International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2018; 5(4): 65-74

Received: 11-05-2018
Accepted: 15-06-2018

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# Insecticidal activity of *Cannabis sativa* L leaf essential oil on the malaria vector *Anopheles gambiae s.l* (Giles)

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**Abstract**
Malaria prevention in sub-Sahara Africa rely on the use insecticide based vector control tools. Because of the rapid development of insecticide resistance in vector populations, essential oils could be a serious alternative to insecticides. The study presents the insecticidal activity of *Cannabis sativa* L leaf essential oil on *Anopheles gambiae s.l.* Characterization of *Cannabis sativa* L leaf essential oil showed the presence of terpenes and aliphatic compounds known to have an insecticidal activity. Bioassay conducted with *An. gambiae s.l* larvae indicated that, lethal concentrations causing 50% and 95% mortality were 0.501%; 1.168% and 0.951%; 2.219% after 24hours; 0.201%; 0.217% and 0.381%; 0.412% after 48 hours exposure (respectively for laboratory and field population). Bioassays conducted with adults indicated knock down times for 50% and 95% of 23±0.7min; 28±1.52 min and 45±0.91min; 55.5±0.95min , respectively for the laboratory and field strains. The lethal concentrations killing 50% and 95% of adult mosquitoes were 0.107%; 0.150% and 0.339%; 0.369%, 24 hours post exposure, for the laboratory and field populations respectively.
The essential oil of *Cannabis sativa* L leaf proved to be effective against *Anopheles gambiae s.l* larvae and adults.

**Keywords:** *Anopheles gambiae s.l, Cannabis sativa* L, essential oil, insecticide, bioassay

## 1. Introduction
In most tropical regions and particularly in Sub-Saharan Africa, vector-borne diseases are serious public health threats that cause hindrance to the socio-economic development of communities. Malaria is one of the most important vector-borne disease affecting 3.3 billion people worldwide. Over 90% of malaria cases are estimated to occur in sub-Saharan Africa [1]. Of those concerned, children under five years old and pregnant women are the most affected [1]. The fight against malaria vectors rely mainly on the use of synthetic insecticides for impregnating bed nets or indoor residual spraying [2]. The overreliance on insecticide based interventions has had as immediate consequence the development of insecticide resistance which now affects all vector species across Africa [3, 4]. Many African countries are affected by this threat and there is fear that this could stop the current momentum of decrease in malaria morbidity and mortality following the increase large scale implementation of LLINs and/or IRS across the continent [5, 6]. The rapid expansion of insecticide resistance could derive from many factors including the massive use of insecticide in public health during the last decade for malaria control, the modification of the environment (urbanisation and pollution) [7-9], the use of similar compounds in both public health and in agriculture, the practice of intensive agriculture over large surface areas and in urban settings with massive and uncontrolled used of pesticides [10-12]. Resistance mechanisms develop by mosquitoes could be either mediated by target sites mutations, cuticle resistance or metabolic resistance [13].
In Cameroon insecticide resistance is largely sprayed in major vector species *An. gambiae s.l* and *An. funestus* [5, 19]. Because of the increasing occurrence of insecticide resistance in the main malaria vectors, the search for new compounds or new integrated strategies for managing insecticide resistance is urgently required [20, 21]. In this context, plant- derived extract or essential oils could be potential alternative for controlling malaria vectors or mosquito- borne diseases [22]. These compounds could offer several advantages: they could increase the number

of compounds available for vector control they are biodegradable less toxic for the environment and could be affordable and easy to use. They could be a source of income for local communities. In Cameroun as in many countries across tropical regions, several studies on essential oils insecticidal activities have been conducted over recent years and proved high potential of plant extracts in the control of pests and mosquitoes [23-26].

In Cameroon, *Cannabis sativa* L is found in forest and savannah areas. It use has been tamed for many years because of it negative effects on human health when it is smoked or inhale.It is used by local communities for many purposes. Flowering leaves and somite are rubbed on the skin to fight worms or to repel bees to collect honey. Aerial parts of *Cannabis sativa* L are burned at night to repel mosquitoes. Yet there is no scientific evidence of it impact in killing mosquitoes. The present study was undertaken to investigate the chemical composition and insecticidal activity of *Cannabis sativa* L leaf essential oil on *Anopheles gambiae s.l* larval and adults' stages.

## 2. Materials and Methods

### 2.1 Plant Material

*Cannabis sativa* L leaves were harvested in the village of Salla in the district of Ayos. The specimen was identified under the number 3614SRFK and classified as belonging to Cannabacea family at the National Herbarium.

### 2.2 Essential oil

The essential oil of the leaves of *Cannabis sativa* L was extracted by hydro distillation using a Clevenger apparatus. The essential oil was dried using anhydrous magnesium sulphate and stored at 4 °C, away from UV rays, before use.

### 2.3 Chemical analysis of *Cannabis sativa* L leaves essential oil using Gas chromatography coupled with mass spectrometry

The determination of retention data and the area percentage of the identified compounds were carried out on a two GC-FID systems.

(A) An Agilent 5890 system equipped with HP-1 (ref: 1909 1 Z-115) column (50m x 320µm; 0.5 µm film thickness). GC oven temperature was kept at 80 °C for 8 minutes and programmed to 220 °C at the rate of 2 °C/min.

(B) An Agilent 6890 system equipped with HP-Innowax (ref: 1909 1 N-216, Agilent Technologies, Santa Clara, CA 95051, USA) column (60 m x320µm,0.5µm film thickness).GC temperature was kept at 60 °C and programmed to 245 °C at the rate of 2 °C/min, then constant at 250 °C for 20 min.

The split ratio was adjusted to 1/100. The injector temperature was 250 °C and the FID detector was kept at 250 °C. The carrier gas was Helium (1.3ml/min).

Gas chromatography- Mass spectrometry was carried out using the first system Agilent 5890 equipped with HP-1column.The mass spectra was recorded in the electron impact mode at 70 eV using the aforementioned chromatographic conditions. Individual components of *Cannabis sativa* L leaves essential oil were identified by their retention index as described in Adams [27] and their mass spectra were interpreted using the WILEY L computer library.

### 2.4 Mosquito's collection

The laboratory strain of *Anopheles gambiae s.l* used for our experiment is the "Yaoundé strain" from the Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC). A field strain collected at the larval stage in Yaoundé near river Ewoé (N $03^0$51'34.9'' and E $011^0$31'3'') was also used. Laboratory strain and field population were reared in the insectary of the Higher Teachers' Training College of the University of Yaoundé I. Larvae were fed with Tetramin Baby Fish Food at a rate of 2.5mg per 100 larvae per day [28, 29]. The pupae were collected in plastic cups and placed in emergence cages. Adults emerging from pupae were fed using a 10% glucose solution. The colony of the laboratory strain were maintained continuously at 25-27 °C and 75-78% relative humidity under photoperiod 12L: 12D. *Anopheles gambiae s.l* third and early four instars larvae and female aged 2-5 days old were used to carry out bioassays.

### 2.5 Bioassay

#### 2.5.1 Bioassay with larvae

Larvae of third and early four instars were used to assess the larvicidal activity of *Cannabis sativa* L leaf essential oil following WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides [30]. Mosquitoes were maintained during one hour in distilled water for observation before running the test. Tests concentrations were prepared by adding 1mL of appropriate concentration of essential oil to disposable test cups containing 99 ml of spring water. Batches of 25 larvae of third and early four instars were transferred from observation cups to test cups. Four replicates were run for each concentration and an equal number of controls were run as well. The control was prepared by adding 1ml of absolute alcohol to 99 ml of spring water. Six different concentrations were used (0.1%; 0.2%; 0.3%; 0.5%; 0.7% and 0.9%). Each test was conducted three times on different days. No food was added to cups during the exposure period. Larvae were considered dead when they were incapable of any movement or not swimming actively when touch. The mortality rate was recorded after 24 and 48 hours of exposure. $LC_{50}$ and $LC_{95}$ were calculated using a log probit approach with WINDL CIRAD-CA version2.0 software.

#### 2.5.2 Bioassay with adults

Adult bioassays were performed with 2-5 days- old non-blood- fed females following the WHO Guidelines [31] with cone using impregnated bed net with the essential oil at the following concentrations: 0.025%, 0.05%, 0.1%, 0.2%, 0.3% and 0.5%. Absolute alcohol was used to dilute the essential oil at the various concentrations and 3ml of each concentration was used to impregnate a portion of bed net (98.47$cm^2$). Due to the high volatility of essential oils, pieces of net prepared as indicated above were dried at room temperature away from sun light for 15 minutes before carrying susceptibility assays [32, 33]. Before each test, female mosquitoes were transferred from the cages to the plastic cup for one hour and specimens with broken legs or unable to fly were discarded and replaced. Twenty replicates of batches of 5 mosquitoes per tube were exposed to each concentration for 1hour**.** Five replicates were

run as control using a portion of net impregnated only with a solution of absolute alcohol. The number of knock down (KD) mosquitoes was recorded at 10 minutes intervals during 1 hour exposure period and $KDT_{50}$ and $KDT_{95}$ (time required for knocking down 50% and 95% of the individuals respectively) estimated with 95% of confident interval. After the exposure period, mosquitoes were transferred back to recovery cups and provided with 10% of glucose solution soaked on cotton pad. Mosquito mortality was recorded 24 hours post- exposure. After the tests, adult mosquitoes from field populations were kept in Eppendorf tubes at -4 °C for identification.

### 2.6 Identication of mosquitoes

Adults of the field population were morphologically identified using the Gillies and Coetzee key [34]. Genomic DNA was extracted according to the Livak protocol [35]**.** Molecular identification of females was conducted according to Santolammazza *et al.* protocol [36].

### 2.7 Data analysis for bioassay

The lethal concentrations inducing 50% and 95% ($LC_{50}$ and $LC_{95}$) mortality was calculated using log probit approach with WINDL CIRAD-CA version 2.0 and STATISTICA software version 6.0 to plot the graphs. The relation between the exposure time, the mortality and the doses was assessed using probit regression.

For the adulticidal effect, the time at which 50% and 95% of adult mosquitoes were knocked down (Knock down time, $KTD_{50}$ and $KDT_{95}$) was calculated using WINDL CIRAD-CA software version 2.0. The relation between the knock down time, mortality and the doses were assessed using probit regression.

## 3. Results

### 3.1 Yield of *Cannabis sativa* L leaf essential oil (table 1)

The table 1 provide general characteristics of the plant and the essential oil.

**Table 1:** The oil yield of *Cannabis sativa* L

| Plant name | Family | Certification number | Leaves weight | Oil weight | Yield |
|---|---|---|---|---|---|
| *Cannabis sativa* L | Cannabacea | 25967SRF/Cam | 3,375g | 1.5g | 0.044% |

The essential oil yield of *Cannabis sativa* L expressed as weigh/weigh (w/w) is very low compared to the oil yield of many aromatic plants. Further studies are requested to assess the influence of the soil, climate, water stress, the collection period, extraction method on the yield.

### 3.2 Chemical composition of *Cannabis sativa* L leaf essential oil

Up to 81 different constituents were identified during analysis (Table 2). The main constituents of *Cannabis sativa* L leaf essential oil are sesquiterpenes (E-β-Caryophyllene (10.72%); Caryophyllene oxide (1.25%); E-β-Farnesene (1.25%) and monoterpenes (Myrcene (31.75%); terpinolene (14.76%); α-pinene (8.12%); β-pinene (3.64%); limonene (1.30%) and E-β-Ocimene (1.10%). Among these compounds: α-pinene; β-pinene; Z-β- ocimene; menthone; E,Z-P-menthen-2-en-1-ol; 1,8-cineol; sabinene; terpinen-4-ol; β-phellandrene; α-phellandrene; Terpinolene; Thujene and linalool are reported to have an inhibitory or biocide activity against several species of bacteria and insects.

**Table 2:** Chemical composition of *Cannabis sativa* L leaf essential oil

| Elution order | Compound | Percentage | Kovat indices | |
|---|---|---|---|---|
| | | | HP-1 | Innowax |
| 1. | Z-3-Hexenol | 0.03 | 842 | 1380 |
| 2. | E-2-Hexenol | 0.02 | 852 | 1408 |
| 3. | Heptanone-2 | 0.03 | 864 | 1182 |
| 4. | Hexenol | 0.03 | 869 | 1351 |
| 5. | Heptanal | 0.03 | 880 | 1185 |
| 6. | 2-Amyl furan | 0.01 | 884 | 1124 |
| 7. | α-Thujene | 0.12 | 923 | 1032 |
| 8. | α-Pinene | 8.12 | 931 | 1035 |
| 9. | Camphene | 0.013 | 944 | 1063 |
| 10. | Octene-1-ol-3 | 0.03 | 961 | 1427 |
| 11. | 6-Methyl-5-heptenone-2 | 0.005 | 964 | 1116 |
| 12. | Sabinene | 0.12 | 967 | 1118 |
| 13. | β-Pinene | 3.64 | 971 | 1132 |
| 14. | 6- Methyl-5-hepten-2-ol | 0.02 | 974 | 1163 |
| 15. | Myrcene | 31.75 | 981 | 1159 |
| 16. | 𝛘-3-Carene | 0.92 | 997 | 1011 |
| 17. | α-Phellandrene | 0.42 | 998 | 1175 |
| 18. | α-Terpinene | 0.31 | 1010 | 1188 |
| 19. | Hexylacetate | 0.02 | 1010 | 1272 |
| 20. | P-Cymene | 0.11 | 1014 | 1200 |
| 21. | Limonene | 1.3 | 1022 | 1203 |
| 22. | 1,8-Cineole+β-phellandrene | 1.29 | 1022 | 1213 |
| 23. | Z-β-Ocimene | 1.10 | 1025 | 1242 |
| 24. | E-β-Ocimene | 9.65 | 1037 | 1247 |

| | | | | |
|---|---|---|---|---|
| 25. | ϒ-Terpinene | 0.23 | 1049 | 1255 |
| 26. | E-Thujan-4-ol | 0.03 | 1053 | 1463 |
| 27. | Fenchone+methyl benzoate | 0.02 | 1072 | - |
| 28. | p-Cymenene | 0.09 | 1072 | 1437 |
| 29. | Terpinolene | 14.76 | 1079 | 1290 |
| 30. | Nonanal | 0.03 | 1083 | 1391 |
| 31. | Linalool | 0.09 | 1086 | 1533 |
| 32. | Z-Thujan-4-ol | 0.03 | 1092 | - |
| 33. | Perillen | 0.02 | 1098 | 1429 |
| 34. | α-Fenchol | 0.07 | 1100 | 1570 |
| 35. | Z-p- Menth-2-en-1-ol | 0.03 | 1114 | 1570 |
| 36. | E-p- Menth-2-en-1-ol | 0.07 | 1114 | 1571 |
| 37. | 4E, 6Z- Allo-ocymene | 0.01 | 1119 | 1375 |
| 38. | Ipsdienol | 0.03 | 1125 | - |
| 39. | Epoxy terpinolene | 0.05 | 1130 | - |
| 40. | Menthone | 0.04 | 1136 | 1465 |
| 41. | Borneol | 0.04 | 1153 | 1719 |
| 42. | P-Cymen-8-ol | 0.12 | 1161 | 1864 |
| 43. | Terpinen-4-ol | 0.07 | 1163 | 1611 |
| 44. | α-Terpineol | 0.04 | 1175 | 1682 |
| 45. | Citronellol | 0.02 | 1212 | 1764 |
| 46. | Hexyl butyrate +methylchavicol | 0.06 | 1224 | - |
| 47. | E-Anethole | 0.10 | 1264 | 1826 |
| 48. | α-Ylangene | 0.20 | 1370 | 1490 |
| 49. | α-Copaene | 0.01 | 1375 | 1491 |
| 50. | β-Elemene | 0.02 | 1386 | 1577 |
| 51. | Isocaryophyllene | 0.23 | 1408 | - |
| 52. | Z-α-Bergamotene | 0.11 | 1410 | 1559 |
| 53. | α-Santalene | 0.14 | 1415 | 1582 |
| 54. | E-β-Caryophyllene | 10.72 | 1419 | 1612 |
| 55. | E-α-Bergamotene | 0.85 | 1435 | 1575 |
| 56. | Allo-Aromadendrene | 0.30 | 1439 | 1620 |
| 57. | α-Guaiene +unidentified compound | 0.04 | 1442 | - |
| 58. | E-β-Farnesene | 1.23 | 1449 | 1663 |
| 59. | ϒ-Elemene+unidentified compound | 0.04 | 1449 | - |
| 60. | α-Humulene | 3.28 | 1452 | 1654 |
| 61. | ϒ- Muurolene | 0.08 | 1473 | 1689 |
| 62. | Selina-4,11-diene | 0.22 | 1475 | 1674 |
| 63. | Selina-4,7(11)diene+E-α-Bisabolene | 0.36 | 1475 | 1688 |
| 64. | β-Selinene | 0.58 | 1480 | 1700 |
| 65. | α-Selinene | 0.41 | 1491 | 1707 |
| 66. | E,E-α-Farnesene | 0.20 | 1496 | 1744 |
| 67. | β-Bisabolene | 0.21 | 1499 | 1727 |
| 68. | 7-Epi-α-Selinene | 0.21 | 1507 | 1764 |
| 69. | ϒ-Cadinene | 0.05 | 1513 | 1740 |
| 70. | 𝛘-Cadinene | 0.09 | 1513 | 1765 |
| 71. | 12-Nor-caryophyll-5-en-2-one | 0.02 | 1515 | - |
| 72. | ϒ-Selinene | 0.24 | 1526 | - |
| 73. | Germacrene B | 0.14 | 1535 | 1823 |
| 74. | Selina-3,7(11)-diene | 0.37 | 1537 | 1783 |
| 75. | E-Nerolidol | 0.15 | 1550 | 2036 |
| 76. | Spathulenol | 0.03 | 1565 | 2107 |
| 77. | Epoxy Caryophyllene | 0.08 | 1563 | - |
| 78. | CaryophylleneOxide | 1.25 | 1567 | 2001 |
| 79. | HumuleneOxide | 0.30 | 1597 | 2047 |
| 80. | Not identified compound | 0.14 | - | - |
| 81. | Not identified compound | 0.18 | - | - |
| | | | | |
| | TOTAL | 98.27 | | |

Analysis performed by gas chromatography with FID detection on 2 columns of different polarity: HP1 and INNOWAX Individual components are identified by their retention index as described in Adams (2012) and their mass spectra are interpreted using the WILEY L computer library. Constituents are presented in the order of elution from the columns.

### 3.3 Larvicidal activity of *Cannabis sativa* L leaf essential oil on *Anopheles gambiae s.l* after 24 and 48 hours exposure

A total of 3600 *An. gambiae s.l* larvae were exposed to different essential oil concentrations (0.1%; 0.2%; 0.3%; 0.5%; 0.7% and 0.9%) and mortality was recorded 24 and 48 hours after exposure. The dose response curve generated after 24 hours exposition to *Cannabis sativa* L leaf essential oil showed important differences between the laboratory and field strain (figure 1). The laboratory strain was found to be more susceptible to the essential oil. The average dilution of essential oil causing 50% and 95% mortality ($LC_{50}$ and $LC_{95}$) were 0.501% and 1.16% respectively for the laboratory strain. For the field strain, doses causing 50% and 95% mortality were 0.951% and 2.219% respectively. The level of susceptibility of the two strains to the essential oil was found to be statistically different (H=3.46; p=0.018).

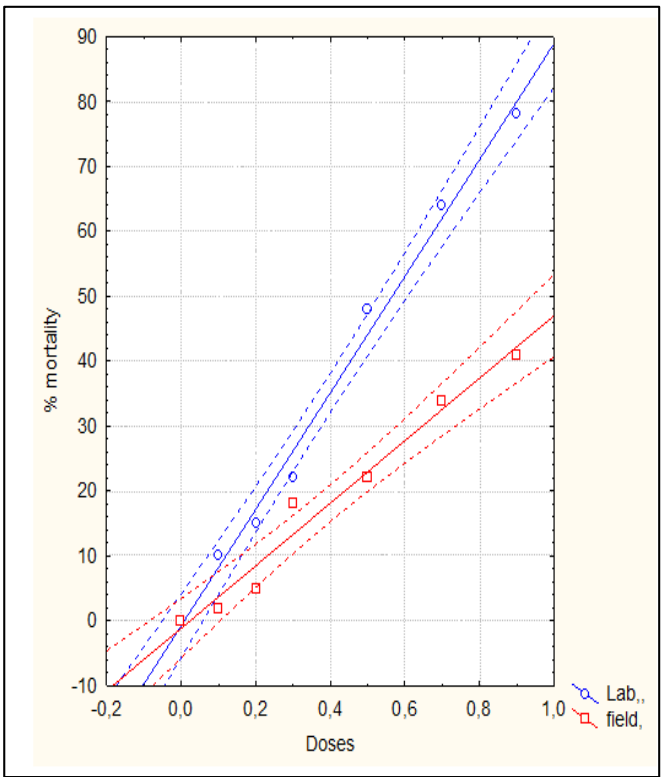


**Fig 1:** Doses-response curve of larvicidal activity of *C.sativa* L leaf E.O on *An. gambiae s.l* after 24 hours

The larvicidal activity of *C. sativa* L leaf essential oil was also assessed on *An. gambiae s.l* larvae after 48 hours exposure (figure 2). From the experiments it appeared that the level of mortality increased with the concentration of essential oils in both the laboratory and field strain. $LC_{50}$ and $LC_{95}$ doses of 0.201%; 0.217% and 0.381%; 0.412% respectively for the laboratory strain and field population were recorded. Laboratory populations were again found to be more susceptible than field population to the essential oil. The tolerance level of larvae after 24 hours exposure was less important than the level after 48 hours exposure and were significantly different for the field population (*H=6.56; p=0.013*). The different equations of regression lines generated (1; 2) following the dose response analysis were respectively (for 24 hours exposure period):

$$Y_{lab}=-0.746+89.71x \quad (1)$$

$$Y_{field}=-1.152+48.171x \quad (2)$$

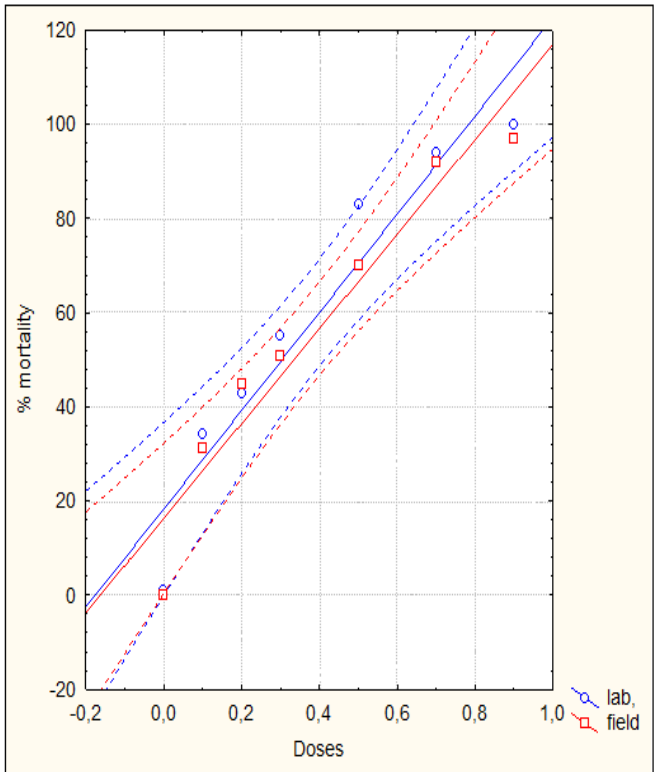


**Fig 2:** Dose-response curve of larvicidal activity of *C.sativa* L leaf E.O on *An. gambiae s.l* after 48 hours

After 48 hours of exposure, the different equations of the regression lines generated were (3; 4):

$$Y_{lab}=18.334+104.31x \quad (3)$$

$$Y_{field}=16.359+100.55x \quad (4)$$

### 3.4 Knock down activity of *Cannabis sativa* L leaf essential oil on adult of *Anopheles gambiae s.l*

During 1 hour, 100 adult female *An. gambiae s.l* were exposed to each concentration of the *C.sativa* L leaf essential oil. At 10 minutes interval, the number of mosquito knock down were counted and recorded. It appeared from the assay that the number of mosquito knocked down increases with essential oil concentration and with the length of exposure (table 3).

**Table 3:** Activity of *C. sativa* L leaf essential oil on adult of *An. gambiae s.l*

| Colonies | Nb of mosquitoes exposed | Nb of replicates | Concentration of essential oil (%) | Mortality after 24H | 95% CI |
|---|---|---|---|---|---|
| Laboratory | 100 | 20 | 0.5 | 100±0.4 | 100-100 |
| | 100 | 20 | 0.3 | 84±0.5 | 79-88 |
| | 100 | 20 | 0.2 | 68±0.6 | 64-73 |
| | 100 | 20 | 0.1 | 54±0.6 | 52-58 |
| | 100 | 20 | 0.05 | 15±0.5 | 12-18 |
| | 100 | 20 | 0.025 | 08±0.4 | 2-11 |
| Field | 100 | 20 | 0.5 | 98±0.6 | 96-102 |
| | 100 | 20 | 0.3 | 86±0.6 | 82-90 |
| | 100 | 20 | 0.2 | 60±0.4 | 55-64 |
| | 100 | 20 | 0.1 | 24±0.4 | 21-27 |
| | 100 | 20 | 0.05 | 10±0.3 | 8-14 |
| | 100 | 20 | 0.025 | - | - |

Considering only the highest concentration of essential oil (0.50%), the $KDT_{50}$ and $KDT_{95}$ calculated with 95% confidence limits (*t=5.268: p=0.003 and t=4.710: p=0.005*) are 23±0.7min; 28±1.52 min and 45±0.91min; 55.5±0.95min respectively for the laboratory strain and field population. The knock down response curve is shown in Figure 3 below.

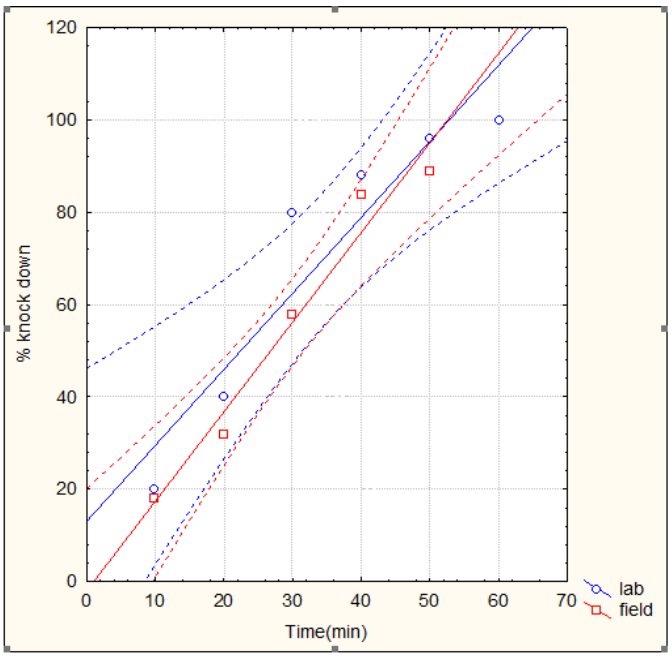


**Fig 3:** Knock down response curve of the activity of *C.sativa* L leaf E.O on *An. gambiae s.l*

Knock down times were different between the two populations of *An.gambiae s.l.* Female adult from the field were less susceptible compare to laboratory strain to *Cannabis sativa* L essential oil(t=5.26; p=0.003 and t=4.71; p=0.005). The different equations generated from the regression analysis are presented below (5;6).

$$Y_{lab}=13.066+1.64x \quad (5)$$

$$Y_{field}=3.133+1.72x \quad (6)$$

### 3.5 Determination of sublethal concentration of essential oils inducing adult mortality

The analysis of the trend of mortality 24 hours post exposure showed an increase in mortality according to essential oils concentration. With dilution of 0.5% mortality rate of 100% was recorded for the laboratory strain whereas mortality rate of 98% were recorded for the field population. The lethal concentration inducing 50% of mortality ($LC_{50}$) and 95% of mortality ($LC_{95}$) for the laboratory strain were 0.107% and 0.150% (t=3.13 p=0.020); for the field strain 0.339% and 0.369% (t=2.54; P=0.043).

The diagnostic dose defined as twice the lethal concentration for 99% of mortality [31] is above 0.50% for the field population. The dose-mortality response curve is shown on the Figure 4.

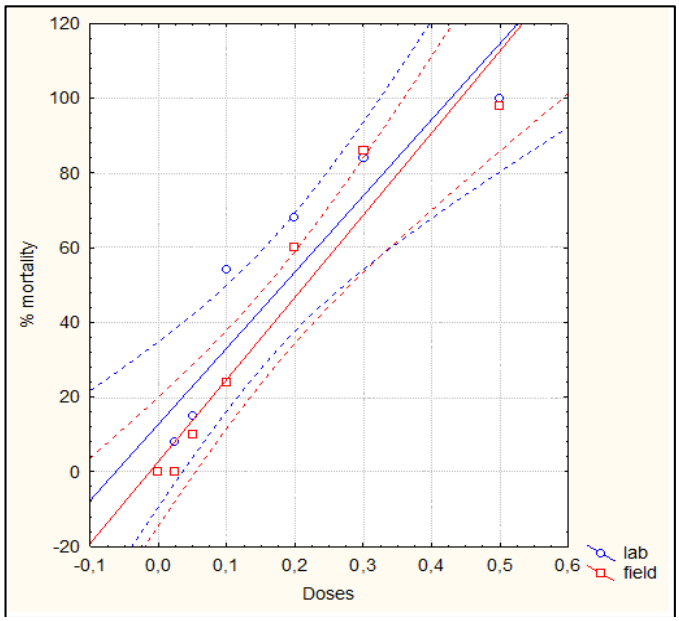


**Fig 4:** Dose response curves of *C.sativa* L leaf essential oil on adult *An. gambiae s.l* females

The essential oil of the leaves of *Cannabis sativa* L is effective both for the laboratory strain as for the wild population of *An.gambiae s.l* (t=3.138, *p=0.020; t=2.544; p=0.043).*

The level of susceptibility of the two populations appeared significantly different (H=3.40; P=0.027), and consequently the doses response curve of individuals in the wild population is below that of the laboratory strain in low concentrations. This reveals that these wild individuals are more tolerant to *Cannabis sativa* L essential activity oil than the laboratory

strain. The different equations of the regression lines of the adulticidal activity (7; 8) are respectively:

$$Y_{lab}=12.789+203.81x \quad (7)$$

$$Y_{field}=2.837+219.69x \quad (8).$$

### 3.6 Identification of field population of *Anopheles gambiae s.l*

Mosquitoes from the laboratory strain are all *An. coluzzii*. A total of 71 adult females from the field population were further processed to identify members of the *An. gambiae* complex. From the analysis, it appeared that 3 (4.23%) were *An. gambiae s.s* and 68 (95.77%) were *An. coluzzii.*

## 4. Discussion

Our study objective was to characterize the chemical composition of *Cannabis sativa* L leaf essential oil and to evaluate its insecticidal properties on the major malaria vector *Anopheles gambiae s.l.* High mortality rate was recorded after exposing both *An. gambiae s.l* larvae and adults to *C. sativa* leaf essential oil. The following supporting the insecticidal activity of the essential oil.

The extraction of essential oil of *Cannabis sativa* L yielded very low concentration 0.044% (w/w). This yield was far less than those reported for the essential oils of *Cympobogon citratus* (1.7%); *Cympobogon giganteus* (1.4%) [3] and *Clausena anisata* (0.24%) [37]. The low yield recorded, could result from the harvesting period of the leaves, the method of growing the plant or the method of extraction. The characterization of essential oil of *Cannabis sativa* L revealed the presence of up to 81 different compounds. Among compounds identified, several with known insecticidal activities on mosquito larval stages were recorded such as α-Pinene; β-pinene; 1,8-cineol and Z-β- ocimene which have been reported to induce high mortality to *Aedes aegypti* [38 ,39], *Aedes albopictus* [40, 41], *Anopheles stephensi* [40, 42] and *Anopheles gambiae* [43] larvae. In addition, menthone and E, Z-P-menthen-2-en-1-ol known for their larvicidal activity on *Culex quinquefasciatus* larvae were also recorded [44]. Some of the compounds characterized such as linalool, *p*-Cymene, 1,8- Cineol, Terpinolene and Thujene were also reported to induce a biocidal or inhibitory activities on several pathogenic Bacteria: Bacteria *Shigella* [45] and different bacteria classes [46, 47]. Yet a certain number of compounds could not be identified further characterization is still required. Although high lethality of larvae was detected 24 hours and 48 hours after exposure, it is not sure that such level of lethality could be recorded in the nature. Further studies are required to assess the residual effect of this compound in the environment. Analysis conducted with adult mosquitoes also showed high lethality of mosquitoes exposed to impregnated net with *C. sativa* L leaf essential oil. It is possible that the induce lethality recorded could have been driven by compounds such as E-p-Menth-2-en-1-ol, 1, 8-Cineol; p-Cymene;α-Pinene and β-Pinene which were reported to have an insecticidal activity against adults *Anopheles stephensi*[42] and *Anopheles gambiae* [3, 43]. Compounds such as Limonene, α-Pinene, 1,8-Cineol ,sabinene,terpinen-4-ol, β-phellandrene and α-phellandrene were all reported to have a significant inhibitory activities on acetylcholinesterase and adenosine triphosphatase on *Sitophilus oryzae* [48]. It is likely that the effect of the essential oil of *Cannabis sativa* L leaf could be amplified by the presence or the synergist effect of different sets of compounds with as major components, Myrcene, Terpinolene, E-β-Caryophyllene, E-β-Ocimenene, α-Pinene,β-Pinene,α-humulene, limonene, E-β-Farnesene [49]. The following needs to be further investigated through both field and laboratory analysis. *Cannabis sativa* L leaf essential was found to induce a rapid knock down effect when adults were exposed to impregnated net. This suggests that, the essential oils which is a cannabinoid substance may affect directly the insect nervous system. Studies conducted in the brain suggested that cannabinoids receptors are mainly located in the basal ganglia, cerebellum and hippocampus all associated with body movement control and coordination and this could rather explain the rapid knock down effect recorded during the study [50].

In human subjects cannabinoids were reported to have various psychological and physiological effects due to their direct effect on the nervous system [51]. Studies conducted on both vertebrate and invertebrates indicated that once in the body, cannabinoids bind to cannabinoid receptors and decrease adenyl cyclase activity, inhibiting calcium N channels and disinhibiting $K^+$ channel which all affect the transmission of nerve impulse [50]. Most of insecticides used in vector control have as target the sodium channel or acetylcholine esterase [13].

Because of the rapid spread of insecticide resistance affecting major malaria vectors [52, 19, 53, 54] and different mode of action of cannabinoids they could be good alternative for the control of resistant mosquitoes.

Comparing the level of susceptibility between field and laboratory populations it appeared that field populations were more tolerant to *C. sativa* L leaf essential oil than the laboratory colony. The following could come from the fact that field mosquito populations are always exposed to different sets of pollutants in the environment which increase overexpression of a certain number of metabolic mechanisms involved with detoxification [55]. Although no investigation of specific mechanisms involved with mosquito tolerance was undertaken. Yet studies conducted in Yaoundé indicated the presence of kdr allele and the overexpression of several detoxification enzymes involved with mosquitoes tolerance to pyrethroids and xenobiotics [56, 57].

## 5. Conclusion

Our study demonstrated high insecticidal effect of *C.sativa* L leaf essential oil against both larvae and adult *An. gambiae s.l* and support the use of this plant for vector control. With the rapid expansion of insecticide resistance to almost all existing insecticides the search for new compounds capable of replacing existing one could be particularly beneficial for the control of a large range of insects vectors responsible for the transmission of diseases across sub-Saharan Africa. Yet for *C. sativa* L further studies are needed for cultivation and extraction process to improve the yield. Also, further field trials and laboratory analysis are required to establish the efficacy of *C. sativa* L leaf essential oil under different conditions and also it efficiency in the control of mosquitoes in the nature.

**6. Acknowledgments**
We thank OCEAC for providing mosquitos' eggs; Pr M. Nyegue for hydro distillation of essential oil; H. Casabianca of the Institute of Analytic Science for chemical analysis of essential oil and F. Zeukeng for his advices.

**7. References**

1. WHO. World malaria report, Geneva: World Health Organization, 2016
2. Zaim M, Aitio A, Nakashima N. Safety of pyrethroid-treated mosquito nets. Med Vet. Ent. 2000; 14:1-5.
3. Bossou AD, Mangelinckx S, Yedomonhan H, Boko PM, Akogbeto MC, De Kimpe N *et al*. Chemical composition and insecticidal activity of plant essential oils from Benin against *Anopheles gambiae* (Giles). Paras Vectors. 2013; 6:337-354.
4. Wanjala CL, Zhou G, Mbugi J, Simbauni J, Afrane YA, Ednah Ototo E *et al*. Insecticidal decay effects of long-lasting insecticide nets and indoor residual spraying on *Anopheles gambiae* and *Anopheles arabiensis* in Western Kenya. Paras Vectors. 2015; 8:588-598.
5. Etang J, Manga L, Toto JC, Guillet P, Fondjo E, Chandre F. Spectrum of metabolic-based resistance to DDT and pyrethroids in *Anopheles gambiae s.l.* populations from Cameroon. J Vect. Eco. 2007; 32(1):123-133.
6. Okia M, Ndyomugyenyi R, Kirunda J, Byaruhanga A, Adibaku S, Lwamafa DK *et al*. Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of Anopheles gambiae s.s. from different malaria transmission zones in Uganda. Paras Vectors. 2013; 6:130-140
7. Chouaïbou MS, Fodjo BK, Fokou G, Allassane OF, Koudou BG, David JP *et al*. Influence of the agrochemicals used for rice and vegetable cultivation on insecticide resistance in malaria vectors in southern Côte d'Ivoire. Malar J. 2016; 15:426-438
8. Nkya TE, Akhouayri I, Kisinza W, David JP. Impact of environment on mosquito response to pyrethroid insecticides: Facts, evidences and prospects. Insect Biochem. Mol. Bio. www.elsevier.com/locate/ibmb. 2013; 43:407-416.
9. Nkya TE, Poupardin R, Laporte F, Akhouayri I, Mosha F, Magesa S *et al*. Impact of agriculture on the selection of insecticide resistance in the malaria vector*Anopheles gambiae*: a multigenerational study in controlled conditions. Paras Vectors. 2014; 7:480-492.
10. David JP, Ismail HM, Chandor-Proust A, Paine MJI. Role of cytochrome P450s in insecticide resistance: impact on the control of mosquito-borne diseases and use of insecticides on Earth. Phil Trans R Soc B *368:20120429;* 2013, 1-12.
11. Antonio-Nkondjio C, Defo-Talom B, Tagne-Fotso R, Tene-Fossog B, Ndo C, Lehman LG *et al*. High mosquito burden and malaria transmission in a district of the city of Douala, Cameroon. BMC Infect. Dis. 2012; 12:275-283
12. Diniz DFA, Varjal de Melo-Santos MA, de Mendonça Santos EM, Beserra EB, Helvecio E, de Carvalho-Leandro D *et al*. Fitness cost in field and laboratory *Aedes aegypti* populations associated with resistance to the insecticide temephos. Paras Vectors. 2015; 8:662-677.
13. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends in Paras. 2011; 27(2):1-8
14. Djogbénou L, Dabire´ R, Diabate´A, Kengne P, Akogbéto M, Hougard JM *et al*. Identification and Geographic Distribution of the ACE-1R Mutation in the Malari Vector Anopheles gambiae in South-Western Burkina Faso, West Africa. Am. J Trop. Med. Hyg. 2008; 78(2):298-302
15. Edi CV, Djogbe´nou L, Jenkins AM, Regna K, Muskavitch MAT, Poupardin R *et al*.CYP6 P450 Enzymes and ACE-1 Duplication Produce Extreme and Multiple Insecticide Resistance in the Malaria Mosquito *Anopheles gambiae*. PLOS Genetics. 2014; 10(3)e1004236:1-12
16. Weetman D, Steen K, Rippon EJ, Mawejje HD, Donnelly MJ, Wilding CS. Contemporary gene flow between wild *An. gambiae* s.s. and *An. arabiensis*. Paras. Vect. 2014; 7:345-353
17. Wondji CS, Colemana M, Kleinschmidtb I, Mzilahowac T, Irvinga H, Ndulaa M *et al*. Impact of pyrethroid resistance on operational malaria control in Malawi. www.pnas.org/cgi/doi/10.1073/pnas.1217229109 2012; 109(47):1-8.
18. Riveron JM, Chiumia M, Menze BD, Barnes KG, Irving H, Ibrahim SS *et al*. Rise of multiple insecticide resistance in *Anopheles funestus* in Malawi: A major concern for malaria vector control. Malar J. 2015; 14:344-353
19. Antonio-Nkondjio C, Tene Fossog B, Kopya E, Poumachu Y, Menze Djantio M, Ndo C *et al*. Rapid evolution of pyrethroid resistance prevalence in Anopheles gambiae populations from the cities of Douala and Yaoundé (Cameroon). Malar. J. 2015; 14:155-16319.
20. Samuel M, Oliver SV, Coetzee M, Brooke BD. The larvicidal effects of black pepper (*Piper nigrum* L.) and piperine against insecticide resistant and susceptible strains of Anopheles malaria vector mosquitoes. Paras Vectors 2016; 9:238-247.
21. Fontenille D. Écosystèmes, entomologie et lutte anti-vectorielle. RESP. ENV. 2008; (51):55-63
22. Deletre E , Chandre F, Williams L, Duménil C, Menut C, Martin T. Electrophysiological and behavioural characterization of bioactive compounds of the *Thymus vulgaris*, *Cymbopogon winterianus*, *Cuminum cyminum* and *Cinnamomum zeylanicum* essential oils against *Anopheles gambiae* and prospects for their use as bednet treatments. *Paras Vectors.* 2015; 8:316-330.
23. Foko Dadji GA, Tamesse JL, Fankem Boyom F. Adulticidal effect of essentizl extractsfrom *Capsicum annuum* (Solanaceae*) Piper nigrum* (Piperaceae) and *Zingiber officinale* (Zingiberaceae)on *Anopheles gambiae* (Diptera-Culicidae) vector of malaria. J Nama. 2007, 1-12
24. Tchoumbougnang F, Jazet Dongmo PM, Sameza ML, Nkouaya Mbanjo EG, Tiako Fotso GB, Amvam Zollo PH *et al*. Activité larvicide sur *Anopheles gambiae* Giles et composition chimique des huiles essentielles extraites de quatre plantes cultivées au Cameroun. Biotechnol. Agron. Soc. Environ. 2009; 13(1):77-84.

25. Akono P, Belong P, Tchoumbougnang F, Bakwo EM, Fankem H. 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 s.s, vecteur du paludisme au Cameroun. J Appl. Biosci. 2012; 59:4340-4348.
26. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. Food Chem. Tox.www.elsevier.com/locate/foodchemtox 2008; 46:446-475
27. Adams RP. Identification of essential oils by gas chromatography quadrupolemass spectroscopy. Allured Publishing Corporation, 4th Edition, Carol Stream 9. 2012, 698
28. Price DP, Schilkey FD, Ulanov A, Hansen IA. Small mosquitoes, large implications: crowding and starvation affects gene expression and nutrient accumulation in Aedes aegypti. Paras Vectors. 2015; 8:252-266.
29. Vantaux A, Ouattarra I, Lefèvre T, Dabiré KR. Effects of larvicidal and larval nutritional stresses on *Anopheles gambiae* development, survival and competence for Plasmodium falciparum. Paras Vectors. 2016; 9:226-237.
30. WHO. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. Geneva: World Health Organization, 2005
31. WHO. Test procedures for insecticide resistance in malaria vector Mosquitoes. Geneva: World Health Organization, 2013, 1-40
32. Wang X, Li O, Shen L, Yang J, Cheng H, Jiang S *et al*. Fumigant, contact, and repellent activities of essential oils against the darkling beetle, *Alphitobius diaperinus*. J Insect science. 2014; 14(75)
33. Faraone N, Hillier NK, Cutler GC. Plant Essential Oils Synergize and Antagonize Toxicity of Different Conventional Insecticides against *Myzus persicae* (Hemiptera: Aphididae). PLoS ONE. 2015; 10(5):e0127774
34. Giles MT, Coetzee M. A supplement to the Anophelinae of Africa South of the Sahara. South Af. Inst. Med. Res. 1987; 55:146
35. Livak KJ. Organization and mapping of sequence of Drosophila melanogaster X-chromosome and Y-chromosome that is transcribed during spermatogenesis. *Genetics.* 1984; 107:611-634
36. Santalamazza F, Mancini E, Simard F, Qi Y, Tu Z, Della Torre A. Insertion polymorphism of SINE200 retrotransposons within speciation island of Anopheles *gambiae* moleucular forms. Malar J. 2008; 7:163
37. Foko Dadji GA, Bobo Ngowe MP, Nyegue MA, Tamesse JL. Chemical composition and biocide properties of *Clausena anisata* (Rutacea) essential oil against the development stages of the malaria vector *Anopheles coluzzii*. *AJEONP* 2018; 6(1):09-15
38. Lucia A, Audino P, Seccacini E, Licastro S, Zebra E, Masuh E. Larvicidal effect of *Eucalytpus grandis* essential oil and turpentine and their major components on *Aedes aegypti* larvae. J Am. Mosq. Cont. As. 2007; 23(3):299-303
39. Dias CN, Lima Alves LP, Rodrigues KAF, Aranha Brito MC, Rosa CS, Mendonça do Amaral F *et al*. Chemical Composition and Larvicidal Activity of Essential Oils Extracted from Brazilian Legal Amazon Plants against *Aedes aegypti* L. (Diptera: Culicidae). *Evidence-Based* Comp. Alter. Med, *2015*, 1-9
40. Zhu J, Zeng X, Ma Y, Liu T, Qian K, Han Y *et al.* Adult Repellency and Larvicidal Activity of Five Plant Essential Oils Against Mosquitoes. J Am Mosq Cont. Assoc. 2006; 22(3):515-522.
41. Evergetis E, Michaelakis A, Papachristos DP, Badieritakis E, Kapsaski-Kanelli VN, Haroutounian SA. Seasonal variation and bioactivity of the essential oils of two Juniperus species against Aedes (Stegomyia) albopictus (Skuse, 1894). Parasitol Res. 2016, 1-9
42. Nathan SS. The use of *Eucalyptus tereticornis* Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Biores. Tech. 2007; 98:1856-1860
43. Kweka EJ, Lima TC, Marciale CM. Pergentino de Sousa D. Larvicidal efficacy of monoterpenes against the larvae of Anopheles gambiae. Asian Pac J Top Biomed 2016; 6(4):290-294.
44. Pavela R, KAFFKOVÁ K, KUMŠTA M. Chemical Composition and Larvicidal Activity of Essential Oils from Different Mentha L. and Pulegium Species against *Culex quinquefasciatus* Say (Diptera: Culicidae*).* Plant Protect. Sci. 2014; 50(1):36-42
45. Bagamboula CF, Uyttendaele M, Debevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri.* Food Microbio. 2004; 21:33-42
46. Rodriguez Flores C, Pennec A, Nugier-Chauvin C, Daniellou R, Herrera-Estrella L, Chauvina AL. Chemical Composition and Antibacterial Activity of Essential Oils Extracted from Plants Cultivated in Mexico. J Mex. Chem. Soc. 2014; 58(4):452-455
47. Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. Evidence-Based Comp. Alt. Med. 2016, 1-21.
48. Abdelgaleil SAM, Mohamed MIE, Shawir M, Abou-Taleb HK. Chemical composition, insecticidal and biochemical effects of essential oils of different plant species from Northern Egypt on the rice weevil, *Sitophilus oryzae* L. J Pest Sci. 2015, 1-11
49. Mulyaningsih S, Sporera F, Zimmermann S, Reichling J, Wink M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. Phytomed. 2010; 17:1061-1066
50. Kendall DA, Yudowski GA. Cannabinoid Receptors in the Central Nervous System: Their Signaling and Roles in Disease. Front. Cell. Neurosci. 2017; 10:294
51. Skaper SD, Di Marzo V. Endocannabinoids in nervous system health and Disease: the big picture in a nutshell. Phil. Trans. R. Soc. B. 2012; 367:3193-3200
52. Nwane P, Etang J, Chouaïbou M, Toto JC, Koffi A, Mimpfoundi R *et al*. Multiple insecticide resistance mechanisms in *Anopheles gambiae s.l* populations from Cameroon, Central Africa. Paras Vect. 2013; 6:41-55.
53. Antonio‐ Nkondjio C, Poupardin R, Fossog Tene B, Kopya E, Costantini C, Awono‐ Ambene P *et al.*

Investigation of mechanisms of bendiocarb resistance in *Anopheles gambiae* populations from the city of Yaoundé, Cameroon. Malar J. 2016; 15:424-435
54. Muller P, Chouaibou M, Pignatelli P, Etang J, Walker ED, Donelly MJ *et al*. Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in *Anopheles arabiensis* sampled from an area of cotton fields in Northern Cameroon. Mol. Ecol. 2007, 1-11
55. Li X, Schuler M, Berenbaum M. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu Rev Entomol 2007; 52:231-253
56. Tene Fossog B, Poupardin R, Costantini C, Awono-Ambene H, Wondji C, Ranson H *et al* Resistance to DDT in an urban setting: Common mechanisms implicated in both M and S forms of Anopheles gambiae in the city of Yaoundé Cameroon. PLoS One 2013; 8:e61408
57. Antonio-Nkondjio C, Fossog B, Ndo C, Djantio B, Togouet S, Awono-Ambene P *et al* Anopheles gambiae distribution and insecticide resistance in the cities of Douala and Yaounde (Cameroon): influence of urban agriculture and pollution. Malar J. 2016, 10:154.