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Chemical Composition and Ovicidal, Larvicidal and Pupicidal Activity of *Ocimum basilicum* Essential Oil against *Anopheles gambiae*. (Diptera: Culicidae)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FDGA and TJL designed the study. Authors FDGA, NMA, THG, AAPH and NMP preformed the study and the experiments. Authors FDGA and NMP preformed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria remains a serious Public Health issue in the sub-Saharan regions. Although *Anopheles gambiae* (main malaria vector) has developed resistance against commonly used insecticides, the emergence of this resistance as well as the pollution of the environment by these

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chemicals have led to the use of plant-derived products such as essential oils.

Aim: This study aimed to characterize and investigate in laboratory bioassays the ovicidal, larvicidal and pupicidal activities of *Ocimum basilicum* essential oil against aquatic stages of *Anopheles gambiae*, the main malaria vector in Cameroon.

Methods: Essential oils of *O. basilicum* fresh leaves were extracted by hydrodistillation, characterized by gas chromatography coupling mass spectrometry (GC-MS) and tested against *An. gambiae* eggs, larvae and pupal stages in laboratory bioassays.

Results: With an overall yield of 0.31% (w/w), the essential oil of *O. basilicum* fresh leaves revealed the presence of 29 compounds by GC-MS. Monoterpenes were the main group of compounds found with 1-8 Cineol (33.9%), β-Pinene 16.09%), Terpineol (11.21%) and α-Pinene (5.65%) as the main ingredients. These compounds demonstrated an efficient toxic effect against the aquatic stages of *An. gambiae* with no egg hatching after 24 hours exposure at >30 ppm. The LC_{50} values of the essential oil were respectively obtained one hour and 24 hours post-exposure for first (29.41 and 24.7 ppm), second (34.7 and 17.6 ppm), third (34.7 and 20 ppm) and fourth (45.29 and 23.5 ppm) instars larvae and the pupal stage (45.88 and 36.47 ppm) of *An. gambiae*.

Conclusion: The essential oil of *O. basilicum* demonstrated a good efficacy against the aquatic stages of *An. gambiae* and could be suitable for use in mosquito control programme for a Public Health purposes.

Keywords: Essential oil; Ocimum basilicum; Anopheles gambiae; larvicidal activity.

1. INTRODUCTION

Malaria, an infectious disease caused by a parasite genus *Plasmodium* and transmitted to humans by an *Anopheles* female mosquito genus, is endemic in tropical Africa and particularly in Cameroon. It is the most important parasitic disease in Africa which mainly affects children below 5 years and pregnant women [1]. Known as a poverty related disease, malaria does not only kill the African population, it considerably affects their economy by reducing its dynamism and hampering children's education [2]. The efforts undertaken at the international level to eradicate malaria are relayed to the national level by a strong commitment of the Cameroonian state through the ECSD (Strategic Document for Growth and Jobs) with the goal of reducing the mortality rate associated to malaria to less than 10% by 2035 [2]. Efforts made by Scientists to eradicate and decrease the prevalence of this scourge are hampered by many concerns which include: The increased resistance of *Plasmodium parasites* to antimalarial drugs, the resistance of mosquito species to synthetic insecticides, and the poor sanitary conditions that promote human contacts with mosquitoes species [3,4]. Among these are the economic issues such as the incapacity to afford basic drugs, effective insecticides and mosquito bednets. Moreover, there is no effective vaccine available against malaria [5]. Vector resistance to conventional insecticides and pollution vis-à-vis the environment [3,4,6-9] have directed researches towards new and more effective bioinsecticides which are environmentally safe (biodegradable) and generally based on plant extracts and essential oils. The development of insect resistance and side- effects associated with synthetic insecticides, make plant extracts and essential oils the focus of intense research efforts. Various plant species including *Ocimum basilicum* have been used to cure or fight against many disease pathogens and vectors. *O. basilicum* (basil) for instance is widely used in systems of traditional medicine for treating digestive disorders (such as stomach ache and diarrhea), kidney complaints, and infections. In Africa, basil is used for treating cough and various types of fever. Interestingly, the leaves and seeds of basil are used locally to make an insecticide that protects stored crops from beetle damage. Essential oils are natural products characterized by effective biological activities as well as long and safe use for both the environment and human populations. Since they are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme [10], they could lead to development of new classes of possible safer insect control agents. Several studies have described the insecticidal effect of the essential oils of the *Ocimum* genus against pathogens related diseases and vectors [11-18]. However, most of these studies have investigated the effectiveness of the essential oils and there are few studies describing the efficacy of each active ingredient of these oils against disease pathogens and vectors. This may be due to the high cost related to the characterization and purification of the oil active components. Various plant extracts have been used worldwide to fight against adult and aquatic stages of malaria vectors [11-13,16,18-21]. Fighting against aquatic stages of malaria vectors could be better since the larvae are relatively confined to a geographical area and unable to escape the effects of insecticides [15]. The insecticidal effect of *O. basilicum* leaf essential oil has been tested against several disease vectors in Cameroon and elsewhere. However, there are few studies describing the larvicidal effects of the essential oil of this plant species against *An. gambiae*. In addition, none of these studies has evaluated its ovicidal and pupicidal potentials. It will therefore be suitable to evaluate its activities against all the developmental stages of pathogen or vector related-diseases in order to identify the most sensitive stage to fight against these diseases. In this study, we aim at characterizing and investigatinge in laboratory bioassays the ovicidal, larvicidal and pupicidal activities of *O. basilicum* essential oil against aquatic stages of *An. gambiae*, the main malaria vector in Cameroon. This essential oil has previously showed important insecticidal activity against adult female *An. funestus ss.* mosquitoes in Cameroon [16].

2. MATERIALS AND METHODS

2.1 Laboratory Rearing of Pre-imaginal Stages of *Anopheles gambiae*

Prior to laboratory activities, *An. gambiae* immature eggs were obtained from OCEAC and incubated for hatching in the zoology laboratory of the Higher teacher training College of University of Yaoundé I. Eggs were kept 24 hours in Petri dishes for maturation and were subsequently divided for ovicidal tests and hatching at 26-28℃, 70-80% RH and 12:12 L/D. Eggs for 100 first instars larvae were maintained in spring water supplemented with 30 mg Tetramin baby fish food in 4.5 dm³ basins as described by Foko et al. [22]. Larvae and pupae were reared and daily fed in the same conditions, and were used for bioassays in order to test their sensitivity to *O. basiclicum* essential leaf oil.

2.2 Plant Harvest and Extraction of Essential Oil

Fresh leaves of *O. basilicum were* harvested in 2013 from Nkolondom (11° 28'N and 3°58' E), a sub-urban area located in the Central Region of Cameroon. The plant material was identified and registered under the number "*O. basilicum* (15866/SRF/CAM)" at the Cameroon National Herbarium, and transported to the Microbiology laboratory of University of Yaoundé I for extraction of essential oils.

Essential oils were extracted by hydrodistillation in Clevenger type apparatus for 5 h 25 min as described by Nyegue [23]. Briefly, fresh leaves of *O. basilicum* were weighed (22.3801 kg) and introduced into the ball with adequate quantity of water. The oil gathered by decantation at the end of the distillation was filtered, dried on a column of anhydrous Sodium Sulfate, and introduced into dark glass bottles and stored in a refrigerator at 4℃ prior to analysis.

2.3 Characterization of *Ocimum basilicum* Essential Oil by Gas Chromatography (GC) and GC Coupling Mass-spectrometry (GC-MS)

The GC and GC-MS were carried out as recently described by Riwom et al. [24]. Briefly, a Variant CP 3380 gas chromatograph equipped with a flame ionization detector (FID) adjusted at 250℃ and coupled to two types apolar column (silica capillary, polar HP-5 J and W) (Agilent (5%-phenyl-95% methyl polysiloxane) of capillary column (30 mm x 0.25 mm thickness and film thickness of 0.25 µm) and Supecowax 10 (polyethylene glycol, Supelco Inc, Bellfonte, PA) fused capillary (internal diameter 30 mm x 0.25 mm, 0.25 µm film thickness) was used for a percentage determination of oil components. The temperature of the column was programmed at 220℃ with a Split mode of injection (split ratio: 1:100). The gas used was Nitrogen with a flow rate of 0.8 ml/min. The detector temperature was 250℃. The temperature was then programmed at 50℃ to 200℃ at a ramp of 5℃/ min and then maintained at 200℃ for 10 minutes. The apparatus was controlled by a computing system containing the COPPASS software that ensures its functioning and the evolution of the chromatographic analyses. The Volume injected was 1 µl and the Adams formula was used to calculate the retention indices relative to the retention time of a series of n-alkanes [25].

Mass spectra obtained from the GC–MS analysis on a Hewlett-Packard (GC 5890 series II) instrument equipped with a HP-5 (5% phenyl-95% methyl polysiloxane) fused capillary silica column (30 mm x 0.25 mm internal diameter and 0.25 µm film thickness) and interfaced with another fused silica capillary DB-Wax (30 mm x

0.25 mm internal diameter, and 0.25 µm film thickness) was used with helium as carrier gas at a flow rate of 0.6 ml/min. The GC analytical parameters were: split, 1:10 (1 µL of a 10:100 CH_2Cl_2 solution), ionization voltage 70eV; electron multiplier 1460eV, mass scan range 35-300 a.m.u, scan rate 2.96 scan/s and injection of 0.1 µl of pure essential oil. The percentage composition of essential oil was computed by the normalization method from the GC-FID peak areas, assuming an identical mass response factor for all compounds. Identification of the oil components was based on their relative retention index in comparison with published data [25].

2.4 Laboratory Bioassay Activities

The essential oil of *O. basilicum* was prepared in a test tube and diluted in absolute alcohol to constitute a stock solution at 500 ppm. Several dilutions were then performed to obtain the desired concentrations of 0, 10, 20, 25, 30, 40, 50 and 100 ppm.

Two hundred individuals of each aquatic stage of *Anopheles gambiae* (eggs, the four instars larvae and nymphs) were used for the bioassay tests. Each test tube was filled with 99 ml spring water and 1 ml of the required concentration of essential oil according to WHO protocol [26]. Each test was replicated three times before validation and the oil was replaced with water in each control tube. The hatching and the mortality rates in each test was read after 1 hour and 24 hours of contact with the essential oil. The mosquitoes were considered dead if they were immobile and unable to reach water surface.

2.5 Statistical Analysis

Data was entered in Excel datasheet of Microsoft Word 2007 and analyzed using SAS software version 9.1. The Chi square test was used to calculate the frequency of tested variables and the Kruskal Wallis and Wilcoxon tests were used to determine and compare the average mortality rates of individuals tested. The LC_{50} value (the concentration at which 50% of the larvae/pupal were immobilized) was calculated to evaluate the exact efficacy of the essential oil toxicity. The *p-value* was set at 5%.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of *O. basilicum* Essential Oil

The average yield of 0.31% (w/w) was obtained for essential oils of *O. basilicum* fresh leaves. This yield is higher than the 0.11% (w/w) obtained by Akono et al. [14] on the same plant and the divergence could be explained by the difference in the climatic conditions of the two regions. In fact, leaves used by Akono and colleagues were harvested in a coastal region of Douala which has a dry climate than the area of Nkolondom where our leaves were harvested. The yield obtained in this study is lower than those obtained by Tchoubougnang et al. [12] with *O. canum* (0.59%), *O. graticimum* (o.60%), *Cymbopogon citrates* (0.67%) and *Thymus vulgaris* (0.95%). Although these plants were harvested in different localities, the yield was calculated on the base of their dry weight in contrast to the fresh weight that was considered in this study. The difference could also be attributed to the gap between the ages of the plants.

In order to determine the chemical composition of *O. basilicum* essential oil, the GC-MS revealed 29 peaks corresponding to the main ingredients of the oil (Fig. 1). In recent studies with the same plant, Azhari et al. [15] obtained 13 components with an average yield of >1%, while Babatunde et al. [18] obtained less than ten compounds. As these authors used the same extraction method as in this study, the difference might be attributed to the geographical origin as well as the age of each plant. This also shows that there is no link between the number of active components of the essential oil and the yield of the extraction. Similar to Azhari et al. [15], Monoterpenes were found as the main component of *O. basilicum* essential oil with 84.3% proportion (Table 1). Hydrocarbonated and oxygenated monoterpenes were respectively found at 56.71 and 27.51%. Although Sesquiterpenes were the lowest group of compounds found as previously described by Tchoumbougnang et al. [12], their rate in this study was higher than that obtained by these authors. The active components found included 1-8 Cineol (33.9%), β-Pinene (16.09), Terpineol (11.21%), α-Pinene (5.65%) and α –Farmesene (5.26%). These results correlate with those obtained by Ndoye [27] whose chemical profile consisted of 1-8 Cineol (66.1 and 70%); β-Pinene (7.4 and 6.6%); Linalool (4.9 and 0.1%) and α-Pinene (4.4 and 4.0%). However, they differ from those found by Tchoumbougnang et al. [12] who mainly obtained linalool (56.3%), Limonene (10.9%) and β-Humene (3.5%) in *O. canum* and p-Cymene (32.1%) and Thymol (24.3%) in *O. graticimum* essential oils. These results show that our plant with those studied by Ndoye [27] constitute a chemotype 1-8 Cineol, while that of Tchoumbougnang et al. [12] harvested in Moutenguene constitutes a chemotype Linalool. We can explain this divergence by the difference between the two plant species and the fact that plants chemotype 1-8 Cineol were harvested relatively at the same period. According to Mohammedi [28], the chemical composition of plant essential oils varies not only in period but also depends on the place of harvest. This mismatch of data could also be ascribed to the genetic variability of the plant species. Overall, the main constituents of essential oils mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones are responsible both for the fragrance and for the biological activity of aromatic and medicinal plants. For instance, Cineol, Pinene, and Terpineol derivatives have shown high antibacterial, antifungal and antiviral activities [29]. They are also remarkable chemical components offering strong therapeutic properties that have been well researched. These properties suggest that using these oils during a cold or flu would help reduce pain, mucus and headaches. They can reduce swelling (great for sinus infections), muscle spasms, and spastic coughing [30,31]. The high quantity of these components found in *O. basilicum* leaf essential oil therefore makes this plant an attractive ingredient helpful for medicinal blending and for quality assurance.

3.2 Ovicidal Activity of *O. basilicum* Essential oil against *An. gambiae*

The hatchability of *An. gambiae* eggs was zero after one hour exposure to *O. basilicum* essential oil (Table 2). This rate did not vary when the concentration of essential oil increased to 30ppm 24 hours post-exposure. Overall, the egg hatching rates varied significantly among the concentrations tested with an LC_{50} of 13.33 ppm after 24 hours exposure to *O. basilicum* essential oil (p=0.026, H=21.95). Our results correlate with those obtained by Ramar et al. [32] who described the ovicidal effect of 10 plant essential oils against *Culex quinquefasciatus* eggs in the laboratory. Similar results were observed on the ovicidal activity of six essential oils against *Callosobruchus maculatus* eggs in Togo [33]. Elumalai et al. [34] showed that *O. basilicum* and *Zingiber officinale* essential oils have ovicidal action against *Spodoptera litura* (Lepidoptera: Noctuidae). Indeed, the observations of these authors adequately show that essential oils are important inhibitors for the development of arthropod eggs. These ovicidal activities could be explained by their penetrating power or the direct toxicity of their active components [35]. The toxicity of *Acorus Calamus* essential oil vapors against *Callosobruchus chinensis* L eggs was reported by Schmidt et al. [36], and suggested a sterilizing action of the compound against the insect eggs. The same conclusion could be drawn regarding our oil for certain concentrations due to the absence of an outbreak even at very low concentrations.

3.2.1 Larvicidal activity of *O. basilicum* essential oil against *An. gambiae*

It is clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts [37,38]. Overall, this study demonstrated the toxic and insecticidal effect of *O. basilicum* essential oil against larval stages of *An. gambiae* in laboratory bioassays. Using the overlapping of the standard errors of the LC_{50} values, the mortality rates of all *An. gambiae* aquatic stages differ significantly between the oil concentrations (Fig. 2). The mortality rates of the mosquitoes larvae increased with the concentrations of *O. basilicum* essential oil as previously described by Murugan [39]. These authors suggested that whatever the mosquitoes larval developmental stage, the mortality rate increases with the concentration of the essential oil. Our observations also correlate with studies described by Kuppusamy and Murugan [40] and Okigbo et al. [13].

Regarding the mosquitoes first instars larvae, the minimal concentrations to obtain 100% mortality were 100 ppm and 40 ppm respectively for one hour and 24 hours exposure to *O. basilicum* essential oil (Table 3). The mortality rates of *An. gambiae* first instars larvae correlated with the concentrations of the essential oil with a statistical difference after one hour (p=0.002, H=22.52) and 24 hours (p=0.002, H=22.25) exposure. The toxic effect was observed with LC_{50} of 29.41 ppm and 24.7 ppm respectively after one hour and 24 hours exposure to essential oil extracted from *O. basilicum* fresh leaves. Similar trends were noticed in studies described by Sengottayan et al. [41] and where first instars larvae were most vulnerable to extracts of *Melia azedarach* against *An. stephensi*. Nasir et al. [42] also described the same effect on immature stages of *Aedes aegypti* using several different extracts. All these data therefore highlight the vulnerability of early

Table 1. Chemical composition of essential oils of *O. basilicum* leaves

Elution order	Chemical compounds	Retention Indices (RI)	Yield (%)
Hydrocarbonated monoterpenes			**27.51**
1	Thujene	931	0.46
2	α-Pinene	940	5.65
3	Sabinene	980	2.67
4	β-Pinene	987	16.09
5	Myrcene	995	2.28
6	Limonene	1023	0.36
Oxygenated monoterpenes			**56.71**
7	1-8Cineol	1048	33.9
8	γ-Terpinene	1065	2.08
9	p-Cymene	1074	0.3
10	Linalol	1095	0.62
11	Borneol	1097	0.4
12	Cis β-Terpineol	1104	0.18
13	Terpine-4-ol	1176	2.44
14	O-Methyl Thymol	1187	4.76
15	Terpineol	1202	11.21
16	geranyl acetate	1224	0.19
17	Ascaridol oxyde	1226	0.45
18	Geraniol	1253	0.18
Hydrocarbonated sesquiterpenes			**13.56**
19	β-Elemene	1388	1.13
20	β-Carophyllene	1435	0.84
21	Trans α-bergamotene	1446	1.33
22	Humulene	1470	2.85
23	Germacreme D	1497	1.21
24	γ-Cardiene	1536	0.94
25	α-Farmesene	1553	5.26

developmental stages of mosquitoes to plant extracts and could help in the fight against malaria vectors with no effect on both humans and the environment. In fact, fighting against the early developmental stages of mosquitoes species helps to avoid the dissemination of mosquitoes population and is more practical and cost less as they are confined in a specific environment.

According to the mortality rate, the larvicidal potential of *O. basilicum* essential oil on second instars larvae of *An. gambiae* was higher than that observed with the first instars larvae after 24 hours exposure. The inhibitory effect was observed with LC_{50} values of 34.7 ppm and 17.6 ppm respectively after one hour and 24 hours exposure to the essential oil (Table 4). The mortality rates of *An. gambiae* second instars larvae correlated proportionally and varied significantly with the test concentrations after one hour (p=0.0023, H=22.23) and 24 hours (p=0.0039, H=20.93) exposure to the essential oil. Similar trends were obtained after exposure of *Eucalyptus globules*, *Azadirachta indica* and *Menthapiperita* to *Ae. aegypti* second instars larvae [42].

Third instars larvae of *An. gambiae* showed similar sensitivity with second instars larvae to *O. basilicum* essential oil after one hour exposure (Table 5). This trend seemingly varied after 24 hours exposure. The minimal concentrations to obtain 100% mortality were respectively 100 ppm and 40 ppm after one hour (LC_{50} = 34.7 ppm) and 24 hours (LC_{50} = 20 ppm) exposure to the essential oil. The mortality rates varied significantly after one hour and 24 hours post-exposure (p=0.005, H=22.07) to *O. basilicum* essential oil. Amer and Mehlhorn [11] obtained a mortality rate of 86.7% after 24 hours exposure of *O. basilicum* essential oil against *Ae. aegypti* third instars larvae in 50 ppm. This mortality is lower than that obtained in this study. Although the *O. basilicum* leaves used by these authors were harvested in Cameroon, this difference in mortality rates might suggest that *An. gambiae* is more sensitive than *Ae. aegypti* against *O. basilicum* essential oil; or, this is only due to the fact that our oil is more toxic according to the

LC_{50} values which are lower than that obtained by Amer and Mehlhorn [11]. In addition, Minijas and Sarda [43] showed that crude extracts containing saponin produced higher mortality in the larvae of *An. gambiae* than in the larvae of *A. aegypti*. In contrast, Novak [44] described that anophelines were less sensitive than aedines against several volatile oils. *O. basilicum* essential oil seems be the most effective against *An. gambiae* in comparison to data obtained by Babatunde et al. [18] with *O. canum*. Our data also suggest that young larval stages could be more sensitive to the oil activities than old stages as previously described by Babatunde et al. [18] with *O. canum* essential oil.

The fourth instars larvae of *An. gambiae* were less sensitive to *O. basilicum* essential oil comparing to the pre-imaginal stages. The minimal concentrations to obtain hundred percent mortality were 100 ppm and 40 ppm respectively after one hour and 24 hours exposure to the essential oil (Table 6). The inhibitory activity was observed with LC_{50} values of 45.29 ppm and 23.5 ppm respectively for 1 hour and 24 hours exposure. As with the first pre-imaginal stages, the mortality rates correlated significantly with the oil concentrations after one hour (p=0.0023, H=22.23) and 24 hours (p=0.0039, H=20.93) exposure. In contrast to these observations, Tchoumbougnang et al. [12] obtained high concentrations of essential oils when working with others species of the *Ocimum* genus including *O. canum* (LC_{50}>200 ppm) and *O. gratissimum* (LC_{50}>150 ppm) against *An. gambiae*. *O. basilicum* might be most effective against *An. gambiae* than the others species of the same genus, and could be more suitable to fight against aquatic forms of malaria vectors in endemic areas.

3.2.2 Pupicidal activity of *O. basilicum* essential oil against *An. gambiae*

The nymphal stages of *Anopheles gambiae* were less sensitive to *O. basilicum* essential oil comparing to the larvae developmental stages. The minimal concentration to obtain 100% mortality was 100 ppm after one hour and 24 hours exposure to *O. basilicum* essential oil (Table 7). The mortality rates correlated significantly with the essential oil concentrations after 1 hour (p=0.0027, H=21.82) and 24 hours (p=0.0027, H=21.85) exposure. The toxic effect was observed with LC_{50} of 45.88 and 36.87 ppm respectively for 1 hour and 24 hours exposure.

These data are similar to those described by Nasir et al. [42] who showed that old mosquitoes stages have a physiology that allows them to better withstand high concentrations of oil insecticides. In addition, the shell of old mosquitoes developmental stages is more solid and might decrease their sensitivity to the activity of the essential oil and the survival capacity [19]. The pupal stages of mosquitoes species are therefore not suitable to evaluate the efficacy of biological insecticides as well as the chemical insecticides.

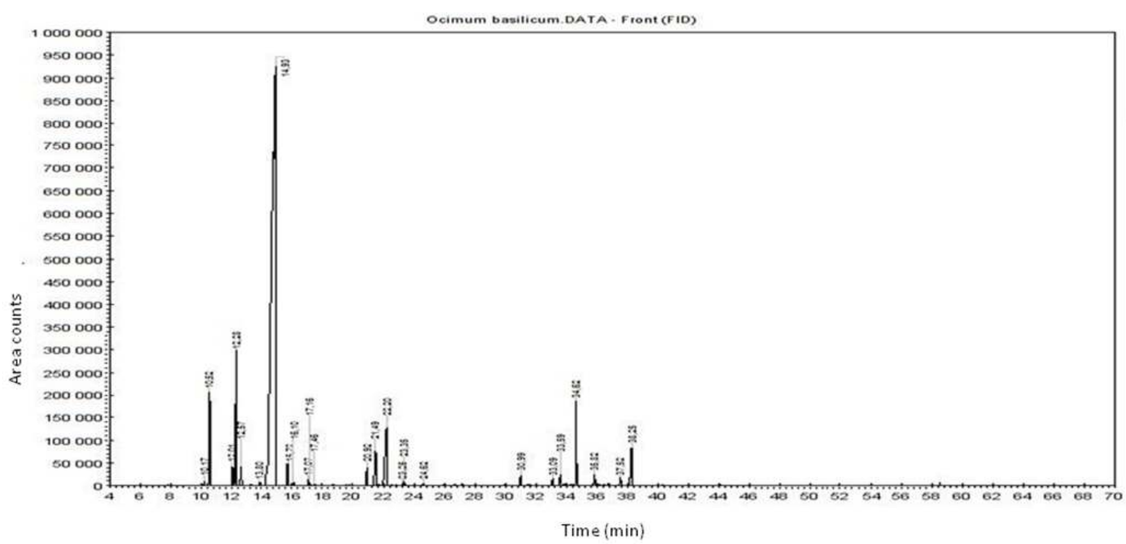

Fig. 1. Gas chromatography profile of *O. basilicum* leaf essential oil

Table 2. Hatching rates of *An. gambiae* eggs according to the concentration of *O. basilicum* essential oil

Concentrations of essential oil (ppm)		100	50	40	30	25	20	10	0	p-value	H-value
Hatching rates (%)	After 1 hour	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	>0.05	0.00
	After 24 hours	0±0	0±0	0±0	1.32±5.7	9.32±1.5	21.32±1.5	94.68±0.58	97.52±0.58	0.0026	21.95
P-value		>0.05	>0.05	>0.05	0.50	0.06	0.06	0.05	0.05		
Z-score		0.0	0.0	0.0	-0.67	-1.8550	0.0593	-1.885	-1.885	-	-

Hatching rates are explained by mean±SD (standard deviations). P-value <0.005 are statistically significant

Table 3. Mortality rates of *An. gambiae* first instars larvae after exposure to *O. basicilicum* essential oil

Concentrations (ppm)		100	50	40	30	25	20	10	0	***p-value***	***H-value***
Mortality rates (%)	After 1 hour	100±0	100±0	90.64±1.53	81.32±1.51	26.68±2.87	20.33±2.52	1.32±5.7	0±0	0.002	22.5215
	After 24 hours	100±0	100±0	100±0	94.68±1.15	73.32±3.51	46.68±3.91	5.32±1.5	0±0	0.002	22.2528
P value		>0.05	>0.05	0.0636	0.0765	0.0809	0.1212	>0.05	>0.05	-	-
Value-Z		0.00	0.0	-1.85	-1.77	-1.75	-1.549	-0.7	0.0		

Mortality rates are explained by mean±SD (standard deviations). P-value <0.005 are statistically significant

Table 4. Mortality rates of *An. gambiae* second instars larvae after exposure to *O. basilicum* essential oil

Concentrations (ppm)		100	50	40	30	25	20	10	0	p-value	H-value
Mortality rates (%)	After 1 hour	100±0	97.32±1.15	84±2.0	57.32±3.05	58.64±2.52	54.68±2.26	0±0	0±0	0.00 23	22.229
	After 24 hours	100±0	100±0	100±0	98.68±1.53	96±1.00	88±1.73	2.64±0.58	0±0	0.0039	20.93
p-value		>0.05	>0.05	0.0636	0.072	0.08	0.076	>0.05	>0.05	-	-
Z-score		0.00	-0.667	-1.8550	-1.7979	-1.7457	-1.7712	-1.3176	0.00	-	-

Mortality rates are explained by mean±SD (standard deviations). P-value <0.005 are statistically significant

Table 5. Mortality rates of *An. gambiae* third instars larvae after exposure to *O. basilicum* essential oil

Concentrations (ppm)		100	50	40	30	25	20	10	0	p-value	H-value
Mortality rates (%)	After 1 hour	100±0	94.68±1.15	88±1.73	52±2.18	18.68±1.53	13.32±1.53	1.32±0.58	0±0	0.0023	22.2935
	After 24 hours	100±0	100±0	100±0	96±1.0	77.32±2.51	69.32±2.51	1.32±0.58	0±0	0.005	22.0675
p-value		>0.05	0.1876	0.0593	0.0765	0.0809	0.080	>0.05	>0.05	-	-
Z-value		0.0000	-1.3176	-1.8856	-1.7712	-1.7457	-1.7457	0.0000	0.00	-	-

Mortality rates are explained by mean±SD (standard deviations). P-value <0.005 are statistically significant

Table 6. Mortality rates of *An. gambiae* fourth instars larvae after exposure to *O. basilicum* essential oil

Concentrations (ppm)		100	50	40	30	25	20	10	0	p-value	H-value
Mortality rates (%)	After 1 hour	100±0	97.32±1.15	84±2.0	58.64±2.52	57.32±3.05	54.68±2.26	0±0	0±0	0.0023	22.229
	After 24 hours	100±0	100±0	100±0	98.68±1.53	96±1.00	88±1.73	2.64±0.58	0±0	0.0039	20.93
p-value		>0.05	0.50	0.0636	0.072	0.08	0.076	0.187	>0.05	-	-
Z-score		0.00	-0.667	-1.8550	-1.7979	-1.7457	-1.7712	-1.3176	0.00	-	-

Mortality rates are explained by mean±SD (standard deviations). P-value <0.005 are statistically significant

Table 7. Mortality rates of *An. gambiae* nymphal stage after exposure to *O. basilicum* essential oil

Concentrations (ppm)		100	50	40	30	25	20	10	0	p-value	H-value
Mortality rates (%)	After 1 hour	100±0.00	78.68±1.15	57.32±2.52	40±2.36	2.64±0.58	1.32±0.58	0±0	0±0	0.0027	21.82
	After 24 hours	100±0	96±1.0	93.32±1.15	41.32±1.63	38.68±2.52	9.32±1.53	4±1.0	0±0	0.0027	21.85
p-value		>0.05	0.076	0.0765	>0.05	0.0765	0.1157	0.197	>0.05	-	-
Z-score		0.00	-1.77	-1.771	0.0000	-1.771	-1.573	-1.291	0.0	-	-

Mortality rates are explained by mean±SD (standard deviations). P-value <0.005 are statistically significant

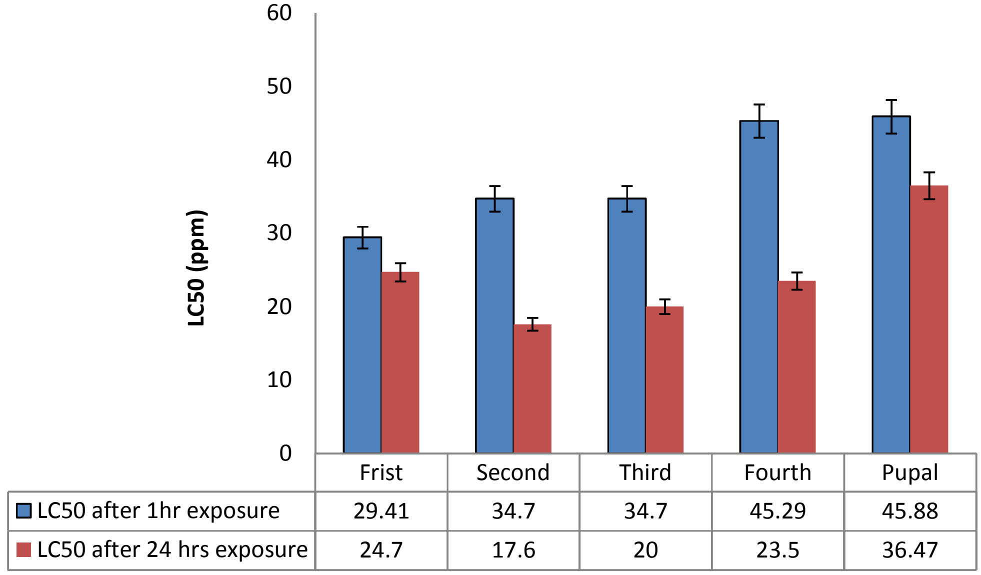

Fig. 2. Letal concentration (LC_{50}) of the larvicidal activity of *O. basilicum* essential oil against pre-imaginal (first, second, third and fourth) and pupal stages of *An. gambiae* in laboratory bioassays

LC_{50} values are given in ppm with standard error bars at 5%

4. CONCLUSION

The finding of this study clearly demonstrated the effectiveness of *O. basilicum* leaf essential oil against aquatic developmental stages of *An. gambiae* in laboratory bioassays. This essential oil was very rich in active components (29 chemicals) and inhibited the eggs hatching at very low concentrations. The larvicidal potential was greater against second and third instars larvae which stand as the most suitable stages to investigate the toxic effect of biological insecticides. Further studies including the efficacy of individual ingredient of *O. basilicum* essential oil, the mode of action and the synergism with the biocides under field condition are needed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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