*, *Citrus grandis*, *Citrus sinensis* and *Citrus medica* were obtained by hydro-distillation using a Clevenger type

apparatus. Their colors ranged from yellow to light yellow. Fresh leaves of *Citrus medica* have an essential oil content (0.30%) about two folds higher than those of *Cinnamomum zeylanicum* (0.15%) and *Citrus sinensis* (0.13%). The leaves of *Citrus grandis* (0.07%) had the lowest recorded content essential oil (table 1). These yields are not consistent with those obtained with leaves of plants of the same species harvested in Yaounde by Jazet [20]. Moreover, they are lower than those obtained with the pericarp of fruits of the same species, harvested in Edea (Cameroon) [3]. The yield variability observed here could be related to the operated organ, period of harvest, soil and climatic factors, pathophysiological state of the plant and distillation time [20, 36, 37].

Analysis of the chemical composition of essential oils of *Cinnamomum zeylanicum* showed a high contain of sesquiterpene hydrocarbon molecules (78.53%), mostly ß-cubebene (78.10%). Botrydiol (0.11%) was the minority compound. These results differ from those obtained with samples from India and Madagascar [33, 34]. *Cinnamomum zeylanicum* from India showed high content of eugenol while coumarin and cineole were minority compounds. That from Madagascar had cinnamaldehyde as majority compound and eugenol as minority compound. From the above, essential oils of *Cinnamomum zeylanicum* have strong chemical composition heterogeneity depending on the geographic location of harvesting sites.

Analysis of the chemical composition of essential oils leaves of *Citrus grandis*, *Citrus sinensis* and *Citrus medica* showed a high content of oxygenated monoterpenes. Their major compounds were cis-decahydro-naphthalene (16.09%), ß-pinene (20.69%) and Z-ß-ocimene (33.03%) respectively (table 2). These results differ from those found by Jazet *et al*. [21]. These authors carried out studies on the chemical composition of *Citrus sinensis* harvested in Yaoundé (Cameroon); they found sabinene (35.4%) and Δ^3 carene (10.2%) to be the major compounds. Very little work has been done on the chemical composition of the leaves of these species of citrus. Most of available literature works concern pericarps of fruits and they indicate that limonene is the major component not only in essential oils form pericarps of *C. grandis*, *C. sinensis* and *C. medica*, but also of *C. aurantifolia* and *C. limon* [5, 12, 23, 25]. There is therefore appears a difference between major compounds of essential oils of the leaves and those of pericarps of Citrus species. Furthermore, there are qualitative and quantitative differences in the chemical composition of the leaves of the studied plant species. This observation indirectly suggests differences in the biological activity of essential oils from these plant species.

Laboratory tests have shown that essential oils of *Ci. zeylanicum*, *C. grandis*, *C. sinensis* and *C. medica* possess remarkable larvicidal and nymphocidal properties vis-à-vis *Anopheles gambiae* s.l., with various toxicity levels (Tables 3 and 4). These toxic potential results from the fact that each essential oil is composed of molecules with varying degrees of proven insecticidal effect. However, the values of LC_{50} and LC_{95} resulting from larvicide tests showed that the essential oil of *Ci. zeylanicum* is the most toxic, followed by those of *C. grandis, C. medica* and *C. sinensis* (Table 5). The high toxicity of essential oil of *Ci. zeylanicum* on *Anopheles gambiae* s.l. larvae could be due to the presence of compounds such as cinnamaldehyde, cinnamate and beta-cubebene. On evaluating the insecticidal effect of essential oil of the leaves of *Ci. zeylanicum* on *Anopheles tessellatus*, *Culex quinquefasciatus* and *Aedes aegypti*, Radhika *et al*. [32], showed that its toxicity is due to the toxic power of cinnamaldehyde. Moreover, other authors have demonstrated the insecticidal properties of the pure form of this molecule [19, 31]. The low content of the essential oil of *Ci. zeylanicum* in cinnamaldehyde suggests that the toxicity of this molecule is enhanced by those of cinnamate and beta-cubebene. Beta-cubebene, the major compound in our essential oil has excellent insecticidal properties. This insecticide potential was demonstrated on larvae of *Aedes albopictus* [26]. The repulsive property shown by cinnamate molecule could also be a factor boosting the toxic potential *Ci. zeylanicum* oil. The repellent property of cinnamate was demonstrated on *Ceratitis capitata* pests [27].

The high contain of essential oils of *Citrus* leaves in oxygenated monoterpenes observed in our results might explain the toxicity of these oils on *An. gambiae* s.l. larvae. Several studies have previously shown the toxic nature of this group of compounds on certain stages of mosquito development [4, 8, 15]. In some citrus species, these compounds would act in synergy with other so-called minor compounds to increase the potential toxicity of the essential oil on mosquito larvae [24, 30]. For example, alpha-pinene, a minor compound contained in essential oils form Citrus leaves has been showed by some authors as having remarkable toxic properties. So did Cheng *et al*. [11], who attributed the effectiveness of the essential oil of *Citrus arizonica* on the larvae of *An. stephensi*, to the synergistic action of limonene and α-pinene.

Beyond the toxic potentialities of essential oils of Citrus leaves and *Ci. zeylanicum*, it should be noted the ability of molecules to diffuse through the integument of larvae and nymphs of *An. gambiae* s.l. Table 5 shows a difference in the toxicity of essential oils vis-à-vis the 2 development stages of *An. gambiae* s.l. For example, *Ci. zeylanicum* which presents the highest larvicidal activity however has the lowest nymphocide activity (tables 3,4,5). This result shows that, although active against the larvae, the essence of *Ci. zeylanicum* would have difficulties crossing the integumentary barrier of *An. gambiae* nymphs, for full effect. Furthermore, the Kruskal Wallis H test showed significant higher sensitivity of larvae to essential oils, compared with respect to the *An. gambiae* s.l. nymphs (table 6). This may be due to the mechanism of action of essential oils on these development stages. In the larvae, the oils have two ways of penetration; oral and integumentary ways. By cons, as pupae do not feed, the only available way for this stage is integumentary. This analysis is consistent with those of Seye *et al*. [35] and Boyom *et al* [7].

This research showed that the essential oil of *Cinnamomum zeylanicum* is rich in sesquiterpene hydrocarbon compounds mostly ß-cubebene while essential oils of *Citrus grandis*, *Citrus sinensis* and *Citrus medica* are rich in cis-naphthalene-Decahydro, ß-pinene and Z-ß-ocimene respectively. Together with minority compounds, these molecules possess interesting larvicidal and nymphocidal properties vis-à-vis *Anopheles gambiae* s.l. Developing biocides from these molecules would be contributive for a lasting solution to the many problems posed by the use of synthetic insecticides.

Table 1: Details on essential oils

Plant				Harvest		Characteristics of essential oils		
Family	**Species**	**Organ**	**Mass (g)**	**Site**	**Date**	**Colour**	**Mass (g)**	**Yield (%)**
Lauraceae	*Ci. zeylanicum*	Leaves	3000	Kekem	11/05/15	Yellow	4.43	0.15
Rutaceae	*C. grandis*	Leaves	3600	Obala	17/05/15	Light-yellow	2.51	0.07
Rutaceae	*C. medica*	Leaves	3050	Yabassi	17/05/15	Light-yellow	8.99	0.30
Rutaceae	*C. sinensis*	Leaves	3400	Obala	19/07/15	Light-yellow	4.48	0.13

Table 2: Chemical composition of essential oils.

N°	KI*	Compounds	*Ci. zeylanicum (%)*	*C. grandis (%)*	*C. sinensis (%)*	*C. medica (%)*
Monoterpenes			7.58	50.23	86.66	92.61
Monoterpene Hydrocarbons			2.62	3.91	39.5	37.2
1	935	3-Methyl cyclohexanol	-	-	1.21	-
2	936	α-Pinene	0.36	0.91	0.11	0.43
3	952	Camphene	0.15	-	-	-
4	**977**	**ß-Pinene**	**-**	**-**	**20.69**	**-**
5	981	Myrcene	0.18	-	-	-
6	987	Furfuryl d'acetate	-	-	-	3.49
7	1007	δ-3-Carene	0.27	-	-	-
8	1014	α-Terpinene	-	-	7.45	0.25
9	1031	Limonene	1.21	3	5.02	-
10	1034	Z-ß-Ocimene	0.45	-	-	33.03
11	1046	E-ß-Ocimene	-	-	5.02	-
Oxygenated Monoterpenes			4.96	46.32	47.16	55.41
12	975	Octen-3-ol	-	7.71	-	-
13	1090	Linalol	0.31	-	-	-
14	1104	α-Fenchocamphorone	-	-	18.62	2.76
15	1124	α-Campholene aldehyde	-	-	0.99	-
16	1125	Norboneol acetate	-	1.55	-	-
17	1135	Citronellal	-	-	-	0.22
18	1141	Camphor	-	0.65	-	-
19	1153	Pinocarvone	-	-	-	1.76
20	1180	Iso-geranial	0.14	10.04	-	1.23
21	1184	α-Terpineol	-	-	12.1	-
22	1192	Myrtenol	-	1.5	-	-
23	1193	Myrtenal	0.55	-	-	-
24	1197	δ-Terpineol	-	-	0.13	-
25	1204	Verbenone	-	0.29	-	-
26	1224	Nerol	-	7	-	-
27	1227	Citronellol	-	-	3.52	-
28	1233	Acetate de bornyle	-	-	-	4.49
29	1241	Genariol	-	-	1.37	-
30	1249	Geraniol	-	-	-	13.36
31	1252	Geranial	-	-	3.02	-
32	1262	Acetate de sabinyle	-	-	-	5.23
33	1270	Nonenal	-	2.12	2.77	-
34	1272	Linalol acetate	0.76	-	-	-
35	1281	Neryl formate	-	-	-	20.52
36	1286	Hydroxy citronellal	1.25	-	-	-
37	1287	3-Octenol propanoate	-	0.72	-	-
38	1288	Thymol	-	-	0.65	-
39	1306	Carvacrol	-	0.69	-	-
40	1307	Nonenol acetate	-	-	0.3	-
41	1320	3-Methylpentenoate	-	-	0.48	-
42	1358	Nonalactone	-	2.57	-	-
43	1384	lsobornyl propanoate	-	-	-	5.01
44	1397	Z-Trimenal	-	-	2.96	-
45	1428	Dictamnol	-	2.91	-	-
46	1434	Neryl acetone	1.11	-	-	-
47	1466	Ethyl Cinnamate	0.22	-	-	-
48	1483	Geranyl propanoate	-	0.53	-	-
49	1546	ß-Silphiperfolan-6-ol	-	-	-	0.2
50	1596	Fokienol	-	1.35	0.25	-
51	1598	Juniperol	-	-	-	0.63
52	1599	Hydro-cinnamaldehyde	0.62	-	-	-
53	1665	Lyral	-	2.93	-	-
54	1679	Elemol-acetate	-	0.6	-	-
55	1716	E-Nerolidyl-acetate	-	0.96	-	-
56	1731	Zerumbone	-	1.21	-	-
57	1894	Catalponone	-	0.99	-	-
Sesquiterpenes			78.64	18.32	8.07	4.83
Sesquiterpene Hydrocarbons			78.53	13.77	5.47	4.83

58	961	Verbenene	0.43	-	-	-
59	1363	α-Cubebene	-	-	-	2.04
60	1379	α-Copaene	-	-	0.85	-
61	1387	ß-Cubebene	78.10	-	-	-
62	1396	ß-Elemene	-	3.62	-	-
63	1398	Cyperene	-	-	-	0.45
64	1429	ß-Copaene	-	-	1.82	-
65	1432	Coumarin	-	-	-	2.34
66	1462	(Z)-ß-Farnesene	-	1.03	-	-
67	1463	α-Acoradiene	-	-	0.55	-
68	1529	δ-Cadinene	-	-	0.76	-
69	1622	α-Corocalene	-	0.66	0.14	-
70	1650	Isoamyl geranate	-	-	0.4	-
71	1697	Acorenone B	-	8.46	0.95	-
72	1794	Bergamotol-acetate	-	-	-	-
73	1949	Pimaradiene	-	-	-	-
Oxygenated Sesquiterpenes			0.11	4.55	2.6	0.00
74	1360	Eugenol	-	-	1.62	-
75	1577	Spathulenol	-	-	0.21	-
76	1638	α-Cadinol	-	-		-
77	1663	α-Eudesmol	-	-	0.57	-
78	1666	ß-Bisabolol	-	-	-	-
79	1689	Botrydiol	0.11	-	-	-
80	1769	ß-Bisabolenal	-	3.02	0.2	-
81	1789	ß-Bisabolenol	-	1.53	-	-
Aromatic Compounds			13.04	17.44	2.71	0.00
82	1099	Cis-decahydro-naphthalene	-	16.09	-	-
83	1106	Ethylphenyl Alcool	13.04	-	-	-
84	1151	Pentyl-benzene	-	1.35	2.46	-
85	1752	Cinnamyl-acetate	-	-	0.25	-
86	1818	Trihydroxy-benzaldehyde	-	-	-	-
Linear compounds			0.14	5.24	1.51	0.37
87	1068	Meta-tolualdehyde	-	2.81	1.51	-
88	1074	ρ-tolualdehyde	0.14	-	-	-
89	1201	Decanal	-	-	-	0.01
90	1464	Dodecenal	-	-	-	0.36
91	1930	Dictamine	-	0.69	-	-
92	1936	Undetermined	-	1.74	-	-
Total (%)			99.4	91.23	98.95	97.81

KI: Kovats Index **%**: Percentage-: Absent compounds

Table 3: Mortality rate (%) of stage IV larvae after 10h of exposition to various concentrations of essential oils (95% CI)

	Essential oils	**Concentrations* (95% CI)**				
		400	**200**	**100**	**50**	**Control**
Mortality rates (%) of stage IV *An. gambiae* s.l. larvae	*Ci. zeylanicum*	100 (100-100)	100 (100-100)	70 (22.9-113)	0 (0-0)	0 (0-0)
	C. grandis	97 (90.9-103.1)	91 (72.7- 09.3)	69 (59.4-78.5)	5 (1.2-9.2)	0 (0-0)
	C. medica	100 (100-100)	87 (61.9- 12.1)	12 (8.9-20.4)	2 (1.7-5.7)	0 (0-0)
	C. sinensis	95 (85.4-104.5)	87 (61.9-12.1)	25 (11.9-38.1)	3 (0.2-6.2)	0 (0-0)

* Unit in ppm

Table 4: Mortality rate (%) of pupae after 10h of exposition to various concentrations of essential oils (95% CI)

	Essential oils	**Concentrations (ppm)**				
		400	**200**	**100**	**50**	**Control**
Mortality rates (%) of *An. gambiae* s.l. pupae	*Ci. zeylanicum*	30 (17,8-42,2)	20 (20-20)	4 (-8,7-16,7)	0 (0-0)	0 (0-0)
	C. grandis	100 (100-100)	40 (32,6-47,3)	24 (9,3-38,7)	0(0-0)	0 (0-0)
	C. medica	94 (82,9-105,0)	81 (74,9-87,1)	58 (31,7-84,2)	22 (18,3-25,7)	0 (0-0)
	C. sinensis	100 (100-100)	100 (100-100)	54 (34,9-73,1)	0 (0-0)	0 (0-0)

Table 5: LC_{50} and LC_{95} of essential oils obtained from 10h of exposition of *Anopheles gambiae* s.l. stage IV larvae and pupae

Essential oils	**Stage IV larvae**		**Pupae**	
	LC_{50} (ppm)	**LC_{95} (ppm)**	**LC_{50} (ppm)**	**LC_{95} (ppm)**
Ci. zeylanicum	98.95	159.80	489.20	1820.78
C. grandis	103.08	218.59	145.18	408.64
C. medica	123.96	278.44	92.76	249.70
C. sinensis	136.98	342.54	78.41	126.11

Table 6: Comparison of the sensitivity of *Anopheles gambiae* s.l. stage IV larvae and pupae exposed to various concentrations of essential oils for 10h (Kruskal Wallis H-test, $p<0.05$).

Essential oils	Concentration (ppm)	Stage		H	P
		Mature larvae	Pupae		
Ci. zeylanicum	400	25±0*	7.5±1.9	6.1	0.01
	200	25±0	5±0	7	0.008
	100	17±7.1	4±8	5.7	0.02
	50	0±0	0±0	-	-
C. grandis	400	24.25±1	25±0	2.29	0.13
	200	22.75±2.9	10±1.2	5.53	0.02
	100	17.25±1.5	6±2.3	5.74	0.02
	50	1±0.8	0±0	4	0.05
C. medica	400	25±0*	23±1.7	-	-
	200	21.75±3.9	20.25±1	0.19	0.65
	100	3±1.4	14.5±4.1	5.46	0.02
	50	0.5±0.6	5.5±0.6	5.6	0.02
C. sinensis	400	23.75±1.5	25±0	2.28	0.13
	200	21.75±1.9	25±0	6.1	0.01
	100	6.25±2.1	13.5±3	5.46	0.02
	50	0.75±0.5	0±0	4.2	0.04

(*) = X± Standard Deviation X: Mean obtained

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