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Chemical composition and residue activities of *Ocimum canum* Sims and *Ocimum basilicum* L essential oils on adult female *Anopheles funestus* ss

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1 SUMMARY

Essential oils obtained by hydrodistillation of the leaves of *Ocimum canum* (Camphor basil) and *Ocimum basilicum* (Sweet basil) were analyzed and their residual activity on adult *Anopheles funestus* ss determined. The extraction yields showed that the leaves of *Ocimum canum* are four times richer in essential oil (0.44%) than those of *Ocimum basilicum* (0.11%). Analysis by (GC) and (GC / MS) revealed that these oils are monoterpenic (83.4 to 92.4%). The oxygenated monoterpenes are predominant in the essential oil of *Ocimum canum* (63, 3%), while the monoterpene hydrocarbons are mainly in essential oil of *Ocimum basilicum* (56.2%). The major components identified in essential oil of *O. canum* are linalool (53.8%) and limonene (22.2%). The essential oil of *O. basilicum* is distinguished by the predominance of compounds such as linalool (18.9%), limonene (30.9%) and β-phellandrene (15.3%). Bioassay tests done by the World Health Organization standard protocol revealed that these essential oils have remarkable adulticidal properties on *An. funestus* ss. The essential oil of *O. canum* (LC50 = 45.61 ppm, one hour after exposure piece of nets to product) is the most efficient, followed by the essential oil of *O. basilicum* (LC50 = 84ppm). Besides, these studies have shown that the effectiveness of these species decreases significantly with time (LC50 for *O. canum* = 45.61, 62.53, 311.23 respectively one hour, five days and 10 days after exposure piece of nets to the product. LC50 for *O. basilicum* = 84; 171.7, 397respectively one hour, 5 days and 10 days after exposure piece of nets to the product). This result explains the low residue activity of these species on adult females of *An. funestus*. This low residue would be the fact of the highly volatile molecules of these species, and allows guiding insecticidal use of these essential oils in the form of aerosol bombs.

2 INTRODUCTION:

The use of plants in the fight against insects has been long known (Crosby *et al.*, 1966). The pyretre, nicotine, rotenone and tobacco have long been used as control agents against insects in some regions of sub-Saharan Africa (Brahim *et al.*, 2006). This control practice today has renewed interest with many problems posed by the use of synthetic insecticides in vector control campaigns. Indeed, besides their high cost, synthetic insecticides have a broad spectrum of action and generally act on non-target organisms (Barbouche *et al.*, 2001). They also cause some resistance in treated insects (Georghiou *et al.*, 1975; Sinegre *et al.*, 1977). Insect resistance to insecticides commonly used would be one of the causes of the upsurge of diseases transmitted by insects such as yellow fever, trypanosomiasis and malaria in tropical Africa. In the specific case of malaria, several studies in Cameroon have demonstrated the phenomenon of vector resistance to pyrethroids (Biboga *et al.*, 2007; Etang *et al.*, 2007, 2006 and 2003; Chouaibou *et al.*, 2006). This makes the epidemiological situation of malaria in this country worrying despite significant curative and preventive measures taken by the authorities to control the disease. Thus, to overcome the problems posed by these chemical formulations, the establishment of new alternatives to control mosquitoes is further encouraged. Several studies in this sense carried out in Cameroon have underlined the role played by the essential oils of plants from the African pharmacopoeia in the fight against mosquitoes. Recently, the insecticidal properties of essential oils of *Capsicum annuum* (Pipper), *Zingiber officinale* (Common ginger) and *Piper nigrum* (Black pepper) have been demonstrated in all stages of *Anopheles gambiae* sl (Foko *et al.*, 2011). Before, Njan Lôga *et al.* (2007) have demonstrated the effectiveness of essential oils from six local plants on *Anopheles gambiae* adult. Akono *et al.* (2012) have then tested the effectiveness of *Ocimum basilicum* and *O. canum* essential oils on female adult of *Anopheles funestus* ss. However, for more successful control operations from essential oils, further work aiming to assess the duration of residue of volatile essences should be conducted. Such work which unfortunately are not common in the literature, help to inform the user of essential oils for better efficiency of the latter during the vector control operations.

This work aims to determine the chemical composition and to assess the duration of residue of essential oils of plants of *Ocimum canum* Sims and *Ocimum basilicum* L on adult female *Anopheles funestus* ss.

3 MATERIAL AND METHODS

3.1 Plant harvesting, extraction and chemical analysis of essential oils: The plant material was collected in June 2009 in an experimental field of the city of Douala (Cameroon). The identification of collected specimens was made at the National Herbarium in Yaoundé (Cameroon) under the following numbers: *Ocimum basilicum* (6899SRFcam) *Ocimum canum* (13497SRFcam) *Capsicum annuum* (43073/HNC). Fresh leaves of Ocimum plants have previously been washed with water source, cut then subjected to hydrodistillation using a Clevenger type apparatus during 5 h (Table 1). The essential oil gathered by decantation at the end of the distillation was filtered on a column of anhydrous sodium sulfate and then introduced into dark glass bottles tightly closed and stored in a refrigerator at about 4 ° C. Gas chromatography and gas chromatography – mass spectrometry (GC and GC-MS) analyses were carried out using an Agilent 6890N gas chromatograph apparatus equipped with a flame ionization detector (FID) and coupled to a quadrupole Agilent 5973 network mass selective detector working in electron impact mode at 70 eV (scanning over 35-350 amu range). The gas chromatograph was equipped with two fused silica capillary columns HP-1 (PDMS, 50 m × 0.2 mm i.d., film thickness: 0.33 µm). The analytical parameters (identical for GC and GC-MS analyses unless specified) were the following: The carrier gas was helium at a flow rate of 1 mL/min. The oven temperature was programmed from 60 to 250 °C at 2 °C/min and held isothermal for 40 min. The injector (split mode, ratio 1/100) temperature was 250 °C. The FID temperature was set at 250 °C, and in the GC-MS analyses, the temperatures of the ion source and transfer line were 170 and 280 °C, respectively. To remove some coelutions, another series of analysis were performed with the same analytical parameters as above, but with a HP-5 capillary column. In both cases, Retention indices (RI) were determined from the retention times of a series of n-alkanes with linear interpolation. The constituents of the essential oil were identified by comparison of their mass spectral pattern and retention indices (RI) with those of pure compounds registered in commercial libraries and literature data and with a laboratory-made database built from authentic compounds. Quantitative data were obtained by internal standardization using nonane as internal standard. For a given compound, its relative response factor (RRF) was predicted according to its chemical class (Costa *et al.*, 2008).

Table 1: Data on the extraction of essential oils from plants studied

Plant				Harvesting		Essential oils		Extraction yield (%)
Family	Specie	Organ	Mass (g)	Place	Date	Color	Mass (g)	
Lamiaceae	*Ocimum canum* Sims	leaf	16350	Douala	8/06/09	Light yellow	71,77	0,44
Lamiaceae	*Ocimum basilicum* L	leaf	16350	Douala	14/06/09	Light-yellow	18,99	0,11

3.2 Rearing of larvae of *An. funestus* ss: Strain of *An. funestus* ss used in these tests was those domesticated at the insectary of the Laboratory of Zoology, University of Yaoundé I (Cameroon). The larvae were reared in plastic boxes (20x10x10 cm). Larval density was 100 larvae for 100cl water source. The food used was a tetra baby fish food according to Desfontaine (1991). Pupae obtained were introduced in cubic cages (30x30x30 cm) wrapped in a mosquito net each, while awaiting a possible emergency. Adults obtained were fed with a 10% sucrose solution. The average temperature inside the insectary, kept constant by continuously operating radiator, was 28.2 ° C ± 0.9 ° C with a relative humidity of 80%.

3.3 Adulticidal tests and determination of the duration of residue **of essential oils:** Adulticidal tests took place in November 2009 at the Insectarium Nkolbisson (Yaoundé) according to the standard protocol of the World Health Organization (1985). From the crude essential oil of each sample of plant and varying amounts of acetone (organic solvent), following test concentrations were obtained: 250 ppm, 200 ppm, 150 ppm, 100 ppm, 50 ppm. Pieces of mosquito net were cut to the size of 0.03 m^2 one. Twelve (12) pieces were used in this experiment due to 6 fragments corresponding to the 5 essential oil concentrations and the control piece. Each piece of net was introduced in the diluted solution, then after 15 minutes of impregnation, it was removed and spread out on a bench. Drying lasted 45 minutes. The dry piece of mosquito net was finally set on the basis of an OMS- cone with adhesive tape. The whole was mounted in a wooden device which the inclination angle with the normal is about 45 °. Twenty adult female *Anopheles funestus* ss were removed from the cages and then introduced into each cone whose upper openings were immediately clogged with cotton wool to prevent the release of mosquitoes. Three minutes after contact with the piece of net, female mosquitoes was introduced using a mouth aspirator in plastic cups covered piece of mosquito not impregnated. These mosquitoes were fed a 10% sucrose solution. Mortality of adult mosquitoes was observed after 24 hours. For each concentration of essential oil, the tests were repeated 7 times to minimize errors and have an effective statistically acceptable. The base of the witness cone was covered with a mosquito net impregnated fragment only with acetone. At the end of the experiment, the impregnated pieces of mosquito nets were kept each in a dry Petri dish and kept open in order to evaluate their length residue effect on the adult female *Anopheles funestus* ss. Residue effect tests were held for 15 days to the rhythm of one test every 5 days according to the protocol described above. The four tests were held 1 hour, 5 days, 10 days and 15 days after impregnation of pieces of mosquito net to different concentrations of diluted essential oils. Each test was also repeated 7 times.

4 RESULTS

4.1 Essential oils yields: The hydrodistillation of *O. canum* and *O. basilicum* leaves, of equal masses (16 350g) shows that the leaves of *O. canum* are four times richer in essential oil than those of *O. basilicum.* These essential oils are all light yellow color (Table 1).

4.2 Chemical analysis of essential oils: Chemical analysis shows that the sample of essential oil from the leaves of *O. canum* is composed of 15 molecules divided into class monoterpene hydrocarbons (9 molecules) and that of sesquiterpene

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hydrocarbons (6 molecules). This volatile oil is mainly monoterpene (92.4%), with a preponderance of oxygenated compounds (63.3%) dominated by linalool (53.8%) (Table 2). The essential oil from the leaves of *O. basilicum* includes 23 molecules divided into class monoterpene hydrocarbons (14 molecules) and that of sesquiterpene hydrocarbons (9 molecules). This essential oil is dominated by monoterpenes (83.4%); with a proportion of hydrocarbon monoterpenes (56.2%) far exceeds that of oxygenated monoterpenes (27.2%). The main constituents are limonene (30.9%) and β-phellandrene (15.3%) for the hydrocarbon fraction, linalool (18.9%) and thymol (6.5%) for the oxygenated fraction (Table 3).

Table 2: Chemical composition of the essential oil from the leaves of *Ocimum canum.*

Monoterpenes		Sesquiterpenes	
Hydrocarbon (29,1%)	**Oxygeneated (63.3%)**	**Hydrocarbon (4,6%)**	**Oxygenated (2,4%)**
α-Thujène (1,1%)			
α-Pinène (0,7%)		α-Copaène (0,1%)	
Camphène (0,1%)		β-Eléméne (1,9%)	
β-pinène (0,1%)		β-Caryophyllène (1,8%)	
Limonène (22,2%)	Eugénol (9,5%)	Germacrène D (0,7%)	α-Cardinol (2,4%)
γ-Terpinéne (1,3%)	Linalol (53,8%)	δ-Cadinène (0,1%)	
Terpinolène (3,6%)			
Total	**92,4%**	**7%**	

Table 3: Chemical composition of essential oil from leaves of *Ocimum basilicum.*

Monoterpenes		Sesquiterpenes	
Hydrocarbon (56,2%)	**Oxygenated (27.2%)**	**Hydrocarbon (12,4%)**	**Oxygenated (2,6%)**
α-Thujène (0,9%)			
α-Pinène (0,3%)			
Camphène (2,1%)		α-Copaène (3,9%)	
Sabinène (0,3%)		β-Eléméne (1,8%)	
β-Pinène (0,8%)	Linalol (18,9%)	β-Caryophyllène (0,4%)	α-Cardinol (2,6%)
p-Cymène (2,6%)	Thymol (6,5%)	Germacrène D (1,1%)	
β-Phéllandrène (15,3%)	Carvacrol (1,8%)	β-Bisabolène (0,3%)	
Limonène (30,9%)		(E, E)-α-Farnésène (2,1%)	
(Z)-β-Ocimène (2,1%)		δ-Cadinène (1,0%)	
(E)-β-Ocimène (0,6%)		α-Cadinène (1,8%)	
Terpinolène (0,3%)			
Total	**83,4%**	**15%**	

4.3 Residue activities of essential oils on *Anopheles funestus* adults: Tables 4 and 5 show that the average number mortality of adult female of An. funestus vary with the concentration and the elapsed time after exposure of piece of mosquito net in diluted solutions of essential oils. Indeed, the Kruskal Wallis H test performed with SPSS 19.0 software shows that the average number of mortality decline significantly with elapsed time after exposure of mosquitoes to essential oil (p <0.0001 for *O. basilicum* and p <0.0001 for *O. canum*) and increased significantly with concentration (p <0.0001 for *O. basilicum* and p <0.0001 for *O. canum*). The exploitation of the regression lines (Figures 1 and 2) obtained from the simplified table of Henry that transforms mortality percentages of female mosquitoes in probit has allowed to identify lethal concentrations (LC50) of diluted solutions of essential oils capable of inducing 50% mortality of female mosquitoes. Table VII shows that for the same essential oil, LC50 increase with elapsed time after exposure pieces of mosquito net to the product. This result shows a remarkable loss of efficiency of pieces of mosquito net after 15 days and translates to the low residue of essential oils studied.

Table 4 : Mortality (24h) of adult female of *Anopheles funestus* depending on the duration of residue of pieces of mosquito net impregnated with diluted solutions of *O. canum* essential oil (Kruskal Wallis H test, P <0.05)

	Tests					
Concentrations (ppm)	**Day 1**	**Day 5**	**Day 10**	**Day 15**	**H**	**P**
250	20±0	20±0	11±0.81	2±0.81	26.153	<0.0001
200	20±0	16±0.81	7.85±0.89	1±0.81	25.869	<0.0001
150	16.57±0.53	14.14±1.06	6.42±0.53	0.02±0.01	25.356	<0.0001
100	16.28±0.75	13±0.57	5.85±0.89	0.02±0.02	25.618	<0.0001
50	16±1.15	12±1	5±0.57	0.02±0	25.978	<0.0001
0	0±0	0±0	0.14±0.37	0±0	3.000	0.392
H	26.338	30.325	28.917	25.942	-	-
p	<0.0001	<0.0001	<0.000	<0.000	-	-

Average of 7 tests each covering 20 mosquito adults

Table 5 : Mortality (24h) of adult female of *Anopheles funestus* depending on the duration of residue of pieces of mosquito net impregnated with diluted solutions of *O. basilicum* essential oil (Kruskal Wallis H test, P <0.05)

	Tests					
Concentrations (ppm)	Day 1	Day 5	Day 10	Day 15	H	P
250	20±0	13±0.81	8.42±0.97	0±0	24.11	<0.0001
200	17.28±1.38	10±1.15	6.85±0.89	0±0	23.735	<0.0001
150	15.14±1.06	9.42±0.53	5.14±0.69	0±0	24.068	<0.0001
100	8±0.81	6.85±0.89	4.42±0.97	0±0	22.8	<0.0001
50	6.42±0.78	3.14±1.06	2.42±0.53	0±0	21.774	<0.0001
0	0.14±0.37	0±0	0.14±0.37	0±0	2.560	0.465
H	39. 787	39. 438	38.311	-	-	-
P	<0.0001	<0.0001	<0.0001	-	-	-

Average of 7 tests each covering 20 mosquito adults

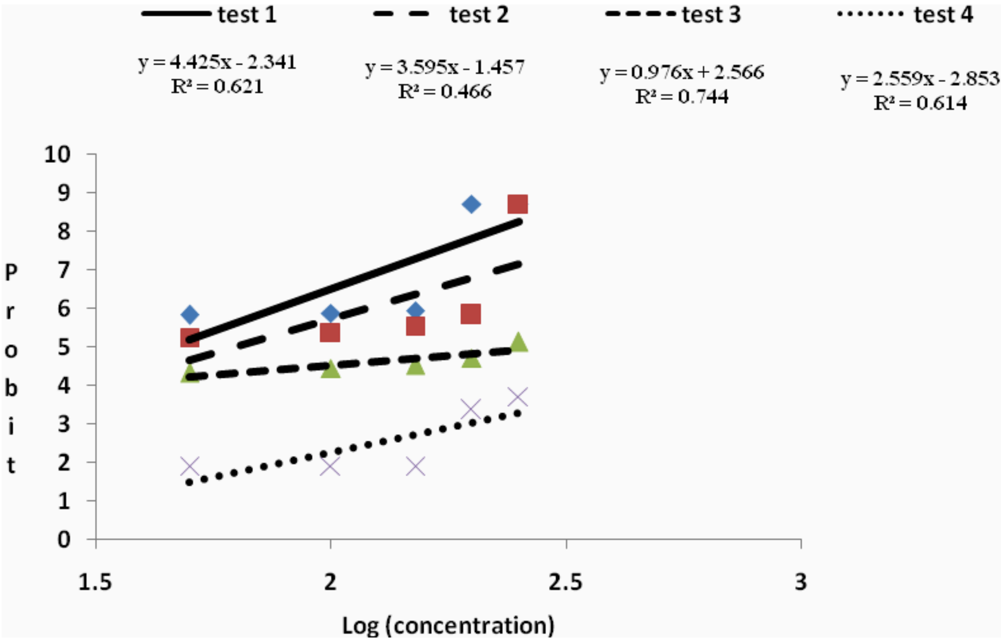

Figure 1: Regression lines of the effects of essential oils from leaves of *Ocimum canum* on *Anopheles funestus* female adult

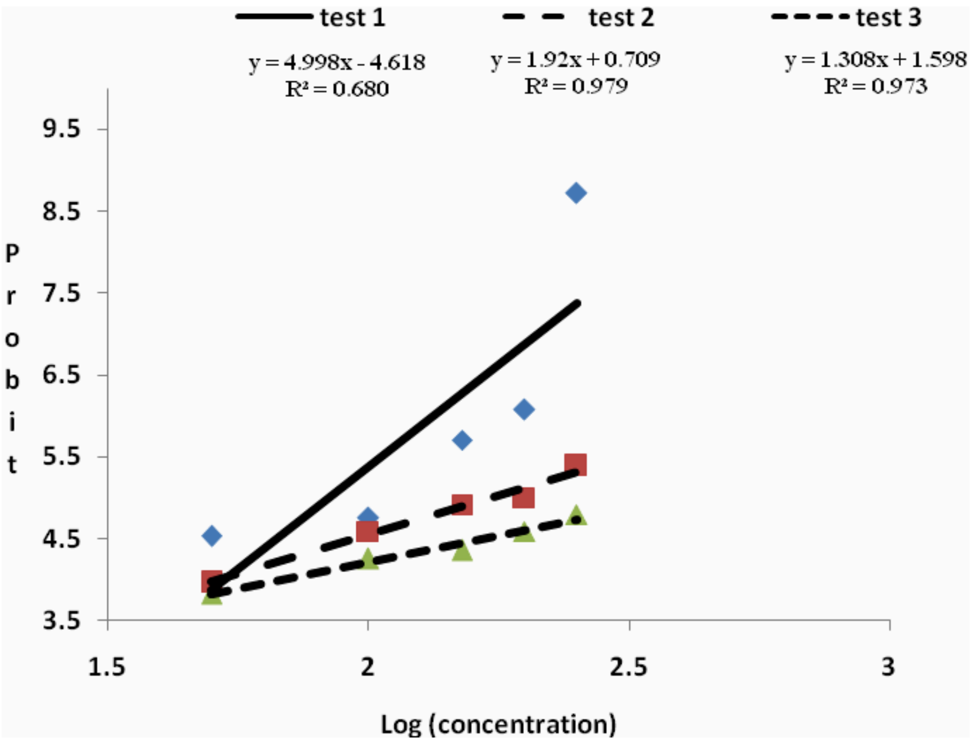

Figure 2: Regression lines of the effects of essential oils from leaves of *Ocimum basilicum* on *Anopheles funestus* female adult

Table 6: Concentrations of essential oils that can induce 50% mortality of mosquitoes LC50 (ppm)

Essential oils	LC50 (ppm)			
	day 1	day 5	day 10	day 15
Ocimum canum	45.61	62.53	311.23	1171
Ocimum basilicum	84	171,7	397	-

DISCUSSION

The results in Table 1 show that the leaves of *Ocimum canum* (0, 44%) have essential oil content 4 times higher than those of *O. basilicum* (0.11%). The extraction yields of the two Ocimum are less than those obtained on the same species and plant organs by Tchoumbougnang *et al.* (2009). This difference is due to the fact that the extraction was done on fresh leaves while our predecessors have done it on dry leaves. This variability in performance may also be related to the harvest period, the soil and climatic factors or the physiopathological state of the plant (Tchoumbougnang *et al.*, 2004; Tchoumbougnang *et al.*, 2005). Chemical analysis of the essential oil of *O. canum* obtained in this work shows that linalool is the major component (53.8%). The volatile oil of the same plant but from Nigeria studied by Ekundayo *et al.* (1989) showed rather high proportion of eugenol (66.4%). The specie of Indian origin studied by Tayal and Dutt (1938) has on the other hand an essential oil rich in citral. These results show some variability of the chemical composition of volatile oils according to geographical origin of plants (Tchoumbougang, 1997). However, the chemical composition of the essential oil of *O. canum* of Cameroonian origin obtained in our work is close to that of Rwandan origin studied by Ntezurubanza *et al.* (1985). These authors obtained an essential oil whose major constituent is linalool (60%). This type of *O. canum* essential oil in which major constituent is linalool was described for the first time by Guenther (1972). The volatile oil of the leaves of *O. basilicum* obtained in our results contains limonene (30.9%) as major compound, followed by linalool (18.9%). It is devoid of eugenol. This sample is thus far from all chemical types previously described in the literature (Nigam *et al.*, 1968; Zola and Garnero, 1972; Akgul, 1989; Mahmout, 1992). Adulticidal tests show a direct relationship between the average number of mortality of *An. funestus* ss adult female, and the time elapsed after exposure of the pieces of mosquito net to the diluted essential oils. Indeed, Tables 4 and 5 show that the insecticidal activity of essential oils from two plants gradually decreases from the first to the 10th day to end at the 15th day. This result reflects the low residue activity of essential oils on *An. funestus* adult. This low residue effect is due to the highly volatile molecules present in the essential oils (Ngamo *et al.,* 2007b). This hypothesis was suggested by Odeyemi *et al.* (2008), in the study of the persistence of the essential oil of *Mentha longifolia* on *S. zemais* (maize weevil). According to these authors, the loss of insecticidal activity of HE *Mentha longifolia* after 6 days on *S. zeamais*, is due to the loss of terpene compounds in the essential oil. However, the duration of residue effect of an essential oil can also be linked to the type of formulation used. According to the work of Pierrard (1986) cited by Keita *et al* (2000), on the activity of the essential oil of dried fruit of *X. aethiopica* on *C. maculatus* (cowpea weevil), the use of the essential oil of *X. aethiopica* alone is not recommended for long-term protection of cowpea seeds. Hence the need to develop a formulation that combines the essential oil to a carrier dilution which would reduce evaporation of the active materials, increase the concentration of oil and extend the effectiveness of the formulation. Moreover, the essential oil of *O. canum* with LC50 always lower than those of *O. basilicum* essential oil (Table 6), is the most efficient. This efficiency is due to the phenolic compounds such as linalool and eugenol identified in this specie. The efficacy of these compounds was demonstrated by Tripathi *et al.* (1985) on fungi such as *Alternaria alternata* and *Colletotrichum capsicii.* Phenolic compounds are also known to have ovicidal, larvicidal, nymphocidal and adulticidal properties against various insect species (Isman, 1999). The effectiveness of *O. canum* can also be the result of its high content in monoterpene oxygenated compounds (63.3%). Park *et al.* (2002), evaluating the effectiveness of the essential oil of *O. gratissimum* on *Callosobruchus chinensis* L. and *Sitophilus oryzae* L. demonstrate that the toxicity of this essential oil is mainly due to its high content of monoterpene oxygenated molecules. Thus the low content of the essential oil of *O. basilicum* in these molecules (27.2%) would be one of the causes of its low toxicity despite the presence in its composition the phenolic compounds such as thymol and carvacrol whose effectiveness has yet been reported by Traboulsi *et al.* (2002) on the larvae of *Culex pipiens.*

CONCLUSION

Ultimately, due to their adulticidal efficiency and their short duration of residue on *An. funestus*, essential oils of leaves of *O. canum* Sims and *O. basilicum* L are proving to be an alternative approach to the use of synthetic insecticides whose cost, the phenomenon of resistance developed in mosquitoes treated and proven action on non-target organisms are far from solving the problem of malaria in Africa. The low residue recorded in our results allows envisaging the insecticidal use of these oils rather in the form of aerosol bomb, but not in the form of impregnated mosquito nets. Thus, we urge African decision makers to promote the use of these plants in the implementation of their national policy of control malaria vectors.

REFERENCES

Akgul A: 1989. Volatile oils composition of sweet basil (*Ocimum basilicum* L) cultivated in turkey. *Nahrung* 33: 87-95.

Akono NP, Belong P, Tchoumbougnang F, Bakwo FEM and Fankem H: 2012. Composition chimique et effets insecticides des huiles essentielles des feuilles fraîches d'*Ocimum canum* Sims et d'*Ocimum basilicum* L. sur les adultes d'*Anopheles funestus* ss, vecteur du paludisme au Cameroun. *Journal of Applied Biosciences* 59: 4340– 4348.

Barbouche N, Hajjem B, Lognay G and Ammar M: 2001. Contribution à l'étude de l'activité biologique d'extraits de feuilles de *Cestrum parqui* L'Hérit. (*Solanaceae*) sur le criquet pèlerin *Schistoceraca gregaria* (Forsk.). *Biotechnol. Agron. Soc. Environ.* 5 : 85–90.

Bigoga J, Manga L, Titanji V, Coetzee M and Leke R: 2007. Malaria vectors and transmission dynamics in coastal south-western (Cameroon). *Malaria Journal* 6: 1- 12.

Brahim A, Saadia O, Fouad M and Saadia M: 2006. Evaluation préliminaire de l'activité larvicide des extraits aqueux des feuilles de Ricin (*Ricinus communis* L.) et du bois de thuya (*Tetraclinis articulata* (Vahl) Mast.) sur les larves de quatre moustiques culicidés : *Culex pipiens* (Linné), *Aedes caspius* (Pallas), *Culiseta longioreolata* (Aitken) et *Anopheles maculipennis* (Meigen). *Biotechnol. Agron. Soc. Environ.*10: 61-71.

Chouaïbou M, Simard F, Chandre F, Etang J, Darriet F and Hougard J: 2006. Efficacy of bifenthrineimpregnated bednets against *Anopheles funestus* and pyrethroids- resistance *Anopheles gambiae* in North Cameroon. *Mal. J.* 5: 1-17.

Costa R, D'Acampora Zellner B, Crupi M L, De Fina M R, Valentino M R, Dugo P, Dugo G and Mondello L: 2008. GC–MS, GC–O and enantio–GC investigation of the essential oil of *Tarchonanthus camphoratus* L. *Flavour and Fragrance Journal* 23: 40-48.

Crosby DG: 1966. Natural pest control agents. *In* Gould, R.F. (Ed.). Natural Pest Control Agents. *Advances in Chemistry*, 53: 1-16.

Desfontaine M, Tchikangwa I., Le Goff G, Robert V and Carnevale P: 1991. Influence de l'alimentation des larves d'*Anopheles gambiae* (Diptera, Culicidae) sur le développement préimaginal en insectarium. *Bulletin de liaison et de documentation de l'O.C.E.A.C.* 98 : 12-14.

Ekundayo O, Laakso I. and Hiltunen R: 1989. Constituents of the volatile oil from leaves of *Ocimum canum* Sims, *Flavour Fragrance J.* 4: 17-18.

Etang J, Manga L, Chandre F, Guillet P, Fondjo E, Mimpfoundi R, Toto JC and Fontenille D: 2003. Insecticide Susceptibility Status of *Anopheles gambiae* s.l (Diptera: Culicidae) in the Republic of Cameron. *J. Med. Entomol.* 40: 491-497.

Etang J, Fondjo E, Chandre F, Morlais I, Brengues B, Nwane P, Chouaïbou M, Djemaï A and Simard F: 2006. First report of the kdr mutations in the malaria vector *Anopheles* *gambiae* from Cameroon. *Am. J. Trop. Med. Hyg.* 74 : 785-797.

Etang J, Manga L, Toto JC, Guillet P, Fondjo E and Chandre F: 2007. Spectrum of metabolic based resistance to DDT and pyrethroids in *Anopheles gambiae* s.l. populations from Cameroon. *J. Vect. Eco.* 32: 123-133.

Foko Dadji GA, Tamesse JL and Fekam Boyom F: 2011. Adulticidal effects of essential oils extract of Capsicum *annuum* (Solanaceae) *Piper nigrum* (Piperaceae) and *Zingiber officinale* (Zingeraceae) on *Anopheles gambiae* (Diptera-Culicidae), vector of Malaria. *Journal of Entomology* 8: 152-163.

Georghiou GP, Ariaratnam V, Pasternak ME and Lin CS: 1975. Organophosphorus multiresistance in Culex quinquefasciatus in California. *J. Econ. Entomol.* 68: 461–467.

Guenther E. 1972. *The essential oils.* Volume 4. Robert E. Krieger Publishing Co., Malabar Florida. Chapters in books.

Isman MB: 1999. *Pesticides based on plant essential oils.* Pestic. Outlook. 2: 68-72.

Keita MS, Vincent Charles, Schmith JP, Rammaswany S. and Belanger A: 2000. Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of stored products Research.* 36: 355-364.

Mahmout Y : 1992. *Contribution à l'étude de quelques aromates et condiments utilisés au Tchad.* Thèse de Doctorat en Chimie, Université de Montpellier II. 120 pp.

Nigam M, Handa k and Rao P: 1968. The essential oil of sweet basil: potential source of linalool in India. *Parfums Cosmétiques Savons* 51: 151-153.

Ngamo TLS, Ngassoum MB, Mapongmetsem PM, Malaisse F, Haubruge E, Lognay G. and Hance T: 2007b. Current post harvest practices to avoid insects attacks on stored grains in northern Cameroon. *Agricultural Journal,* 2 (2): 242- 247.

Njan Nlôga AM, Saotoing P, Tchouankeu JC and Messi J: 2007. Effect of essential oils of six local plants used insecticide on Adults of *Anopheles gambiae*, Giles, 1902. *Journal of Entomology* 6: 444-450.

Ntezurubanza L, Scheffer J and Looman A: 1985. Composition of the essential oil of *Ocimum canum* grown in Rwanda, Pharm. *Weekblad Sci. Edi.* 7: 273-276.

Odeyemi O O, Masika P and Afolayan AJ: 2008. Insecticidal activities of essential oil from the leaves of *Mentha longifolia* L. subsp. *capensis* against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). *Biozone Reerach Involved,* 2: 220-225.

Park I., Lee SG and Choi DH: 2002. *Insecticidal* activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtuse* against *Callosobruchus chinensis* (L) and *Sitophilus oryzae* (L.). Journal of stored products research 38: 10-13.

Pellecuer J, Allegrini J and Simeon de Buochberg M : 1976. Sur l'activité de l'huile essentielle de Hyptis pectinata. Révue de l'Institut pasteur de Lyon. 9 : 135-159.

Sinegre G., Jilien JL and Gaven B: 1977. Acquisition progressive de la résistance au chlorpyrifos chez les larves de *Culex pipiens* (L.) dans le Midi de la France. *Parasitologia* 19 : 79–94.

Tayal J and Dutt S: 1938. *Chemical examination of the essential oil of Ocimum canum,* Proc. Nat. Acad. Sci. India. 8 : 120-125.

Tchoumbougnang F : 1997. *Contribution à la détermination des teneurs, des caractéristiques chimiques et des activités antifongiques des huiles essentielles de quelques plantes aromatiques, condimentaires et médicinales du Cameroun.* Thèse présentée en vue de l'obtention du diplôme de Doctorat 3ème cycle en Biochimie. Université de Yaoundé I. 270p.

Tchoumbougnang F, Amvam zollo P, Dagne E and Mekonnen Y : 2004. In vivo antimalarial activity of essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* on mice infected with *Plasmodium berghei*. *Planta medica.* 71: 1-4.

Tchoumbougnang F, Amvam zollo PH, Avlessi F, Alitonou GA, Sohounhloue DK, Ouamba JM, Tsomambet A., Okemy-Andissa N, Dagne E, Agnaniet H, Bessiere JM and Menut C: 2005. Variability in the chemical compositions of the essentials oils of five Ocimum species from Tropical African Area. *Journal of Essential Oil Research* 18: 194-199.

Tchoumbougnang F, Dongmo PMJ, Sameza ML, Mbanjo EGN, Fotso GBT, Zollo PHA and Menut C: 2009. Larvicidal activity against *Anopheles gambiae* Giles and chemical composition of essential oils from four plants cultivated in Cameroon. *Biotechnologie, Agronomie, Societe et Environnement* 13: 77-84.

Traboulsi AF: 2002. Insecticidal properties of essential plant oils against the mosquito *Culex pipiens molestus* (Diptera: Culicideae). *Pest. Manage. Sci*, 58: 491-495.

Tripathi R, Banerji R, Sharma M, Balasubrahmanyam V and Nigam S: 1985. Essential oil from a new strain of *Ocimum gratissimum* against betelvine pathogenic fungi, *Agric. Biol. Chem*. 44: 2277-2282.

World Health Organization: 1985. Bioessay method for the titration of bacillus sphaericus: consultation on the development of Bacillus sphaericus as a microbial larvicide. 3 : 85-95.

Zola A and Garnero J: 1972. Contribution à l'étude de quelques essences de basilic de type européen, *Parfums Cosmétiques Savon*s 3 : 15-19.

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