International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2018; 5(1): 61-66

Received: 26-11-2017
Accepted: 29-12-2017

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Toxic effect of *Chenopodium ambrosoides*, *Hyptis suaveolens* and *Lippia adoensis* leaf methanol extracts and essential oils against fourth instar larvae of *Anopheles gambiae* (Diptera: Culicidae)

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Abstract
Nowadays, controlling insect vectors using plant products is the utmost encouraged in the mosquito pest management to the detriment of synthetic insecticides which are environmentally non-biodegradable and harmful for human and livestock. The present investigation aimed to evaluate the effectiveness of *Chenopodium ambrosoides*, *Hyptis suaveolens*, and *Lippia adoensis* leaf methanolic extracts and essential oils against fourth instar larvae of *Anopheles gambiae* in the laboratory. Plant extracts were dissolved in 1 ml of methanol and doses of 125, 250, 500 and 1000 ppm for methanol extracts and 200, 100, 50 and 25 ppm for essential oils were prepared in the volume of 100 ml with tap water in the 250 ml plastic cups. Twenty five fourth instar larvae were transferred to each solution dose and larval mortality was recorded after 24 h post-treatment. In results, all the plant products tested have shown their dose-dependent toxic effect against on *An. gambiae* larvae. Tested at 200 ppm, essential oils of each plant caused 100% mortality of larvae. The essential oil of *C. ambrosoides* (LC_{50} = 6 ppm after 18 h) was the most potent compared to *H. suaveolens* (LC_{50} = 19.20 ppm) and *L. adoensis* (LC_{50} = 75.63 ppm) after 24 h post-exposure. At the highest dose of 1000 ppm, all plant extracts exhibited 100% mortality of *An. gambiae* larvae. Among the plant extracts, the methanolic extract of *L. adoensis* with LC_{50} = 94.71 ppm was revealed to be the most effective compared to *H. suaveolens* (LC_{50} = 132.01 ppm) and *C. ambrosoides* (LC_{50} = 204.56 ppm) extracts 24 h post-treatment. From these results, *H. suaveolens*, *L. adoensis* and *C.ambrosoides* leaf methanolic extracts and essential oils could be used as a promising and eco-friendly approach in the vector control programs.

Keywords: larvicidal, plant methanolic extracts, essential oils, *Anopheles gambiae*

1. Introduction
Mosquitoes (Diptera: Culicidae) are well known as the best vectors of diseases causing illness and death in many developing countries. They are involved in the transmission of the most important diseases including malaria, lymphatic filariasis, Japanese encephalitis, dengue and yellow fever [1].

Worldwide, an estimated 212 million cases of malaria with 429 000 deaths from malaria globally in 2015 was reported by WHO [2]. However, 90% of malaria cases and 92% of deaths, majority in children aged under 5 years, were reported in the WHO African Region. In Africa, *Anopheles gambiae* Giles is the major vector of *Plasmodium falciparum*, responsible of 68% of deaths occurred in the continent. In Cameroon, 8 million malaria cases were reported with 21.000 deaths [2].

Since miscellaneous cases of drug resistance in the treatment of malaria have been reported, and moreover the absence of malaria vaccine, the best method of preventing the disease remains vector control measures. Therefore, the most commonly recommended methods for preventing the disease is eliminate immature stages in their breeding sites or killing and repelling adult mosquitoes [3]. Currently, synthetic residual insecticides are largely used for mosquito-borne disease control program to kill mosquito larvae at the breeding sites or to exterminate or prevent adult mosquitoes from human bites [4]. Unfortunately, the repeating and misuse of these synthetic chemicals has led to the development of mosquito resistance to these pesticides [5]. Besides, these chemical insecticides operationally costly, environmental pollution and deleterious effects on non - target organisms [6]. These demerits of synthetic

chemicals have created the need for developing safer alternative approaches to control disease vectors. The use of plant materials as insecticide has a long history. Saxena [7] described more than 1500 plant species belonging to 235 families having potential insecticidal properties.

The plant species *Chenopodium ambrosioides* L belonging to the Chenopodiaceas family is an indigenous perennial plant largely distributed in Cameroon [8]. The plant is widely used in traditional medicine to treat intestinal parasites, nervous infections, cough, pulmonary obstruction, typhoid, influenza, skin and kidney infection, anti- inflammatory [9]. As insecticide, the plant essential oils possessed larvicidal and repellent properties against *An. gambiae* and *An. arabiensis* [10,11]. The essential oil of the plant was also effective against the maize weevils *Sitophilus zeamais* [12].

Hyptis suaveolens L. (Laminaceae) is an annual sub-shrub, distributed in the tropic of West Africa [13]. Several studies reported the medicinal uses of this plant [14, 15, 16]. Previous research documented toxic and repellent activity of this plant against cowpea borers [17], *Sitophilus* species and *Callosobruchus maculates* [18]. Mosquitocidal activity of the plant was reported against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* [19, 20, 13].

The species *Lippia adoensis* Hochst (Verbanaceae) is an herbaceous plant distributed throughout West Africa [21]. The plant has been used in traditional medicine to treat several diseases [22, 23, 24]. Extracts of the plant were reported to be a promising fumigant against a number of insect pests of cultivated crops [25]. The plant possesses a pediculocidal and scabicidal activities against body lice, head lice and scabies' mites [26]. Nukenine *et al*. [27] reported its efficacy against *Sitophilus zeamais* Motsch. Mosquitocidal activity of the plant against *Aedes* spp and *Anopheles arabiensis* was also reported [28, 29].

This study was aimed to evaluate larvicidal activity of *Chenopodium ambrosoides*, *Hyptis suaveolens* and *Lippia adoensis* leaf methanolic extracts and essential oils against fourth instar larvae of *Anopheles gambiae* mosquito species.

2. Materials and Methods

2.1 Plant materials

2.1.1 Harvesting and processing

The green leaves of *C. ambrosoides* and *H. suaveolens* were collected at Dang (University of Ngaoundéré campus), Vina Division, Adamawa region, Cameroon in March 2016, while the leaves of *L. adoensis* were collected from Mbe in the Vina Division of the Adamaoua region of Cameroon in June 2016. Leaves were dried at room temperature and then pulverised in powder using mortar until the powder passed through a 0.4 mm mesh sieve. The powder was stored in opaque containers inside a refrigerator at -4℃ until needed.

2.1.2 Preparation of plant methanolic extracts

From the collection of plant powder, 500 g for each plant was weighed and extracted for 72 h by cold maceration in 2.5 L of methanol (Sigma Aldrich), shaking twice a day (morning and afternoon) in the laboratory of Chemistry, University of Ngaoundéré. To obtain the methanol extract, 500 g of powder of each plant were macerated in 2500 ml of methanol for 3 days at room temperature and then the maceration was filtered using Whatman No.1 filter paper. The residue of maceration was rinsed and filtrated several 3 times with fresh methanol until a clear phase was obtained. The filtrate was summited to Rotary Evaporator apparatus to obtain a residue called crude extract. The crude extract was stored in a refrigerator at 4 °C until needed for bioassay. The yield of extraction was determined following the formula:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of plant powder used}} \times 100$$

2.1.3 Extraction of essential oil

One kilogram (200 g) leaf powder of each plant species was used separately for essential oil extraction. Each plant powder was subjected to hydrodistillation process for 3 hours using a Clevenger apparatus. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at -4°C in refrigerator until needed for bioassay.The yield of oil obtained from plant materials was calculated following as following.

$$\text{Oil yield (\%)} = \frac{\text{Weight of essential oil obtained}}{\text{Weight of plant powder used}} \times 100$$

2.2 Mosquito rearing

The eggs of *Anopheles gambiae* were collected from the main culture of OCEAC, Yaoundé, Cameroon, and reared according to the protocol of WHO [30] in insectarium of the laboratory of Biological of the University of Ngaoundéré. The larvae were fed with TetraMin® (Tetra GmbH, Germany). Well Water was use for breeding of the aquatic stages of the mosquito in trays. The water in the tray was renewed every other day avoid water pollution resulting from the presence of the nutritional powder.

2.3 Larvicidal test

The larvicidal activity of *H. suaveolens* and *L. adoensis* leaf methanol extracts and essential oils was assessed against fourth instar larvae of *An. gambiae* following the method described by WHO [31]. The extracts were dissolved in 0.5 ml of Tween-80 and different concentrations of 125, 250, 500 and 1000 ppm of plant extracts and 200, 100, 50 and 25 ppm of plant essential oils were prepared in the volume of 100 ml with tap water in the 250 ml plastic cups. Twenty five fourth instar larvae were transferred into the each test solution prepared and four replicates were maintained for each concentration. Mortality was recorded after 24 h of exposure, during which no food was given to the larvae. Larvae were considered dead if appendages did not move when probed with needle in the siphon or cervical region. Larvae incapable of rising to the surface or not showing the characteristic diving reaction when water was disturbed, were considered moribund and added to the dead larvae for calculating percentage of mortality.

2.4 Statistical analysis

Abbott's formula [32] was applied for mortality correction whenever required. The percentage of mortality data were subjected to the ANOVA procedure using SPSS 16.0. Tukey test (P=0.05) was applied for mean separation. Lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality of *An. gambiae* larvae 24 h after treatment application were determined using Probit analysis (Finney [33]; SPSS 16.0).

3. Results

3.1 Yield plant extraction

The extraction yield of *Chenopodium ambrosoides*, *Hyptis suaveolens* and *Lippia adoensis* methanolic extracts and essential oils is presented in table 1. From 1000g of each plant powder used, the *L. adoensis* methanolic extract yield (9.76%) obtained was high compared to 7.24 and 5.77% methanolic extract yield obtained for *C. ambrosoides* and *H. suaveolens*, respectively. After hydrodistillation of 200 g of each plant powder, the oil yields obtained were 1.8, 0.44 and 1.2% for *C. ambrosoides*, *H. suaveolens* and *L. adoensis*, respectively.

Table 1: Extraction yield (%) of methanolic leaf extracts and essential oils of *Chenopodium ambrosoides*, *Hyptis suaveolens* and *Lippia adoensis*

Extraction type	Plant species	Powder used (g)	Yield (%)
Cold maceration (methanolic extract)	*C. ambrosoides*	500	7.24
	H. suaveolens	500	5.77
	L. adoensis	500	9.76
Hydrodistillation (essential oil)	*C. ambrosoides*	200	1.8
	H. suaveolens	200	0.44
	L. adoensis	200	1.2

3.2 Toxicity of essential oils

The mortality percent of *An. gambiae* larvae exposed to different doses of *Chenopodium ambrosoides*, *Hyptis suaveolens* and *Lippia adoensis* methanolic extracts and essential oils after 1, 6, 12, 18 and 24 hours post-treatment are presented in figure 1. In general, for all plant essential oils tested, the mortality of larvae increased with increasing concentration and exposure time. After 24 h post-treatment with the essential oil of *C. ambrosoides*, 100% mortality of *An. gambiae* larvae was recorded at all doses tested while a significantly ($F_{(5;18)}$ = 227.01; P<0.001) mortality rate of mosquito larvae ranging from 55% at 25 ppm to 100% at 200 ppm and significantly ($F_{(5;18)}$ = 464.05; P<0.001) from 15% (at 25 ppm) to 100% (at 200 ppm) were registered with *H. suaveolens* and *L. adoensis* essential oils, respectively after 24 h post-exposure. After 1 h post exposition, 5, 13 and 0% mortality were recorded at the lowest dose (25 ppm) with *C. ambrosoides*, *H. suaveolens* and *L. adoensis* essential oils, respectively, while 100% mortality of *An. gambiae* larvae was recorded with the highest tested concentration (200 ppm) of the three plant species as well as the positive control applied at 1000 ppm. After 24 h post-treatment, all the plant leaf essential oils tested at the highest dose of 200 ppm caused 100% mortality of mosquito larvae as well as the positive control (1000 ppm). The LC_{50} and LC_{90} values of the plant essential oil decreased with increasing exposure time (Table 1).

The values of LC_{50} and LC_{90} of the three plant essential oils tested of *An. gambiae* larvae decreased with increasing exposure time (Table 2). Among the plant essential oils tested, the *H. suaveolens* with the lowest value of LC_{50}= 47.91 ppm was the most potent on *An. gambiae* larvae compared to *C. ambrosoides* (LC_{50} = 72.26 ppm) and *L. adoensis* (LC_{50} = 339.97 ppm) essential oils after 1 h post-exposure. After that period (1 h), the LC_{90} values recorded were 156.24, 86.47 and 1337.44 ppm for *C. ambrosoides*, *H. suaveolens* and *L. adoensis* leaf essential oils, respectively.

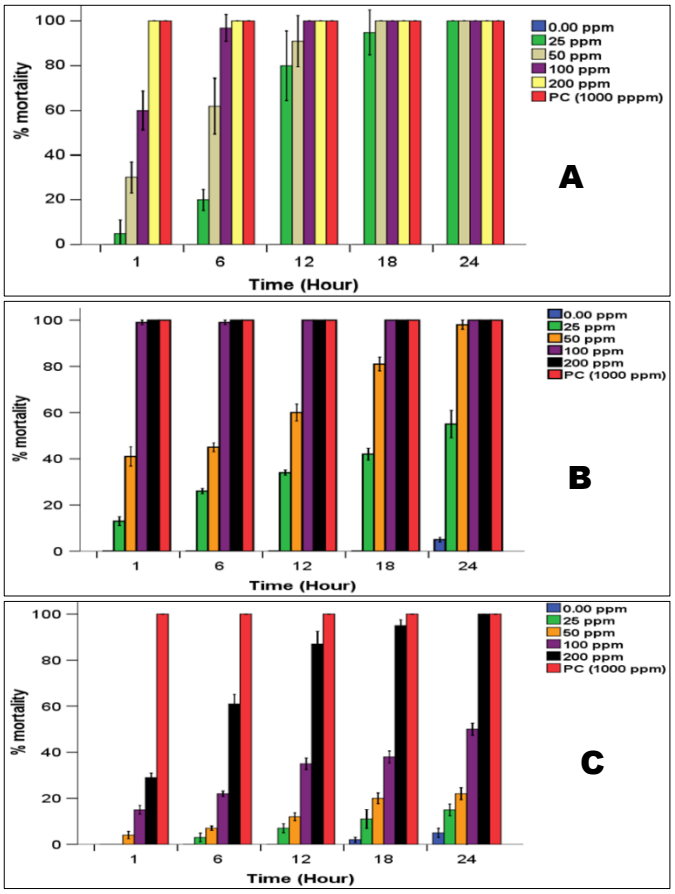

Fig 1: Percentage mortality of *Anopheles gambiae* larvae treated with *Chenopodium ambrosoides* (A), *Hyptis suaveolens* (B) and *Lippia adoensis* (C) leaf essential oils after 1, 6, 12, 18 and 24 hours post-exposition, PC= Positive control (Dichlovos 49%).

Table 2: LC_{50} and LC_{90} (ppm) of *Chenopodium ambrosoides*, *Hyptis suaveolens* and *Lippia adoensis* leaf essential oils against *Anopheles gambiae* larvae after 1, 6, 12, 18 and 24 hours post-exposure.

Plant species	Time (H)	Slope±SE	R^2	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
Chenopodium ambrosoides	1	3.82±0.15	0.48	72.26 (64.37-81.27)	156.24 (132.29-196.09)	90.47***
	6	4.38±0.20	0.53	40.32 (36.79-43.99)	78.99 (70.01-92.55)	57.48***
	12	2.51±0.25	0.53	12.30 (3.06-19.11)	39.75 (29.81-58.08)	121.16***
	18	2.70±0.68	0.54	6.00 (-)	17.87 (-)	69.97***
	24	2.93±0.22	0.54	-	-	76.14***
Hyptis suaveolens	1	4.99±0.22	0.32	47.91 (43.77-52.51)	86.47 (76.22-102.47)	72.79***
	6	3.99±0.18	0.32	42.19 (37.37-47.33)	88.25 (75.26-110.53)	90.08***
	12	3.87±0.19	0.28	35.44 (31.67-39.16)	75.96 (66.24-91.57)	61.63***
	18	4.19±0.24	0.23	28.74 (27.06-30.33)	58.09 (54.19-63.09)	20.33ns
	24	2.93±0.22	0.17	19.20 (13.22-23.80)	52.44 (43.87-68.96)	76.14***
Lippia adoensis	1	2.15±0.17	0.93	339.97(272.76-469.32)	1337.44 (857.67-26.36-97)	21.05ns

	6	2.64±0.14	0.94	171.01(144.26-214.94)	524.96 (374.06-894.84)	58.12***
	12	3.02±0.13	0.96	108.08 (90.67-132.84)	286.71 (213.93-458.83)	110.08ns
	18	2.89±0.12	0.97	90.43 (72.76-116.27)	250.51 (178.11-458.65)	163.50***
	24	3.02±0.12	0.98	75.63 (62.01-93.57)	200.38 (149.26-326.88)	

[ns]P>0.05; **P<0.01; ***: p<0.001; LCL: Lower Confidence Limit; UL: Upper Confidence Limit;

3.3 Effect of plant methanolic extracts against *Anopheles gambiae* larvae

Table 3 presents the mortality percentage of *An. gambiae* mosquito larvae exposed to different doses of *C. ambrosoides*, *H. suaveolens* and *L. adoensis* leaf methanolic extracts and LC_{50} as well as LC_{90} (ppm) of these plant extracts 24 h post-exposure. In general, all plant extracts tested significantly (P<0.05) exhibited a larvicidal activity on the larvae of *An. gambiae* and this activity increased with increasing concentrations. The larval mortality of larvae significantly ($F_{(5;18)}$ = 325.20; P<0.001) varied from 30% (at 125 ppm) to 100% at 1000 ppm with *C. ambrosoides* extract. With *H. suaveolens* extract, the larval mortality significantly ranged from 46% (at 125 ppm) to 100% (at 1000 ppm). Treated with the methanolic extract of *L. adoensis*, the mortality of the larvae varied significantly from 69%at 125 ppm to 100% at the highest dose of 1000 ppm. At the lowest dose of 125 ppm, the mortality percentages of 30, 46 and 69% were recorded with *C. ambrosoides*, *H. suaveolens* and *L. adoensis* leaf methanolic extracts, respectively. However, 100% mortality of larvae was registered with all plant methanolic extracts and the commercial insecticide (Dichlovos 49%) tested at 1000 ppm.

Among the plant extracts, the methanolic extract of *L. adoensis* with LC_{50} = 94.71 ppm was revealed to be the most effective compared to *H. suaveolens* (LC_{50} = 132.01 ppm) and *C. ambrosoides* (LC_{50} = 204.56 ppm) extracts 24 h post-treatment. The LC_{90} values of 638.37, 244.01 and 197.04 ppm were also recorded with *C. ambrosoides*, *H. suaveolens* and *L. adoensis* leaf methanolic extracts, respectively.

Table 3: Mortality Percentage of *Anopheles gambiae* larvae treated with plant methanolic extracts and LC_{50} as well as LC_{90} (ppm) of *Chenopodium ambrosoides*, *Hyptis suaveolens* and *Lippia adoensis* 24 h post-exposure.

Plant species	Conc (ppm)	% mortality	R^2	Slope±SE	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
Chenopodium ambrosoides	0	0.00±0.00e	0.84	2.59±0.12	204.56 (172.06-236.56)	638.37 (522.29-848.43)	56.98***
	125	30.00±3.82d					
	250	62.00±2.58c					
	500	76.00±2.82b					
	1000	100.00±0.00a					
	Dichlovos (1000 ppm)	100.00±0.00a					
	$F_{(5;18)}$	325.20***					
Hyptis suaveolens	0	0.00±0.00d	0.46	4.80±0.31	132.01 (119.66-143.01)	244.01 (221.24-278.68)	29.77**
	125	46.00±2.58c					
	250	90.00±4.16b					
	500	100.00±0.00a					
	1000	100.00±0.00a					
	Dichlovos (1000 ppm)	100.00±0.00a					
	$F_{(5;18)}$	426.67***					
Lippia adoensis	0	00.00±0.00c	0.42	4.02±0.36	94.71 (74.51-109.55)	197.04 (176.28-230.93)	32.77**
	125	69.00±3.41b					
	250	95.00±3.00a					
	500	100.00±0.00a					
	1000	100.00±0.00a					
	Dichlovos (1000 ppm)	100.00±0.00a					
	$F_{(5;18)}$	458.90***					

Mean of mortality ± standard deviation within a column followed by the same letter did not differ significantly according to Tukey test (P= 0.05); **P<0.01; ***: p<0.001; LFL: Lower Fiducial Limit; UFL: Upper Fiducial Limit; Number of replicates: 4

4. Discussion

Mosquito control targeting the larval stage in developing countries seem to be an ideal approach to mosquito control as it eliminates mosquitoes before they reach the stage able to transmit diseases. However, botanicals have been reported as useful for control of mosquitoes. In the present investigation, all plant products (Extracts and essential oils) exhibited a significant larvicidal dose-dependent and exposure time-dependent activity on fourth instar larvae of *An. gambiae*.

The mortality of mosquito larvae might be caused by the secondary metabolites contained in the extracts or essential oils of these plant species. Flavonoids, terpenoids, alkaloids, steroids and phenols are among the metabolites with biological activities against insects [34]. Different plant secondary metabolites such as alkaloids, phenolic, terpenoids are reported to possess biological properties and could also protect plants from insect pests and diseases [35]. Indeed, plant secondary metabolites interfere with the proper functioning of mitochondria more specifically at the proton transferring sites [36]. However, secondary metabolites from different plants species cause physiological and cellular disturbances that include inhibition of acetylcholinesterase, disruption of sodium and potassium ion exchange, and interference of mitochondrial respiration [36]. Moreover, they affect midgut epithelium or gastric caecae and the malpighian tubules in mosquito larvae [37].

In this present study, plant extracts acted at different level of efficacy and *L. adoensis* was the most potent among the two others plant species. The results are comparable to those obtained by Massebo *et al*. [38] in which LC_{50} =17.5 ppm for *C. ambrosoides* and LC_{50}=56.4 for *L. adoensis* were recorded with essential oils of these plants tested on *An. arabiensis* larvae. Similarly, a significant larvicidal activity of *Thymus serpyllum* against *Anopheles stephensi* Liston was reported by [39] with LC_{50}<10 ppm after 24 h of exposure. The adulticidal activity of *L. adoensis* and *C. ambrosoides* essential oils with CL_{50} of 13 and 6.5 ppm, respectively were also reported by [29] against *An. arabiensis* adults. In same way, *C. ambrosoides* tested at 200 ppm exhibited also a significant larvicidal activity against *An. gambiae* larvae with LC_{50} value of 77 ppm, 24 h post-exposure [10]. A significant larvicidal property of *Acalypha ciliata* and *A. ornate* with CL_{50} values of 77.59 and 73.96 ppm, respectively against *An. gambiae* larvae was reported [40]. The variation of the activities among plants could be explained by the variations according to the plant species, the parts of the plant, the geographical location where the plants were grown and the application method.

In general, the toxic effect of essential oils is higher than those of extracts in this present study and could be attributed to their volatile properties with rapid action in insect. Indeed, essential oils are lipophilic in nature and interfere with basic metabolic, biochemical, physiological, and behavioral functions of insects when they are inhaled, ingested or skin absorbed [41]. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine [42] or GABA-gated chloride channels [43] which their disruption results in total breakdown of nervous system in insects.

5. Conclusion

From our results, methanolic extracts and essential oils of *C. ambrosoides, H. suaveolens* and *L. adoensis* exhibited a strong larvicidal activity against *An. gambiae* larvae. However, C. ambrosoides essential oil was the most potent among the plant essential oils tested while *L. adoensis* extract was the most effective among plant extracts applied on mosquito larvae and thus, might be used in the mosquito control program in the potential larval aquatic habitats or breeding sites around human dwellings.

6. Acknowledgements

Authors are grateful to OCEAC for mosquito eggs supplied and facilities for mosquito rearing. Authors acknowledge also the laboratory of Industrial Chemistry Department of the National School of AgroIndustrial Sciences, University of Ngaoundéré, Cameroon for essential oils extraction.

7. References

1. WHO. A global brief on vector-borne diseases. © World Health Organization 2014. WHO/DCO/WHD/2014.1. 2014, 56p.
2. WHO. World Malaria Report 2015: Summary. Geneva: World Health Organization; 2016 (WHO/HTM/GMP/2016.4). Licence: CC BY-NC-SA 3.0 IGO 2016.
3. Killeen GF, Fillinger U, Knols BG. Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high efective coverage. Malarial Journal. 2002; 1:1-8.
4. Joshep CC, Ndoils MM, Malima RC, Nkuniya NHM. Larvicidal and mosquitocidal extracts, a coumrin, isofavonoids and petrocarpans from Neorautanenia mitis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2004; 98:451-455.
5. Katade SR, Pawar PV, Wakharkar RD, Deshpand NR. Sterculia guttata seeds extractives an efective mosquito larvicide. Indian Journal Experimental Biology. 2006; 44:662-665.
6. Mittal PK, Subbarao SK. Prospects of using herbal products in the control of mosquito vectors. ICMR Bull. 2003; 33:1-10.
7. Saxena RC. Botanical pest control. In: Dhaliwal GS, Heinrichs EA, eds. Critical Issues in Insect Pest Management. New Delhi: Commonwealth Publishers. 1998, 155-179.
8. Burkill H. Useful plants of west tropical Africa, 2nd ed. Richmond Surrey, London: Kew Publishing. 1985.
9. Yadav N, Vasudeva N, Singh S, Sharma S. Medicinal properties of the genus *Chenopodium* Linn. Natural Product Radiance. 2007; 6(2):131-4.
10. Bigoga JD, Saahkem PA, Ndindeng SA, Ngondi JL, Nyegue N, Oben JE *et al*. Larvicidal and Repellent Potential of *Chenopodium ambrosioides* Linn Essential Oil against *Anopheles gambiae* Giles (Diptera: Culicidae). The Open Entomology Journal. 2013; 7:16-22.
11. Fekadu M, Mekuria T, Tesfaye B, Meshesha B, Gebre-Michael T. Evaluation on larvicidal effects of essential oils of some local plants against *Anopheles arabiensis* Patton and *Aedes aegypti* Linnaeus (Diptera, Culicidae) in Ethiopia. African Journal of Biotechnology. 2009; 8(17):4183-8.
12. Langsi DJ, Nukenine EN, Fokunang CN, Suh C, Goudoungou WJ. Potentials of essential oils of *Chenopodium ambrosioides* L. and *Cupressus sempervirens* L. against stored maize pest, *Sitophilus zeamais* Motschulsky. Journal of Entomology and Zoology Studies. 2017; 5(2):309-313.
13. Okigbo RN, Okeke JJ, Madu NC. Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* against mosquito larvae. Journal of Agricultural Technology. 2010; 6:703-719.
14. Chitra S, Patil MB, Ravi K. Wound healing activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae). International Journal of Pharmacology and Technology Research. 2009; 1(3):737-744.
15. Koche D, Shirsat R,Imran S, Bhadange DG. Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (MS) India. International Journal of Pharmacology and Bio Sciences. 2010; 1(4):253-256.
16. Anonymous. The Wealth of India. CSIR, New Delhi, ISBN. 2001; 81:230-237.
17. Musa AK, Dike MC, Onu I. Evaluation of Nitta *(Hyptis suaveolens* Poit.) seed and leaf extracts and seed powder for the control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) in stored groundnut. ISSN 1995-896X. 2009; 2(3):176-179.
18. Olotuah OF. Laboratory evaluation of pesticidal activities

of *Hyptis suaveolens* in pest management. International Journal of Agricultural Research. 2013; 8(2):101-106.
19. Cavalcanti ESB, De Morais SM, Ashley ALM, William PSE. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. Memorias do Instituto Oswaldo Cruz. 2004; 99:541-544.
20. Arivoli S, Samuel T. Mosquitocidal activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae) extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Recent Scientific Research. 2011; 2(5):143-149.
21. Abena AA, Diatewa M, Gakossi G, Gbeassor M, Hondi-Assah TH, Ouamba JM. Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. Fitoterapia. 1998; 74:231-236.
22. Pascual ME, Slowing K, Caretero E, Mara KD, Villar A. *Lippia*: Traditional uses, chemistry and pharmacology. A Review Journal Ethnopharmacology. 2001; 76:201-214.
23. Kanco C, Koukoua G, N'Guessan YT, Fournier J, Pradère JP, Toupet L. Contribution à l'étude phytochimique de *Lippia multiflora* (Verbenaceae). Current Review of Chemistry. 2004; 7:1029-1032.
24. Acquaye D, Smith M, Letchamo W, Simon J. *Lippia tea* Centre for New Use Agriculture and Natural Products. Rutgers University, New Brunswick, New Jersey, USA, 2001.
25. Olaifa JI, Erhun WO, Akingbohungbe AE. Insecticidal activity of some Nigerian plants. Insect Science and its Application. 1987; 8:221-224.
26. Oladimeji FA, Orafidiya OO, Ogunniyi TAB, Adewunmi TA. Pediculocidal and scabicidal properties of *Lippia multiflora* essential oil. Journal of Ethnopharmacology. 2000; 72:305-311.
27. Nukenine EN, Adler C, Reichmuth C. Efficacy evaluation of plant powders from Cameroon as post-harvest grain protectants against the infestation of *Sitophilus zeamais* MOTSCHULSKY (Coleoptera: Curculionidae). Journal of Plant Disease and Protection. 2007; 114(1):30-36.
28. Okonkwo CO, Ohaeri OC. Insecticidal potentials of some selected plants. Journal of Chemical and Pharmaceutical Research. 2007; 5(4):370-376.
29. Massebo F, Tadesse M, Balkew M, Gebre-Michael T. Bioactivity of essential oils of local plants against adult *Anopheles arabiensis* (Diptera: Culicidae) in Ethiopia. Advances in Bioscience and Biotechnology. 2013; 4:805-809.
30. WHO. Report of the World Health Organization informal consultation on the evaluation and testing of insecticides. 1996.
31. WHO. Pesticides and their application for the control of vectors and pests of public health importance: World Health Organization, WHO Pesticides Evaluation Scheme. 2006, 113.
32. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economical Entomology. 1925; 18:265-267.
33. Finney DJ. Probit analysis. London: Cambridge University Press, London, United Kingdom. 1971, 68-2.
34. Orozco J, Soto A, Hipolito A. Efecto de repelencia de Crotalaria juncea, Galactia striata y Cymbopogon nardus para el manejo de *Cyrtomenus bergi* (Hemiptera: Cydnidae). Revista de Biologia e Ciencias da Tierra. 2006; 6:179-185.
35. Bilal H, Hassan SA. Plants secondary metabolites for mosquito control. Asian Pacific Journal of Tropical Diseases. 2012, 168-178.
36. Usta J, Kreydiyyeh S, Bakajian K, Nakkash-Chmaisse H. Invitro effect of eugenol and cinnamaldehyde on membrane potential and respiratory complexes in isolated rat liver mitochondria. Food Chemistry and Toxicology. 2002; 40:935-940.
37. David JP, Rey D, Pautou MP, Meyran JC. Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. Journal of Invertebrate Pathology. 2000; 75:9-18.
38. Massebo F, Tadesse M, Bekele T, Balkew M, Gebre-Michael T. Evaluation on larvicidal effects of essential oils of some local plants against *Anopheles arabiensis* Patton and *Aedes aegypti* Linnaeus (Diptera, Culicidae) in Ethiopia. African Journal of Biotechnology. 2009; 8(17):4183-4188.
39. Amer A, Mehlhorn H. Persistency of larvicidal effects of plant oil extracts under different storage conditions. Parasitology Research. 2006; 99:473-477.
40. Aboaba S, Ibrahim K, Omotoso O. Toxicity and mosquito larvicidal activities of the essential oils from the leaves of *Acalypha ornata* and *Acalypha ciliata* in southwest Nigeria. Journal of Vector-Borne Diseases. 2012; 49:114-116.
41. Khater HF. Ecosmart Biorational Insecticides: Alternative insect control strategies, insecticides. Advances in Integrated Pest Management, In Tech. 2012, 708.
42. Enan EE. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Archives of Insect Biochemistry and Physiology. 2005; 59:161-171.
43. Priestley CM, Williamson EM, Wafford KA, Sattelle DB. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. Brazilian Journal of Pharmacognosy. 2013; 140:1363-1372.