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Chemical composition and larvicidal activity on *Anopheles gambiae* s.l. of essential oils of *Ocimum basilicum* and *Ocimum americanum* two plant of Lamiaceae family

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Abstract
As part of the search for new insecticide molecules, the larvicidal activity of essential oils from two aromatic plants from Niger was evaluated on *Anopheles gambiae* s l larvae. The essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS), and the larvicidal tests were carried out according to a WHO protocol (1985). Analysis by gas chromatography (GC) and GC-MS showed that the essential oil of *Ocimum americanum* L. (Dry leafy stems) is mainly composed of camphor (28.1%) and eucalyptol (17.6%). *Ocimum basilicum* essential oil (Dry stems), on the other hand, contains mainly β-linalool (53.18%) and eucalyptol (10.28%). Bioassays showed that *O. canum* essential oil (LD_{50} = 61.50 ppm) is the most active against Anopheles gambiae larvae. The essential oil samples in the present study were less active than deltamethrin (LD_{50} = 2.3 ppm) used as a reference. Despite this difference in activity, essential oils could be of great interest in the formulation of new insecticides.

Keywords: Essential oils, *Anopheles gambiae*, larvicidal activity, malaria, Niger

Introduction
Mosquitoes are responsible for several vector-borne diseases with very considerable impact on human Public Health (Seye *et al.*, 2006; Youssef *et al.*, 2011; Pascal *et al.*, 2011) [18, 19, 17]. According to the WHO, in 2018, over 213 million cases, or 93% of cases, were recorded in the WHO African region, far ahead of the South-East Asian region (3.4%) and the Eastern Mediterranean region (2.1%). Nineteen sub-Saharan African countries and India accounted for almost 85% of all malaria cases worldwide. Six countries alone accounted for more than half of all cases: Nigeria (25%), Democratic Republic of Congo (12%), Uganda (5%), Côte d'Ivoire, Mozambique and Niger (4% each). Mosquito species of the Anopheles genus are responsible for malaria transmission worldwide (Greenwood, 2008) [12].
In Niger, the species *Anopheles gambiae* has long been suspected of transmitting malaria (Julvez *et al.;* 1992) [13]. On the other hand, a strong proliferation of this species has been noted in recent years (Ahadji-Dabla *et al.*, 2014) [4].
The chemical insecticides used in mosquito control are highly effective on culicid mosquitoes, but have a number of drawbacks. In addition to having a detrimental effect on aquatic life, they can be the cause of a number of environmental problems, including the phenomenon of insect resistance to insecticides (Aouinty *et al.*, 2006; Cui *et al.*, 2007; Daaboub et.al, 2008) [6, 7, 8]. The mechanisms of resistance of mosquitoes to insecticides can be either behavioral (flight, camouflage), or related to physiological changes (Cuticular and metabolic) or related to the mutation of the insecticide target protein (Hamon, 1963; Oppenoorth, 1985; Fournier & Mutero 1994) [20, 21, 22]. Thus, researchers and scientists are already trying to find effective and accessible alternatives based on natural products, which are now enjoying renewed interest and growing popularity (El Ouali Lalami *et al.*, 2013) [11]. In Niger, despite the varietal diversity of medicinal and aromatic plants, studies on the insecticidal activity of plant extracts against mosquito larvae are very limited. As part of the valorization of natural bio-insecticides, the present work concerns the evaluation of the larvicidal activity of the essential oil of two aromatic plants from Niger's biodiversity on the *Anopheles gambiae* species.

Methodology
Plant material: The plant material consisted of samples of 2 plants (Table I). Samples were collected in two locations in Niger and shade-dried for 2 weeks (Table I).

Identification and authentication were carried out at the Garba Mounkaila Laboratory in the Biology Department of the Abdou Moumouni University, Niamey.

Table 1: Collected plants

Plant species	Botanical families	Part used	Place of harvest
Ocimum basilicum L.	Lamiaceae	Dry leafy stems	Harobanda rice field
Ocimum americanum L.	Lamiaceae	Dry leafy stems	Garbal (Airport)

Animal material

The larvae of *Anopheles gambiae* s.l. (Stages II and III) are the animal material used in this study. These larvae were collected by sieving in the Saguiya district (Commune V / Niamey / Niger) using a plastic sieve.

The collected larvae, packed in a plastic bucket, are transported to the Biology Laboratory of the Abdou Moumouni University in Niamey. These larvae are carefully rinsed with well water and fed with cookies (a food rich in carbohydrates) for 24 hours (h) before being used for biological tests.

The identification of the larvae was done at the medical and health research center (CERMES/Niamey/Niger).

Methods of study

Extraction of the essential oil: Hydrodistillation using the Clevenger is the technique used for essential oil extraction. Unpulverized dry plant material was used. To achieve this, 100 g of dry plant material was introduced into a 2 L flask. 700 ml of distilled water was added. The flask was fitted with a Clevenger-type apparatus and boiled (100 °C) for two hours (2h). At the end of distillation, the essential oil was separated from the water by liquid-liquid extraction of the distillate with diethyl ether (3 x 10 mL) using a separating funnel. The organic phases (Containing the essential oil) are collected in a tared flask. The diethyl ether was allowed to evaporate at room temperature. The essential oil was then stored in a refrigerator until use.

Analysis of essential oils

Essential oils previously obtained from the two (2) plants in the present study were analyzed, namely *Ocimum canum* (leafy dry stems), *Ocimum basilicum* (Leafy dry stems).

The analysis was carried out at the Ecole Normale Supérieure de Chimie de Montpellier (France), in the AM2N laboratory of the Institut Charles Gerhardt de Montpellier (ICGM UMR 5253 CNRS). For this, 1µl of the sample is injected into the gas chromatograph coupled to a mass spectrometer (SHIMADZU, model QP2010SE), equipped with a Phenomenex Zebron ZB-5ms column 20 m long, 0.18 mm internal diameter and 0.18 µm stationary phase film thickness. The injector is split/splitless, Fast. The regulator is set at 970kPa, the PLC has 150 positions. The mass spectrum (MS) has one ionization mode: electronic impact, scan speed: 50 scans/s, acquisition speed: 10,000 uma/s. The oven temperature is programmed from 50 °C with a step of 2 min, to 280 °C with a gradient of 22 °C. min^{-1} and a final step of 2 min at 280 °C. The carrier gas is helium at a flow rate of 0.7 $ml.min^{-1}$.

Constituent Identification: The identification of the constituents of the essential oils was done on the basis of their retention indices and their mass spectra by comparison with the data of the Shimadzu manufacturer's library (NIST 2008).

Study of the larvicidal activity: Bioassays were performed using a WHO (1985) protocol on the essential oil of each plant. Several preliminary bioassays were conducted and the following concentration ranges were selected: 0; 25; 50; 75; 100; 125; 150; 175 and 200 ppm and 0.3; 0.6; 1.2; 1.5; 3; 6; 9; and 12 ppm respectively for the essential oil samples and the reference product (deltamethrin). Well water is used in the different dilutions. Twenty (20) *Anopheles gambiae* larvae are introduced into each 100 ml Petri dish containing the test solution. The whole set is left to incubate for 48 hours at room temperature. The control consists solely of well water. Dead larvae were counted after 48 hours of exposure. Each analysis was repeated three times.

Percentage mortality and LD50

The mortality rate of *Anopheles gambiae* larvae was calculated by the method of Abbott (1925) [1].

$$\% \, m = \frac{NLM - NLMT}{NTL - NLMT} \times 100$$

% m = percentage of mortality

NLM = number of dead larvae in the test petri dish

NLMT = number of dead larvae in the control

NTL = total number of larvae

The median lethal dose (LD_{50}) is the dose that causes 50% mortality of Anopheles larvae. It was determined using the formula of Dragstedt and Lang (1957) [9].

$$LD_{50} = \frac{50(X2 - X1) + X2Y2 - Y1X1}{Y2 - Y1}$$

X2: higher concentration framing the LD_{50}

X1: lower concentration framing the LD_{50}

Y1: percentage of mortality corresponding to X_1

Y2: percentage of mortality corresponding to X_2

Statistical analysis

Standardized data were subjected to analysis of variance (ANOVA) followed by Tukey's PLSD test at the 5% probability level for the separation of statistically significant means. In order to determine whether there was a significant difference between the different doses of extracts used and, if so, which dose was the most effective in terms of mortality.

Results

Extraction yield of essential oils

Table 2: Yields of essential oil of different plants

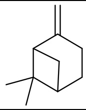
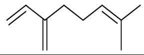
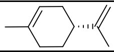
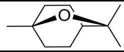
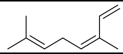
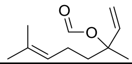
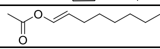
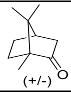
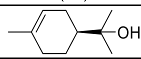
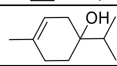
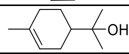
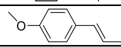
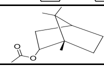
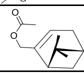
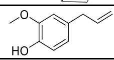
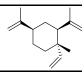
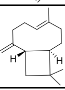
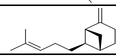
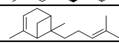
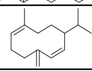
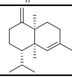
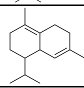
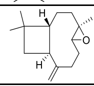
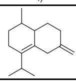
Plant species	Parts used	Yield (%)
Ocimum canum	Dry leafy stems	0,21
Ocimum basilicum	Dry leafy stems	0, 73

Using the Clevenger, the yields of essential oils of the different samples obtained by hydrodistillation are summarized in (Table II). The analysis of this table shows that the best yields are obtained with the samples of *Ocimum basilicum* (0, 73%).

Chemical composition of essential oils of aromatic plants

The results of gas chromatography-mass spectrometry (GC-MS) analysis of the essential oils of *O. canum* and *O. basilicum* are shown in Table III. The analysis of this table shows that the essential oil of *Ocimum canum* of nature monotrepenes (75.2%) and sesquiterpenes (24, 8%), sixteen (16) other constituents were detected and identified whose most important are: eucalyptol (17.6%), camphor (28.2%). As for the sample of *O. basilicum*, fifteen (15) other compounds could be detected and identified. They are of monoterpene nature (91.8%). *O. basilicum* essential oil consists mainly of β-linalool (53.2%) and eucalyptol (10.3%).

Table 3: Chemical composition of the essential oil of the samples of the plants studied

Retention time	Chemical compounds	Pourcentage content (%)		
		O. canum	*O. basilicum*	Chemical structure
	Monoterpenes	75,22	91,83	
4513	*β* -Pinène	---	0,82	
4591	*β*-Myrcène	---	1,37	
4953	*D*-Limonène	1,67	0,61	
5012	Eucalyptol	17,62	10,28	
5097	*β*-Ocimène	---	3,16	
5544	*D*-Linalol	1,95	---	
5564	*β*-Linalol	---	53,18	
5579	Octen-1-ol, acétate	1,85	---	
5983	Camphre	28,17	2,44	
6126	*α*-terpinéol	1,44	---	
6207	L-terpinen-4-ol	2,55	---	
6310	*α*-Terpinéol	7,91	---	
6319	*p*-Propénylanisole	---	9,3	
6932	Bornéol, acétate ??	10,58	2,12	
7175	acétate de myrtényle	1,48	---	
7367	Eugénol	---	8,55	
	SESQUITERPENES	24,78	8,17	
7618	*β*-élémène	---	1,71	
7743	*β*-Caryophyllène	8,78	---	
7878	*α*-Bergamotène	9,42	1,95	
8167	*trans*-*α*-Bergamotène	2,62	---	
8206	Germacrène D	---	0,7	
8378	*γ*-Muurolène	---	1,47	
8391	Cadina-1(10),4-diène	2,01	---	
8806	Oxide de Caryophyllène	1,95	---	
9096	8-Isopropyl-5-méthyl-2-méthylene-1,2,3,4,4a,5,6,7-octahydronaphthalène	---	2,34	
Total		100	100	

Larvicidal activity of essential oils on *Anopheles gambiae* larvae after 48h of exposure

Table V and Figure 1 show the percentage of dead *Anopheles gambiae* larvae as a function of the doses of essential oil and deltamethrin (Reference insecticide) used over 48 hours of exposure. Analysis of these results shows that the essential oil samples studied have a dose-dependent effect on *Anopheles gambiae* larvae. The mortality rate of the larvae is a function of the doses (Or concentrations) of essential oils. The minimum concentrations required to achieve 100% mortality of *A. gambiae* larvae were estimated at 100 ppm for *O canum* and around 200 ppm for *A. gambiae*.

The analysis of the results (figure 2) showed very low LD_{50} for *O canum* (LD_{50} = 61.5 ppm) justifying good insecticidal activities. The best LD_{50} was obtained with *O. basilicum* (LD_{50}=135.4ppm).

On the other hand, essential oils were less active than deltamethrin (LD_{50} = 2.3ppm). Although less active than chemical insecticides, the essential oils in the present study could be of great interest in the field of vector control, since as natural substances they are biodegradable and less polluting than synthetic insecticides.

Table 4: Larvicidal activities of essential oils after 48 hours of exposure

Concentration	*O. canum*	*O. basilicum*
Control	0	0
25 ppm	44 ± 5^a	3 ± 2^b
50 ppm	63 ± 9^a	5 ± 0^b
75 ppm	91 ± 8^a	7 ± 3^d
100 ppm	100 ± 0^a	47 ± 10^c
125 ppm	100 ± 0^a	72 ± 3^c
150 ppm	100 ± 0^a	79 ± 10^c
175 ppm	100 ± 0^a	88 ± 6^b
200 ppm	100 ± 0^a	95 ± 0^a

Means in the same row followed by identical letters do not differ statistically (Tukey PLSD test $p<0.05$).

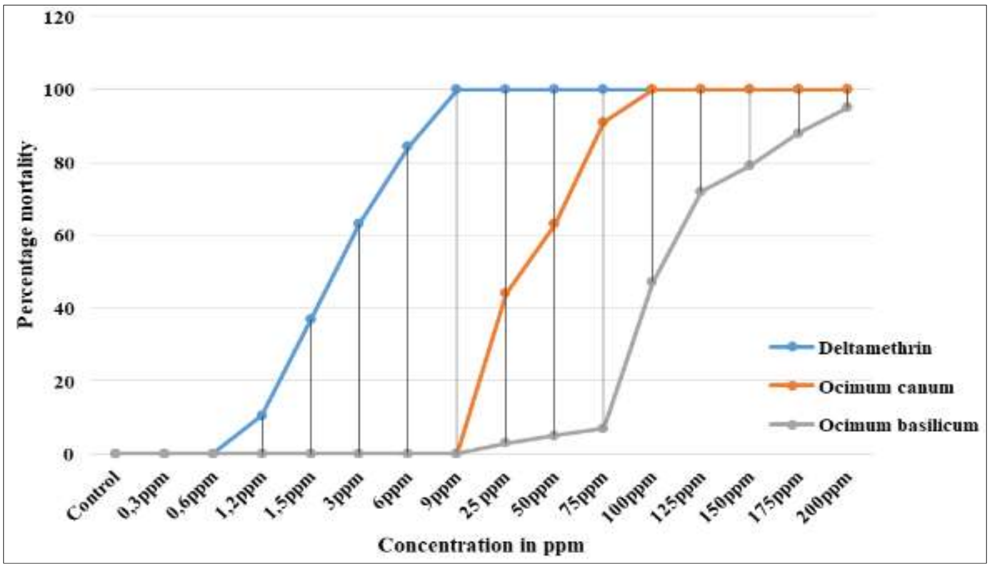


Fig 1: Larvicidal activity of the essential oil of two studied plants and deltamethrin against *Anopheles gambiae* larvae.

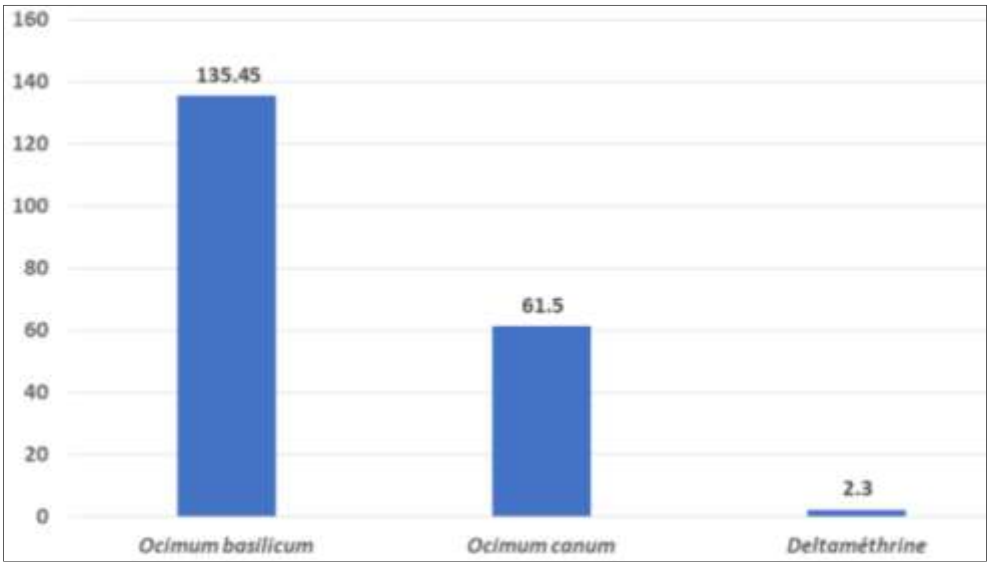


Fig 2: Dose letale 50 (LD_{50}) of essential oil, and deltamethrin

Discussion

Ocimum canum and *Ocimum basilicum* essential oils are mono and sesquiterpenic in nature. The essential oil of *O. canum* consists mainly of camphor (28.2%) and eucalyptol (17.6%). Similar results were reported in Côte d'Ivoire by Kobenan *et al*. in 2018, where eucalyptol was obtained at 41.7% and camphor at 16.9% in *O. canum* essential oil.

β-linalool (53.2%) and eucalyptol (10.3%) are the main compounds in *O. basilicum* essential oil.

Different results were obtained In Cameroon, Aurélie *et al*. in 2016 reported eucalyptol (33.9%), β-pinene (16.1%), and terpineol (11.2%) as majority constituents of *O. basilicum*. In Togo, Akantetou *et al.*, in 2020 reported estragol (85.5%) and eucalyptol (2.2%) as the main constituents of *O. basilicum* essential oil.

Bioassays showed that *O canum* essential oil (LD_{50} = 61.50 ppm) was more active against *Anopheles gambiae* larvae than the *O. basilicum* sample (LD_{50} = 135.45 ppm). These different results obtained are consistent with several previous works (Ntonga *et al.*, 2016, Aurelie *et al.*, 2016 and Dris *et al.*, 2017).

The larvicidal activity observed with the various essential oil samples could be linked to their major compounds. Indeed, the toxicity of terpene compounds towards many insects has been reported (Konstantopoulou *et al.*, 1992).

Conclusion

Given the numerous cases of mosquito resistance observed when synthetic insecticides are used in the preventive fight against malaria, natural molecules with larvicidal or insecticidal properties, which are biodegradable and therefore more respectful of the environment and the ecosystem, appear to be an alternative approach. The present study concerns the larvicidal activity of the essential oil of two (2) aromatic plants from Niger on *Anopheles gambiae*.

GC-MS analysis showed a significant monoterpene content for both plant samples studied. The study showed that the essential oil has significant activity against *Anopheles gambiae* larvae. In fact, the essential oil of *O. canum* (LD_{50} = 61.50 ppm) was more active than that of *O. basilicum* (LD_{50} = 135.45 ppm) against *Anopheles gambiae* larvae. Although essential oils were less active than deltamethrin (the reference compound) in the present study, they could be of great interest in the field of research into new insecticide molecules. These results open up interesting prospects for their use in the production of bio larvicides and insecticides.

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