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Chemical composition and Insecticidal Activity of *Piper umbellatum* leaf. essential oil on the major malaria vector *Anopheles gambiae* S.L and *Culex quinquefasciatus*

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Abstract
The prevention of Arthropod borne diseases is based on the use of synthetic insecticides. Because of the development of vector resistance to the advocated insecticides, plant essential oils could be an eco-friendly alternative against vector resistance. The study present dealt with the chemical composition and insecticidal property of *Piper umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus*.
Piper umbellatum leaf essential oil was extracted by hydro distillation with a Clevenger type apparatus and analysed by GC-MS. Bioassays were performed on third instar larvae and 2-5 days old non-blood fed female adults of the laboratory and field strain of *Anopheles gambiae* S.L and *Culex quinquefasciatus*.
Chemical analysis of *Piper umbellatum* leaf essential oil revealed the presence of terpenes compounds reported effective on insects and various pest. For larvicidal tests, the lethal concentrations killing 50% and 95% of the larvae were 0.51%; 0.61%; 0.09% and 0.91%; 1.09%; 0.23% for 24 h exposure respectively for *Anopheles gambiae* S.L and *Culex quinquefasciatus*. These concentrations were 0.43%; 0.58%; 0.05% and 0.79%; 1.05% and 0.16% for 48h exposure respectively. Knockdown time for 50% and 95% of knocked down mosquitoes were: 05±0.4min; 09±0.2min; 19±0.57min and 40±0.32min; 47±0.54min; 51±0.49min respectively. Concentrations killing 50% and 95% of adult females were: 0.40%; 0.43%; 0.24% and 0.73%; 0.76%; 0.52% respectively.
Piper umbellatum leaf essential oil was effective against the third instar larvae and adult females of *Anopheles gambiae* S.L and *Culex quinquefasciatus*.

Keywords: *Piper umbellatum*, essential oil, insecticide, *Anopheles gambiae* S.L, *Culex quinquefasciatus*

1. Introduction
Malaria remains the most common parasitic disease and the most lethal vector born disease in Sub-Saharan Africa. It is the leading cause of death and among its victims, children under five and pregnant women are its target of choice [1]. This condition is for most families the primary source of expenditure and a hindrance for a socio-economic development. The fight against this endemic disease is made difficult by several factors: the development and rapid expansion of vector resistance on Anopheline species to recommended synthetic insecticides [2, 3, 4], the resistance of *plasmodium* strains to chloroquine, the lack of vaccines against *plasmodium* [5] and the poverty of the countries concerned. The vector activity of *Culex quinquefasciatus* remains significant. This vector transmits virus such as West Nile virus [6], Rift Valley fever virus [7]; Zika virus [8]; Protozoa such as *Plasmodium cathemerium* [9]; *Plasmodium relictum* [10] to vertebrates; Nematodes such as *Wuchereria bancrofti* [11]; *Dirofilaria immitis* [12] and *Brugia malayi* [13].
Vector resistance is generated by several environmental factors that exert a constant selective pressure on vector populations: the release of detergents and pollutants resulting from industrial activity and other anthropogenic activities related to urbanization [14, 15], application of agricultural inputs and herbicides [16] a large scale indoor residual spraying and insecticidal treated nets intervention [17].
Studies conducted elsewhere in Africa and particularly in Cameroon have reported resistance in Anopheline population [18] and highlighted various mechanisms of resistance: metabolic resistance, cuticle resistance and target sites mutations. These resistance mechanisms are largely distributed across Africa [19] and seem to compromise the efficacy of synthetic insecticides [20]. From these unfortunate findings, it is urgent to search new and effective insecticides. Thus, several authors have suggested the use of plant derived extracts and essentials oils as natural insecticides [21, 22]. Tests undertaken in this direction have demonstrated not only the effectiveness of the essentials oils but also many advantages that could be offered by their use in the field [23]. Because unlike synthetic insecticides with ecological consequences [24], plant derivatives are biodegradable and made of several active ingredients. Therefore our study aimed to investigate the chemical composition and insecticidal activity of *P.umbellatum* leaf essential oil against *Anopheles gambiae* S.L and *Culex quinquefasciatus* in the laboratory conditions. Yet exploratory studies had been carried in Cameroun [25, 26].
Piper umbellatum has been used since ancient time by natives for many purposes: in folk medicine to heal the stomach-ache, in the culinary arts as spice, as a repellent for house flies. Leaves of this plant are rubbed on the body to allow picking honey during the day. *Piper umbellatum* extract has the inhibitory effects towards the myotoxic phospholipase of the snake venoms [27]. *Piper umbellatum* hydroethanolic extract does not exhibited toxicity on mice [28].

2. Materials and Methods

2.1 Plant material

The leaves of *Piper umbellatum* were harvested in the morning at the University of Yaoundé I campus. Before hydro distillation, this plant was identified at National Herbarium in comparison with the number of the collector R. Letouzey 2499 of the specimen number 3614SRFK. The specimen was classified as belonging to *Piperaceae* family.

2.2 Essential oil extraction

The extraction of *Piper umbellatum* leaf essential oil took place in the laboratory of the department of Microbiology of the University of Yaoundé I using a Clevenger type apparatus. This extraction was performed in several sessions, each session during 6 hours in order to allow a maximum extraction. The essential oil was collected in a dark bottle, dried using anhydrous magnesium sulphate and stored at 4°C, away from UV rays before use. The mass of plant used and extraction yield are presented in table 1.

2.3 Chemical analysis of *Piper umbellatum* leaf essential oil

The chemical analysis of *Piper umbellatum* leaf essential oil was performed by Gas Chromatography, coupled with Mass Spectrometry. The analysis was performed with two Perkin Elmer instruments, a Clarus 500 GC gas chromatograph coupled to an MS Clarus 500 mass spectrometer.

The column used in chromatography is an Elite 5MS (5% Diphenyl / 95% Dimethyl Polysiloxane stationary phase) 30 m long, with an internal diameter of 0.25 mm and a film thickness of the 0.25 μm stationary phase.

The carrier gas was helium with a flow rate of 0.75 mL / min.

The injector was brought to 300 ° C, the program begins with a step of 3 min at 60 ° C and the temperature increases from 10 ° C / min to 300 ° C where a step of 3 min is performed.

The acquisition of the mass spectrum was carried out on an m / Z range of between 20 and 400 with 0.1 scan / s and an electron ionization energy of 70 eV. The temperature of the source and the transfer line were 250 ° C.

The sample to be analysed was solubilized in a small volume of dichloromethane.

The data processing software was Turbomass 6.1 and the database for the comparison of mass spectra were NIST MS Search 2.0.

2.4 Mosquito's collection

We used for our experiment two strains of *Anopheles gambiae* S.L and *Culex quinquefasciatus*. The laboratory strain of *Anopheles gambiae* S.L is the "Yaounde strain" from the Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC) and the aquatic stages of the wild population were collected in Yaoundé down town, near river Ewoé (N $03^0$51'34.9'' and E $011^0$31'3''). *Culex quinquefasciatus* were sampled at Melen pound behind the Faculty of medicine and Biomedical Sciences of the University of Yaoundé I (N $03^0$85'63.1'' and E $011^0$48'49.4''). Mosquitoes were reared in the insectary of the Teachers' Training College of the University of Yaoundé I. Larvae were fed with Tetramin Baby Fish Food at a rate of 2.5mg for 100 larvae per day [29, 30]. The pupae were collected in plastic cups and placed in emergence cages. Adult from the 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 and *Culex quinquefasciatus* third and early four instars larvae and female aged of 2-5 days old were used to carry out tests.

2.5 Bioassays

2.5.1 Bioassay with larvae

Larvae of third and early four instars were used to assess the larvicidal activity of *Piper umbellatum* leaf essential oil following WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides [31]. Before running the test, larvae were maintained during one hour in distilled water for observation. 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 negative control was prepared by adding 1ml of absolute alcohol to 99 ml of spring water. Six different concentrations were used (0.1%; 0.2%; 0.4%; 0.6%; 0.8% and 0.9%) for *Anopheles gambiae* S.L and five(0.0125%; 0.025%; 0.05%; 0.1% and 0.2%) for *Culex quinquefasciatus*. 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. The lethal concentrations killing 50% and 95% of the larvae (LC_{50} and LC_{95}) were calculated using a log probit approach with WINDL CIRAD-CA software version2.0.

2.5.2 Bioassay with adult

Adult bioassays were performed with 2-5 days- old non-blood- fed females following the WHO Guidelines [32] with cone using impregnated bed net with the essential oil at the following concentrations: 0.1%, 0.2%, 0.4%; 0.6% and 0.8% for *Anopheles gambiae* S.L and 0.05%; 0.1%; 0.2%;0.4%; and 0.6% for *Culex quinquefasciatus*. 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 (98.47cm^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 [33, 34]. Before each test, females 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 female mosquitoes per cone were exposed to each concentration for 1hour**.** Five replicates were run as control using a portion of net impregnated only with 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. Individual with legs broken or not able to fly were considered dead. Mosquito mortality was recorded 24 hours

post- exposure. After the tests, adult mosquitoes of *Anopheles gambiae* S.L from field population and *Culex quinquefasciatus* were kept in Eppendorf tubes at -4°C for identification.

2.6 Mosquito identification

Adult females of the field population of *Anopheles gambiae* S.L were morphologically identified using the Gillies and Coetzee key [35] while *Culex* mosquito were identified using Reuben key [36]. Genomic DNA of *An.gambiae* S.L was extracted according to the Livak protocol [37] and molecular identification of females was conducted according to Santolammazza *et al* protocol [38].

2.7 Data analysis for Bioassays

The lethal concentrations inducing 50% and 95% (LC_{50} and LC_{95}) larval mortality were calculated using log probit approach with WINDL CIRAD-CA software 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 activity, the time at which 50% and 95% of adult mosquitoes were knocked down (Knock down time, KTD_{50} and KDT_{95}) and the LC_{50} and LC_{95} were 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 *Piper umbellatum* Essential oil

The table 1 below provides characteristics of *Piper umbellatum* and his greenish essential oil.

Table 1: The oil yield of *Piper umbellatum*

Plant name	Family	Certification number	Leaves weight(g)	Essential oil weight(g)	Extraction yield
Piper umbellatum	*Piperaceae*	3614SRFK	7390.8g	0.9g	0.012%

The extraction yield of *Piper umbellatum* leaf essential oil is very low and therefore requires a huge mass of plant to obtain a volume of essential oil sufficient for biological assays. A better method of extraction, the judicious choice of the period of harvesting and the control of some edaphic factors could allow a good yield.

3.2 Chemical composition of *Piper umbellatum* leaf essential oil

The chemical analysis of *Piper umbellatum* leaf essential oil revealed up to 24 different compounds comprising 89.60% of the essential oil. Major compounds are sesquiterpenes: copaene (9.63%) β-elemene (10.56%) and γ muurolene (11.37%). Unidentified components comprise 10.40 % of the essential oil.

Table 2: Chemical composition of *Piper umbellatum* leaf essential oil

Elution order	Compounds	Percentage	Kovat retention index	
			Npo.	Po.
1.	α-pinene	6.23	931	1035
2.	camphene	1.83	944	1063
3.	β-pinene	1.83	971	1132
4.	β-myrcene	4.60	981	1161
5.	limonene	1.56	1022	1203
6.	Z-β-ocimene	1.33	1025	1242
7.	E-β-ocimene	2.63	1037	1247
8.	linalool	1.27	1086	1533
9.	L-borneol	2.45	1153	1719
10.	terpinen-4-ol	2.96	1163	1611
11.	p-menth-1-en-8-ol	5.32	1280	-
12.	copaene	9.63	1375	1491
13.	β-elemene	10.56	1386	1577
14.	caryophyllene	1.37	1419	1612
15.	α-caryophyllene	1.18	1453	-
16.	γ-muurolene	11.37	1473	1689
17.	germacrene D	3.92	1480	1708
18.	α-farnesene	1.90	1496	1744
19.	δ-cadinene	2.30	1513	1765
20.	calamenene	1.14	1532	-
21.	z-nerolidol	4.56	1550	2036
22.	caryophyllene oxyde	1.95	1567	2001
23.	α-eudesnol	1.6	1652	2227
24.	phytol	6.1	2116	2613
	Total	89.6%	-	-

Analysis performed by gas chromatography with two Perkin Elmer instruments, a Clarus 500 GC gas chromatograph coupled to an MS Clarus 500 mass spectrometer. Individual components were identified by the data processing software Turbomass 6.1 and the database for the comparison of mass spectra NIST MS Search 2.0.

Among these 24 identified compounds, α-pinene; β-pinene; β-myrcene; linalool and α-caryophyllene are reported effective on various insects and pest.

3.3 Larvicidal activity of *Piper umbellatum* leaf essential oil

The larvicidal potential of *P.umbellatum* leaf essential oil was assessed on mosquito larvae for 24 hours and 48 hours exposure period.

3.3.1 Larvicidal activity of *Piper umbellatum* leaf essential oil on 24 hours

During 24 hours exposure period, larvae of *Anopheles gambiae* S.L and *Culex quinquefasciatus* were facing each concentration of *Piper umbellatum* leaf essential oil. The table 3 presents the number of dead larvae recorded (with confident interval limit of 95.5%) corresponding to each concentration of essential oil.

Table 3: Larvicidal activity of *Piper umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus* on 24 hours

Populations		Nb of Larvae exposed	Nb of replicates	Essential oil concentration (%)	Mortality after 25 H	95% CI
An.gambiae S.L	Lab. strain	100	4	0.8	82±0.6	78-86
		100	4	0.6	59±2.2.224	43-74
		100	4	0.4	28±0.7	23-37
		100	4	0.2	9±0.25	7-10
		100	4	0.1	3±0.4	0.7-1.35
	Field strain	100	4	0.9	90±0.8	85-97
		100	4	0.8	64±04	61-67
		100	4	0.6	44±1.4	33-53
		100	4	0.4	15±0.4	12-18
		100	4	0.2	6±0.28	4-8
		100	4	0.1	1±0.25	1-2
Culex quinquefasciatus		100	4	0.2	76±0.4	73-79
		100	4	0.1	63±0.6	59-68
		100	4	0.05	50±0.8	45-57
		100	4	0.025	30±0.2	28-37
		100	4	0.0125	12±0.2	9-15

Lab: laboratory After 24 hours exposure, dead larvae were recorded and figure 1 was plotted.

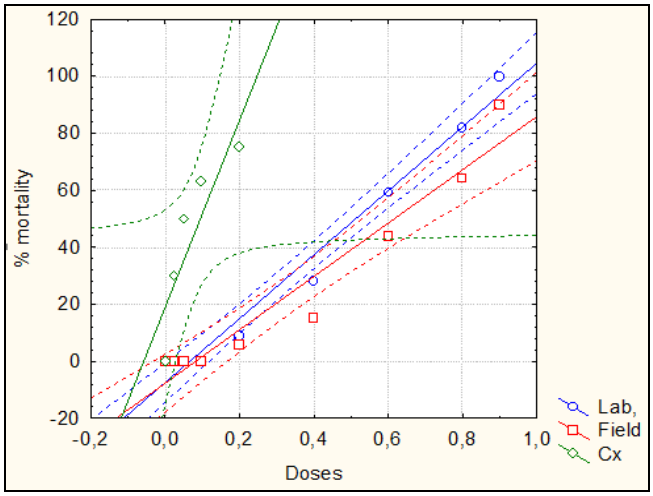

Fig 1: Larvicidal activity of *Piper umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus* after 24 hours

The regressions lines equations of the larval mortality after 24 hours exposure period corresponding respectively to laboratory strain, field population of *An.gambiae* S.L and *Cx. quinquefasciatus* are:

Y_{lab}= *-7.4117+112.0994X;* Y_{field}= *-7.6287+93.5476X and* Y_{Cx}= *19+328X*.

The dose-response analysis showed that the larval mortality increases with essential oil concentration for each population (H=3.25; p=0.02). The lethal concentration killing 50 % (LC_{50}) of mosquitoes larvae after 24 hours exposure are: 0.51%; 0.61% and 0.09% respectively for laboratory strain, field population of *An. gambiae* S.L and *Culex quinquefasciatius*. While concentrations killing 95% (LC_{95}) of larvae are: 0.91%; 1.09% and 0.23% respectively.

3.3.2 Larvicidal activity of *Piper umbellatum* leaf essential oil on 48 hours

When the exposure is extended to 48 hours, mortality still increasing but the lethal concentrations killing 50% and 95% decrease respectively (H=3.3; p=0.03). The table 4 shows the mortality depending on essential oil concentration.

Table 4: Larvicidal activity of *Piper umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus* on 48 hours

Populations		Number of larvae exposed	Number of replicates	Essential oil concentration (%)	Mortality after 48H	95% CI
An.gambiae S.L	Laboratory strain	100	4	0.8	100	100-100
		100	4	0.6	83±1.1	74-89
		100	4	0.4	39±1.9	26-52
		100	4	0.2	19±0.7	14-24
		100	4	0.1	5±0.2	3-6
	Field population	100	4	0.9	98±0.2	96-100
		100	4	0.8	80±0.4	77-83

		100	4	0.6	57±0.8	52-63
		100	4	0.4	29±0.4	26-32
		100	4	0.2	10±0.2	8-12
		100	4	0.1	2±0.2	0-4
Cx.quinquefasciatus		100	4	0.2	100	100-100
		100	4	0.1	84±0.4	81-87
		100	4	0.05	66±0.6	62-70
		100	4	0.025	43±0.4	40-46
		100	4	0.0125	17±0.4	14-20

The regression lines equations of the larval mortality after 48 hours exposure are respectively:

Y_{lab}= *-5.0201+125.9126X*; Y_{field}= *-5.4336+95.2528X* and Y_{Cx}= *26.2+432X*

After 48 hours exposure, lethal doses killing 50% of larvae are: 0.43%; 0.58% and 0.05% (respectively) while lethal doses killing 95% of the larvae are: 0.79%; 1.05% and 0.16% respectively for the laboratory strain, field population of *An.gambiae* S.L and *Cx.quinquefasciatus*. The larvae of *Cx. quinquefasciatus* and the laboratory strain of *An. gambiae* S.L are more susceptible to *Piper umbellatum* leaf essential oil than the larvae of the wild population of *An.gambiae* S.L.

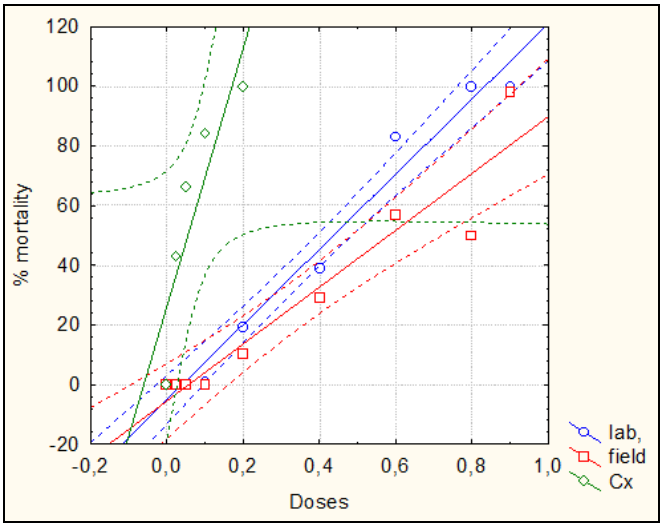

Fig 2: Larvicidal activity of *Piper umbellatum* Leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus* after 48 hours

3.4. Knock down activity of *Piper umbellatum* leaf essential oil on *An.gambiae* S.L and *Cx.quinquefasciatus*

During one hour, considering only higher concentrations, at 10 minutes interval, the number of mosquito knocked down was recorded. It appears that the knock down activity evolves with the duration of exposure for each population (H=3.41; p=0.02).

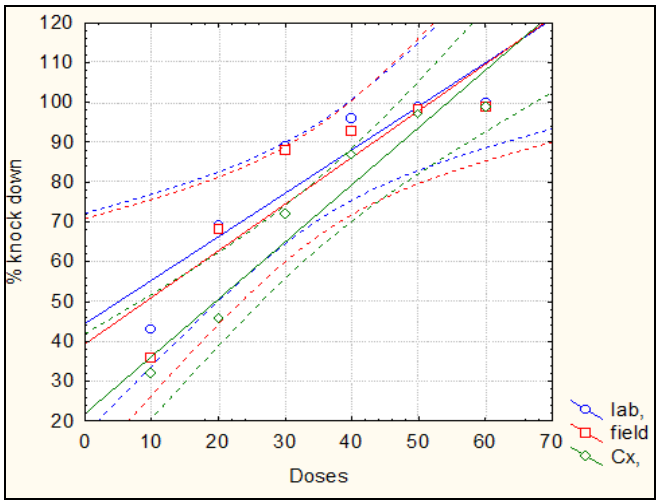

Fig 3: Knock down activity of *Piper umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus*

The knock down time for 50%(KDT_{50}) of mosquito knocked down are $05\pm0.4min$; $09\pm0.2min$ and $19\pm0.57min$ respectively for laboratory strain, field population of *Anopheles gambiae* S.L and *Culex quinquefasciatus*; while the knock down time for 95% (KDT_{95}) of mosquito knocked down are (with 95% confidence interval): $40\pm0.32min$; $47\pm0.54min$ and $51\pm0.49min$ respectively.

The regression lines equations generated are: Y_{lab}= *44.46+1.0914X*; Y_{field}= *39.33+1.171X* and Y_{Cx}= *21.36+1.43X* respectively.

3.5-Adulticidal activity of *Piper umbellatum* leaf essential oil on *Anopheles gambie* S.L and *Culex quinquefasciatus*

Piper umbellatum leaf essential oil exhibited toxic effect 24 hours post exposure both to *An.gambiae* S.L and *Cx. quinquefasciatus*. The number of dead adult mosquitoes increases with doses (H=4.6; p=0.034) for each population.

Table 5: Adulticidal activity of *Piper umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus*

Populations		Number of larvae exposed	Number of replicates	Essential oil concentration (%)	Mortality after 24H	95% CI
An.gambiae S.L	Laboratory strain	100	4	0.8	100	100-100
		100	4	0.6	84±0.28	81-87
		100	4	0.4	42±0.28	40-44
		100	4	0.2	30±0.28	28-32
		100	4	0.1	8±0.40	5_11
	Field population	100	4	0.8	99±25	98-101
		100	4	0.6	78±2.8	76-80
		100	4	0.4	39±0.47	36-42
		100	4	0.2	17±0.25	26-32
		100	4	0.1	3±0.47	0-6

Cx.quinquefasciatus	100	4	0.6	99±0.28	98-101
	100	4	0.4	80±0.4	77-83
	100	4	0.2	66±0.28	64-68
	100	4	0.1	22±0.86	15-27
	100	4	0.05	7±0.25	6-9

Adult of *Culex quinquefasciatus* are more susceptible to *Piper umbellatum* leaf essential oil than adult of *Anopheles gambiae* S.L. However, adults of the field population of *An.gambiae* S.L appear more tolerant to the essential oil activity (H=6.93, p=0.012) than the laboratory strain.

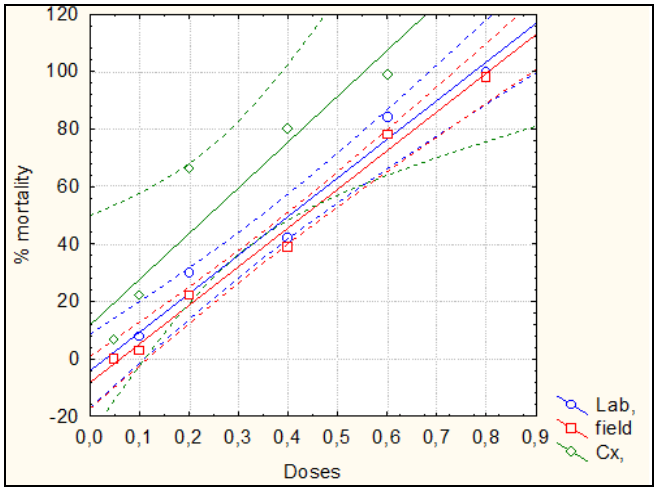

Fig 4: Adulticidal activity of *Piper umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus*

The different regression lines equation of mortality managed for the adulticidal activity of *P.umbellatum* leaf essential oil are:

$Y_{lab}=-4.147+134.36X$; $Y_{field}=-8.228+134.59X$ and $Y_{Cx}=11.742+159.47X$ respectively

The lethal concentrations killing 50%(LC_{50}) of adult mosquitoes are: 0.40%; 0.43% and 0.24% respectively for the laboratory strain, field population of *An.gambiae* S.L and *Cx.quinquefasciatus*; while the lethal concentrations killing 95%(LC_{95}) of adult mosquitoes are: 0.73%; 0.76% and 0.52% respectively.

3.6-Identification of the field population of *Anopheles gambiae* S.L and *Culex quinquefasciatus*

Mosquitoes from the laboratory strain are all *An.coluzzii*. Further analysis was performed with 71 adult females of the field population to identify the members of *An.gambiae* complex. The analysis reveals that 3 (4.23%) were *An.gambiae s.s* and 68 (95.77%) were *An.coluzzii*. The morphological analysis of 200 adult mosquitoes from the breeding site of *Cx.quinquefasciatus* showed that 4 (2%) were *An.coluzzii*; 14(7%) were *Cx.perfuscus* and 182 (92%) were *Cx.quinquefasciatus*.

4. Discussion

The extraction yield of *P.umbellatum* leaf essential oil is very low (0.012%) in comparison with the yield of others plants treated under the same conditions. The study conducted with plant cultivated in Cameroon exhibited a yield of 0.59% to 0.95% [39]. Many factors could be advocated to explain this result: botanical specificity, harvesting period and the method of extraction. Further study might elucidated this result and improve the extraction yield.

The chemical analysis of the essential oil revealed a mixture of many components of which up to 24 different compounds were identified. Others components comprising 10.4% of the essential oil remain undetermined. More sophisticated apparatus for analysis could allow the determination of these compounds. The chemical composition of *P.umbellatum* leaf essential oil shows the presence of monoterpenes and sesquiterpenes reported effective against insects and bacteria. These findings explain the effectiveness of *P. umbellatum* leaf essential oil on *An. gambiae* S.L and *Cx.quinquefasciatus*. Thus, many studies demonstrated the efficacy of monoterpenes: α-pinene, β-pinene, myrcene, limonene, linalool and borneol against the third instar larvae of *An.gambiae* S.L [40], *Culex pipiens pallens*, *aedes aegypti*, *Ochlerotatus togoi* [41] *Aedes albopictus* [42]. In addition, sesquiterpenes: β-caryophyllene and β-elemene are reported toxic against the third instar larvae of *An. subpictus*, *Ae. aegypti* and *Cx. tritaeniarhynchus* [43], caryophyllene oxide and germacrene D against *An. anthropophagus* [44]. These compounds are also known to be effective against Protozoa and Bacteria. Therefore β-pinene, myrcene and limonene are reported effective against *Trypanosoma brucei brucei* [45]. Monoterpenes linalool and limonene are affective against *Staphylococcus aureus*, *Staphylococcus epidermis* and *Escherichia coli* [46]. The efficacy of *P. umbellatum* leaf essential oil on mosquito larvae suppose many pathway. One of the actions might consist on the morphological damages on the larvae exoskeleton by inhibition of chitin synthesis and thus reduce the deposition of cuticle layers [47]. The knockdown activity on adult mosquitoes that occurs shortly after contact with the impregnated mosquito net reflects the action of the essential oil compounds on adult mosquitoes. Indeed the sudden paralysis that occurs, involves a direct action on the insect nervous system. This effect increases with the dose of essential oil and the exposure time. The mortality that follows 24 hours post exposure shows the toxicity of the essential oil of *P. umbellatum*. Several authors have demonstrated the effectiveness of the compounds found in *P. umbellatum* leaf essential oil on adult mosquitoes. Thus, compounds such as linalool, germacrene D are effective against *An. subpictus* [43], *An. gambiae* [48]; α-pinene, limonene, β-ocimene against *An. stephensi*, [49]; terpinen-4-ol, germacrene D against *An. anthropophagus*; myrcene against *An.gambiae* [39] and *An. funestus* [50]; caryophyllene oxide against *An.gambiae* [51]. Terpinen-4-ol, α-pinene and β-pinene are effective against the stored product insects *Sitophilus zeamais* and *Tribolium castaneum* [52]. *Piper umbellatum* leaf essential oil compounds, α-pinene and β-pinene inhibit the Acetylcholinesterase activity [53]. Other studies have shown the repulsive properties of Phytol, one of the important compounds of the essential oil of *P. umbellatum* on *An. gambiae* major vector of malaria. Further studies could focus on field trials in order to well establish the efficacy of

P. umbellatum leaf essential oil and to fix the volatile active compounds of essential oil on the impregnated materials. The insecticidal activity of *P. umbellatum* leaf essential oil is dosage and time dependent. The current study suggests the possibility of developing bio insecticides based on plant essential oils.

5. Conclusion

Our study focused on the chemical composition and insecticidal activity of *P. umbellatum* leaf essential oil on *Anopheles gambiae* S.L and *Culex quinquefasciatus*. The chemical analysis of the essential oil revealed up to 24 compounds, some of which are effective against *An.gambiae* S.L and *Cx.quinquefasciatus*. This findings make it possible to suggest the use of *P.umbellatum* essential oil in the formulation of new biodegradable insecticides, which are eco-friendly for the environment and less expensive. This could be a source of income for local people. Further studies may identify unknown compounds of the essential oil and improve the extraction yield. Field trials are necessary to confirm and to well establish the effectiveness of *P.umbellatum* leaf essential oil.

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