Journal of Diseases and Medicinal Plants
2024; Vol. 10, No. 2, pp. 29-39
https://doi.org/10.11648/j.jdmp.20241002.12

Review Article

Effects of Essential Oils of *Vepris heterophylla* (Rutaceae) and *Xylopia aetiopica* (Annonaceae) on Stage II and IV Larvae of *Anopheles gambiae* Sensu Stricto Giles 1902

Ndevenna Ndawakai[1], Bouba Th éophile[1], Saotoing Pierre[1,*], Nukenine Nchiwan Elias[2]

[1]Department of Life and Earth Sciences, Higher Teachers' Training College, University of Maroua, Maroua, Cameroon
[2]Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Ngaoundere, Cameroon

Abstract

Objective: In order to control malaria through the reduction of *Plasmodium* spp vector populations, a study of the sensibility of *A. gambiae* ss stage II and IV larvae to *V. heterophylla* and *X. aetiopica* essential oils was carried out as well as their combined effect evaluated in the University of Ngaoundere, Adamawa region Cameroon from january to february 2020. Methodology: The tests consisted in evaluating the mortality of *A. gambiae* ss larvae in the presence of diluted solutions of the essential oils following a WHO (2005) methodology. Results: Bioassays revealed that the essential oils of both plants have remarkable insecticidal properties. At 250ppm, essential oils of *V. heterophylla* and *X. aetiopica* induced 100% mortality of stage II larvae after 18h of exposure. In stage IV larvae, the same concentration killed 100% of the larvae after 24h of exposure. The combined effect of the essential oils of the two plants on stage II and IV larvae of *A. gambiae* ss varied according to the proportion of combination made. In stage II larvae, the combination of *V. heterophylla* 50% and *X. aetiopica* 50% essential oils showed better efficacy (LC_{50}=42.62ppm; LH_{50}=03hr23min27sec), followed by that of *V. heterophylla* 25% and *X. aetiopica* 75% (LC_{50}=44.24ppm; LH_{50}=03hr31min21sec) and that of *V. heterophylla* 75% and *X. aetiopica* 25% (LC_{50}=53.66ppm; LH_{50}=05hr07min59sec). A similar trend was observed in stage IV larvae. Conclusion and application: In sum, due to their proven insecticidal properties against *A. gambiae* ss, the combination of essential oils of *V. heterophylla* and *X. aetiopica* should be highly recommended for the development of natural biocides.

Keywords

Anopheles gambiae ss, Essential Oils, *Vepris heterophylla*, *Xylopia aetiopica*, Combined Effects, Cameroon

1. Introduction

In the World, the number of people affected by malaria in 2021 is estimated at 247 million compared to 245 million in 2020 with approximately 619,000 deaths, 67% of which are in children under five years of age [1]. Africa unfortunately continues to bear the heavy burden with 234 million cases and 593,000 deaths in 2021 [1]. Cameroon is classified in 2021, among the eleven most affected countries with at least 4000 deaths of which 2700 in children under five years old [1]. The Far North Region Cameroon leads the mortality rate with 32% of deaths [2]. However, the government and international

*Corresponding author: psaotoing@gmail.com (Saotoing Pierre)

Received: 11 January 2024; **Accepted:** 22 February 2024; **Published:** 30 May 2024

partners continue to lead the fight, through the distribution of Long-Lasting Impregnated Mosquito Nets [3]. In spite of the special attention given by both national and international authorities to fight against malaria in Cameroon, the morbidity rate related to this disease continues to rise [4]. Numerous obstacles stand in the way of the scientific community's efforts to control malaria, including the phenomenon of resistance of vector agents to synthetic insecticides, the resistance of *Plasmodium* spp. to antimalarial drugs [5], poor hygiene conditions in some localities favoring human-mosquito contact, and problems of access to primary health care due to the high cost of new antimalarial drugs and insecticides [6]. In response to this situation, the World Health Organization recommends the introduction of impregnated mosquito nets and indoor spraying with synthetic insecticides [7]. Unfortunately, most of these chemicals have adverse effects on humans, other animals and the environment due to their accumulation in the natural environment [8]. Similarly, the regular use of chemical substances to control malaria vectors in some regions induces the appearance of the phenomenon of mosquito resistance to these substances [9]. Given these limitations, it is important to reorient vector control towards the evolutionary stages of the vector, i.e. the larval stage, and above all to use available local plants as an intermediate and more acceptable solution for these populations [4]. In order to contribute to this research, essential oils of *V. heterophylla* and *X. aetiopica* were used to determine their larvicidal activities on *A. gambiae* sensu stricto, the main vector of malaria in Sub-Saharan Africa. The general objective of this work is to contribute to the fight against malaria by reducing the populations of vectors of *Plasmodium* spp.

2. Materials and Methods

2.1. Collection of Plant Leaves

Figure 1. *Vepris heterophylla.*

The plants used in this work and identified at the Yaoundé National Herbarium under the numbers 13770SRFK for *V. heterophylla* (figure 1) and 25091SRFK for *X. aetiopica* (figure 2) were collected in Ngaoundéré in June 2019, a period during which development and floristic diversity are at their maximum. The aerial parts (leaves) of the plants were removed and then dried in the shade in a dry and ventilated place for 10 days. After drying, they were selected and stored in a dark and humid place to be used for the preparation of essential oils.

Figure 2. *Xylopia aetiopica.*

2.2. Extraction of Essential Oils

The method adopted by Diksha *et al*. [10] was used for extraction of the essential oils in the laboratory of Industrial Chemistry and Bio-resources of the National School of Agro-Industrial Sciences (ENSAI), University of Ngaoundere. The dried powdered leaves of each plant was subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus, to give yellow-clear oil for *Vepris heterophylla* and *Xylopia aetiopica*, (Figure 3). The oil was allowed to stand for 2 h, to be clear, and then it was collected carefully after draining out condensed water. Distillates of essential oils were dried over anhydrous sodium sulphate, filtered and stored at -4 ℃ in freezer until needed for analyses and bioassays. The yield of the essential oil obtained from plant materials was calculated as using the formula below [11]:

$$Extraction\ Yield = \frac{Weight\ of\ essential\ oil}{Weight\ of\ plant\ material\ used} x100$$

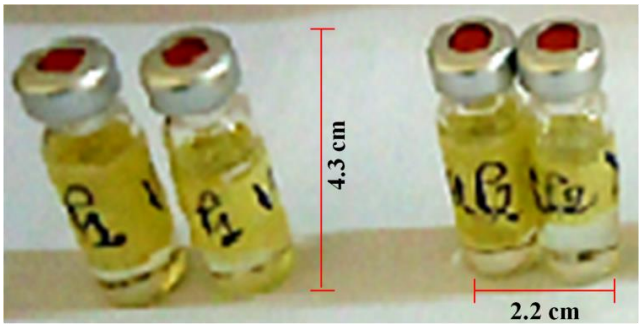

Figure 3. *Essential oils extracted (P1: Vepris heterophylla; P2: Xylopia aetiopica).*

2.3. Dilution of the Essential Oil Stock Solutions

The choices of the concentrations of essential oils were based on several preliminary tests. The concentration of the stock solutions was 0.5g/L (500ppm). From these stock solutions of essential oils of both plants, dilutions were made in acetone to obtain experimental concentration ranges. Four (4) concentrations were chosen from the stock solution concentration in geometric progression (0.25g/L, 0.125g/L, 0.062g/L and 0.03125g/L) ready for testing (Table 1).

Table 1. *Dilution of stock solutions.*

Solutions	CEO (SS)	CEO/2	CEO/4	CEO/8	CEO/16
Concentrations (g/L)	0.5	0.25	0.125	0.0625	0.03125
Concentrations (ppm)	500	250	125	62.5	31.25

CEO: Concentration of Essential Oil; SS: Stock solution; g/L: gram per liter; ppm: part per million

2.4. Breeding of the *A. gambiae* Sensu Stricto Strain

The eggs of *A. gambiae* ss were provided by the Organization for the Coordination of Endemic Diseases in Central Africa (OCEAC) based in Yaoundé-Cameroon to be maintained in rearing to obtain larvae for biological tests. Rearing was carried out at the Entomology Laboratory of the University of Ngaoundéré in January 2020. The eggs were soaked in plastic trays containing untreated natural well water. A few hours (18h - 24 h) after soaking the eggs, they hatched into stage I larvae that visibly measured 2 mm in length. Stage I larvae developed after 48 h into stage II larvae. After 4 days, the larvae had reached stage IV (figure 4). The larvae were fed a nutriment powder consisting of shrimps and crushed cookies. To avoid any pollution resulting from the presence of the nutrient, the water used for larval rearing was renewed every two days [12]. The average temperature inside the insectarium was kept constant (28.2 ℃ ± 0.9 ℃) by a heater running continuously and the relative humidity was 80%.

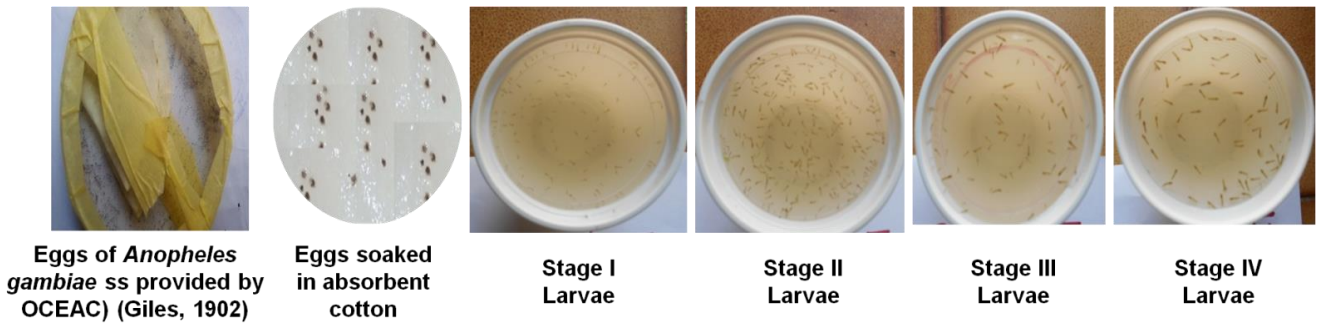

Figure 4. *Rearing of A. gambiae ss larvae.*

2.5. Determination of the Larvicidal Effect of the Essential Oils of the Two Plants

The determination of the larvicidal effect of the essential oils of *V. heterophylla* and *X. aetiopica* was made by biological tests which consisted in evaluating the mortality of the larvae of stage II and IV of *A. gambiae* ss in the presence of the diluted solutions of the essential oils according to a methodology inspired by the protocol of the World Health Organization [13].

2.5.1. Sensibility Testing of Stage II and IV Larvae to Essential Oils

Twenty-five (25) larvae of stage II and IV were collected with a pasteur pipette and put in small transparent plastic boxes of dimension 10 x 6 x 3.6 cm, each containing 99 mL of water supplemented with 1mL of each solution so diluted in the previously prepared boxes supplemented with a volume of bedding water up to 100mL, total volume [14]. The same number of larvae was placed in a negative control (acetone) box containing 99 mL of distilled water without any trace of essential oils, supplemented with 1mL of acetone [15]. Temephos

was used as a positive control. Three replicates were performed for each dilution as well as for the negative control.

2.5.2. Combined Effect of the Essential Oils of the Two Plants

The realization of the combined effect of the essential oils of the two plants on the larvae of stage II and IV of *A. gambiae* ss which obeyed the protocol of World Health Organization is presented in Table 2.

Table 2. *Combination of essential oils (P1+P2) on stage II and IV larvae.*

Combinaisons	P1+P2	P1+P2	P1+P2
Proportions	P1: 25% + P2: 75%	P1: 75% + P2: 25%	P1: 50% + P2: 0%

P1: Vepris heterophylla P2: Xylopia aetiopica

2.6. Data Analysis

Statistical analyses were performed using R software. We also used the following tests:

1) Kruskal-wallis H test for simultaneous comparison of more than two means;
2) Student's t-test for paired series to compare two means (mean mortality of stage II larvae and mean mortality of stage IV larvae;
3) Pearson R correlation tests to establish the relationship between LC_{50} and corresponding LH_{50}.

The curves and regression lines were made in Excel 2007. The determination of LC_{50} and LH_{50} were done by the formula of Finney (1971) [16].

$$LC_{50} = \log 10^{-1}\left(\frac{5-b}{a}\right) \quad LH_{50} = \log 10^{-1}\left(\frac{5-b}{a}\right)$$

3. Results and Discussion

3.1. Yield of Essential Oils of *Vepris heterophylla* and *Xylopia aetiopica*

The essential oils of *V. heterophylla* and *X. aetiopica* obtained by hydrodistillation presented yields recorded in Table 3. The essential oil of *X. aetiopica* presented a high yield (0.92%) compared to that of *V. heterophylla* which gave a low yield (0.52%). The results obtained show that our yields of essential oil are very low. This would be related to the fact that the extraction was done on dry leaves while the other studies were done on fresh leaves [17]. This variability in yield could also be related to the time of harvesting, edaphic and climatic factors or the physiopathological state of the plant, as well as the type of extraction technique and recovery steps [4]. According to Chemat *et al.* [18], the variation is influenced by the length of time the plant is kept after harvesting and can vary from 0.4% after eight days to 1.45% if the extraction of essential oils is performed on the same day the plant is harvested.

Table 3. *Yields of essential oils of Vepris heterophylla and Xylopia aetiopica.*

Plant species	Part taken/Location	Mass (g)	Volume of oil (mL)	Yield (%)
V. heterophylla	Leaf/Ngaoundéré	2000	10.4	0.52
X. aetiopica	Leaf/Ngaoundéré	2000	18.4	0.92

3.2. Toxicity of Essential Oils Tested Separately on Stage II and IV Larvae

The results illustrated in figures 5 and 6 show the toxicity of the essential oil of *V. heterophylla* towards stage II and IV larvae of *A. gambiae* ss. From these figures, it appears that the percentage of mortality is proportional to the doses and times. Mortality in the negative control box is zero, confirming the toxicity of the essential oils of both plants [19]. At the first hour of exposure, mortality varied with concentration between 24% and 84% for stage II larvae and between 6% and 61% for stage IV larvae. A non-significant difference was found between the mortality rates observed (t = 2.8284, df = 3, p-value = 0.06628). This result suggests that there is an age-relate difference in larval sensibility to the essential oils of the two plants, with stage II larvae being less tolerant than stage IV larvae. This may be explained by the hardening of the cuticle of the preimaginal or immature stages during their development, which makes the active ingredients of the essential oils impervious. Indeed, other experimentation carried out by Aouinty *et al.* [20] has shown that a resistance of stage IV larvae compared to stage II larvae could be explained by the development or maturation of cuticles in stage IV larvae. The results are similar to those of Aouinty *et al.* [20] who showed that the LC_{50} varied according to plant extracts, Culicidae species and larval development

stage and that immature larvae were more sensitive to extracts than mature larvae. The highest concentration induced 100% mortality at the 6th hour of exposure in stage II larvae unlike stage IV larvae in which, the essential oil induced 100% at the 12th hour of exposure. Mortality of stage II and IV larvae increased gradually and exceeded 50% for all concentrations at the 12th hour of exposure. In the same vein, Matu (2011) [19] showed that the essential oil obtained from the leaves of *V. heterophylla* contains about 35 compounds, mainly alkaloids, triterpenes, and flavonoids responsible for the larvicidal effects. Indeed, other experimentation carried out by Matu (2011) [19] has shown that a methanol extract of the leaves of *V. heterophylla* exhibits very strong insecticidal activities with an LC_{50} = 204.7 µg/mL and the essential oil of this plant provides significant protection of stored grains against *Tribolium castaneum*, the red flour tribolium. In another study carried out by Sidibe *et al*. [21], freshly harvested leaves of the plant yielded an essential oil containing mainly sabinene (3-12%), δ-cadinene, β-elemene, β-caryophyllene, germacrene-D-4-ol and α-cadinol.

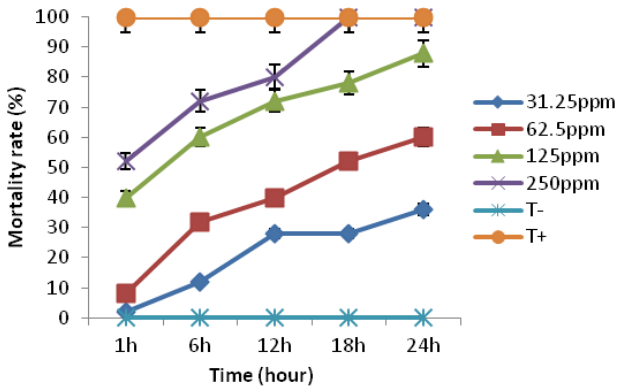

Figure 5. *Mortality rate of stage II larvae to essential oil of V. heterophylla.*

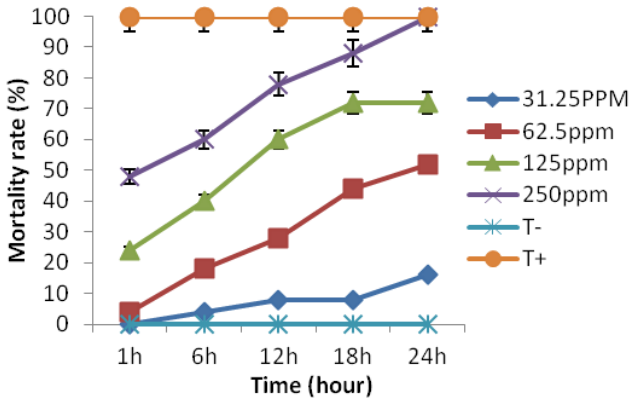

Figure 6. *Mortality rate of stage IV larvae to essential oil of V. heterophylla.*

Figures 7 and 8 illustrate the toxicity of the essential oil of *Xylopia* tested on stage II and IV larvae of *A. gambiae* ss. It is also evident from these figures that the mortality rate is dose and time dependent. At the first hour of exposure, mortality ranged from 14% to 44% for stage II larvae and from 12% to 54% for stage IV larvae as concentrations increased. The highest concentration induced 100% mortality at the 12th hour of exposure for stage II and IV larvae. Larval mortality increased progressively and exceeded 50% for all concentrations at the 12th hour of exposure for stage II larvae and at the 24th hour of exposure for stage IV. The resistance of stage IV larvae compared to stage II larvae could be explained by the development or maturation of cuticles in stage IV larvae. The results are similar to those of Aouinty *et al.* [20] who showed that the LC_{50} varied according to plant extracts, Culicidae species and larval development stage and that immature larvae were more sensitive to extracts than mature larvae.

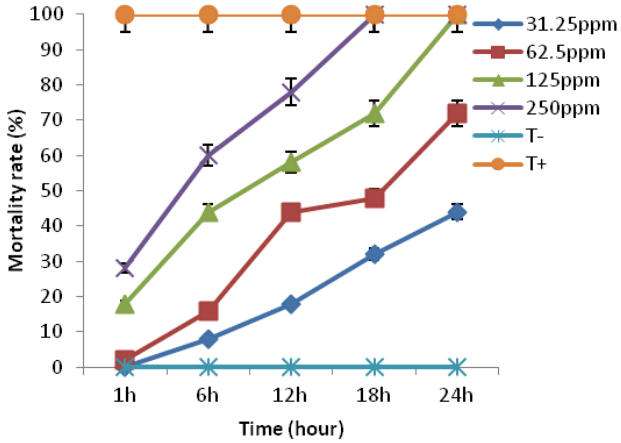

Figure 7. *Mortality rate of stage II larvae to essential oil of X. aetiopica.*

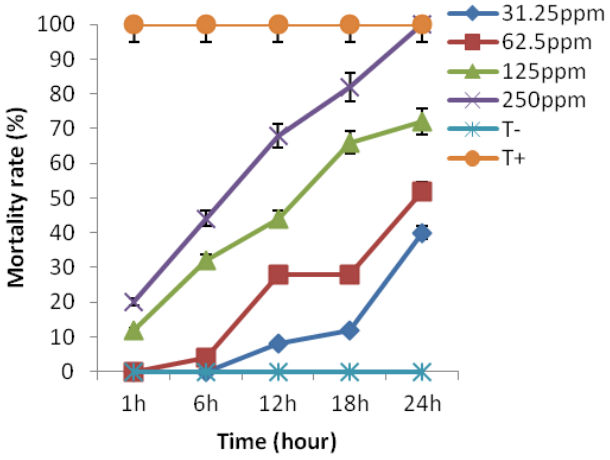

Figure 8. *Mortality rate of stage IV larvae to essential oil of X. aetiopica.*

A non-significant difference was also noted between these observed mortality rates (t = 1.03, df = 3, p-value = 0.391).

Koffi *et al.* [22] in their work on the chemical composition and insecticidal activity of the essential oil of the fruits of *X. aethiopica* (Dunal) A. Rich (Annonaceae) on *Callosobruchus maculatus* performed GC and GC/MS analysis of the essential oil of *X. aetiopica* and identified 43 compounds accounting for more than 95% of the essential oil composition: β-pinene (31.92%), germacrene-D (13.04%), α-pinene (10.28%), sabinene (7.88%), and 1,8-cineole (4.87%). Koffi *et al.* [22] revealed that essential oil has an interesting insecticidal activities against *Callosobruchus maculatus*, a cowpea pest in storage, because at the dose of 7 µL/L essential oil tested, the mortality rate is more than 98% after 24 hours of application.

Determination of the lethal concentration 50 (LC_{50}) and lethal hours 50 (LH_{50}) of the essential oils of *V. heterophylla* and *X. aetiopica* on stage II and IV larvae of *A. gambiae* ss was carried out according to the method of Finney (1971) [16] from regression lines obtained by transforming the percentages of mortality into probit after 24 hours of exposure as a function of the decimal logarithm of the concentrations and hours. Table 4 shows the regression equations, coefficients of determinations, and the different lethal concentration 50 (LC_{50}) and corresponding lethal hours 50 (LH_{50}) of the essential oils of the studied plants.

Table 4. *Regression equations, correlation coefficient, LC_{50} and LH_{50} of the essential oils of the studied plants.*

EO	stages	Regression equation	r	LC_{50} (ppm)	t-test	Regression equation	r	LH_{50} (hr/min/sec)
P1	II	y = 1.8867x + 1.3849	0.98	82.43^{***}	t_1	y =0.8612x + 4.2761	0.98	06hr55min38sec
	IV	y = 2.3233x + 0.1652	0.99	120.49^{***}		y = 0.7944x + 4.0418	0.96	16hr04min35sec
P2	II	y = 1.6467x + 1.7105	0.99	99.45^{ns}	t_2	y = 1.3327x + 3.7065	0.96	09hr20min42sec
	IV	y = 1.6967x + 1.271	0.99	157.68^{ns}		y = 1.2206x + 3.4648	0.95	18hr06sec09sec

EO: Essential oil; P1: Vepris heterophylla; P2: Xylopia aetiopica; R: correlation coefficient. ***: very highly significant (P<0.001); t1 = 8.1924, df = 6, p-value = 0.0001783; nsP>0.05, ns non-significant; t2 = 0.74589, df = 6, p-value = 0.4839

The analysis of the different results shows that the essential oil of *V. heterophylla* has LC_{50} values of 82.43ppm (stage II) and 120.49ppm (stage IV). A very highly significant difference was releaved between these lethal concentrations (t = 8.1924, df = 6, p-value = 0.0001783). For the essential oil of *X. aetiopica*, the LC_{50} values are 99.45ppm and 157.68ppm for stage II and IV respectively. A non-significant difference was noted between the two lethal concentrations (t = 0.74589, df = 6, p-value = 0.4839). The lethal times obtained follow the same order of reactivity with 06hr55min38sec for the essential oil of *V. heterophylla* tested on stage II larvae of *A. gambiae* ss, 09hr20min42sec for the essential oil of *X. aetiopica* tested on stage II larvae, 16hr04min35sec for the essential oil of *V. heterophylla* tested on stage IV larvae and 18hr06sec09sec for the essential oil of *X. aetiopica* tested on stage IV larvae. In view of the LC_{50}s and LH_{50}s obtained, therefore, the essential oils of *V. heterophylla* (LC_{50} = 82.43ppm; LH_{50} =06hr55min38sec) and *X. aetiopica* (LC_{50} = 99.45ppm; LH_{50} = 09hr20min42sec) acted more effectively on stage II larvae in terms of concentration and time. A highly positive correlation ($0.95 \leq r \leq 0.99$) between LC_{50}s and LH_{50}s. Indeed, a fraction that has a low efficacy or then a high LC_{50} will also have a high LH_{50} [4]. Our results corroborate those of Saotoing *et al.* (2014) [23] who were able to verify this correlation with the extract of *Khaya senegalensis* (LC_{50}=2.79g/m^2, LH_{50} = 14hr51min19sec) and *Azardirachta indica* (LC_{50} = 2.77g/m^2, LH_{50} = 11hr56min42sec) essential oils on *A. gambiae* adults.

3.3. Combined Effect of Essential Oils on Stage II Larvae

The combination of P1 25% + P2 75% extracts on stage II larvae (Figure 9) showed that all concentrations induced 100% mortality after 12 h of exposure while the positive control induced 100% mortality after 1 h. Mortality rates varied with the different concentrations and duration of exposure to the product. For the combination of P1 75% + P2 25% extracts (Figure 10), except for the smallest concentration (31.25ppm), the other concentrations induced 100% mortality after 24h of exposure for the 62.5ppm and 125ppm doses. The highest concentration killed 100% of the larvae after 18h of exposure. Mortality rates varied with the different concentrations and duration of exposure to the product. Combined with 50% (Figure 11), the highest concentrations 250ppm and 125 ppm induced 100% mortality after 6 h and 12 h of exposure, respectively. The smallest concentrations 62.5ppm and 31.25ppm induced 100% mortality after 18h of exposure. A non-significant difference was noted between these observed mortality rates (χ^2 = 0.066265, df = 2, p-value = 0.9674). The evolution of mortality rates of mosquito larvae tested with plant leave extracts was also reported in the results of Diallo *et al.* [24]. Dichloromethanic extracts of *Cussonia barteri* from Mali were toxic to *Anopheles gambiae* sl and *Aedes* larvae at the concentration of 500 mg/L and toxicity increased with

increasing concentrations and duration of exposure [24]. The combination P1 50% +P2 50% was more effective than combinations P1 75% +P2 25% and P1 25% +P2 75%. This means that in the combinations, *V. heterophylla* and *X. aetiopica* essential oils showed an additive and synergistic effect on stage II *Anopheles gambiae* ss larvae. Synergistic effects of plant extracts are also reported by Akono *et al*. [25].

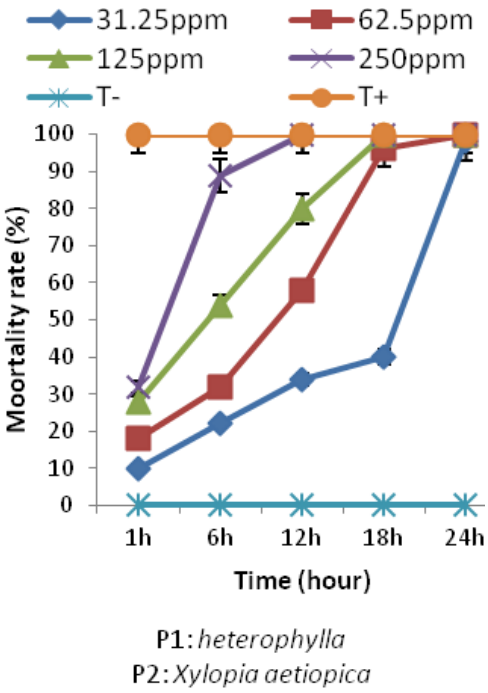

Figure 9. *Stage II larval mortality at P1 25% + P2 75% combination.*

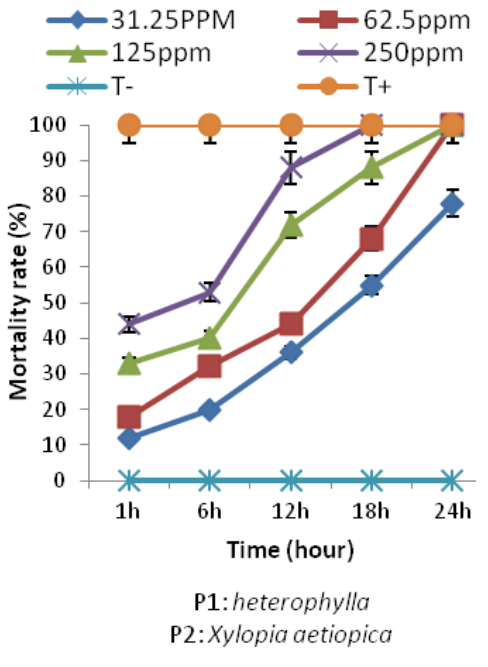

Figure 10. *Stage II larval mortality at P1 75% + P2 25% combination.*

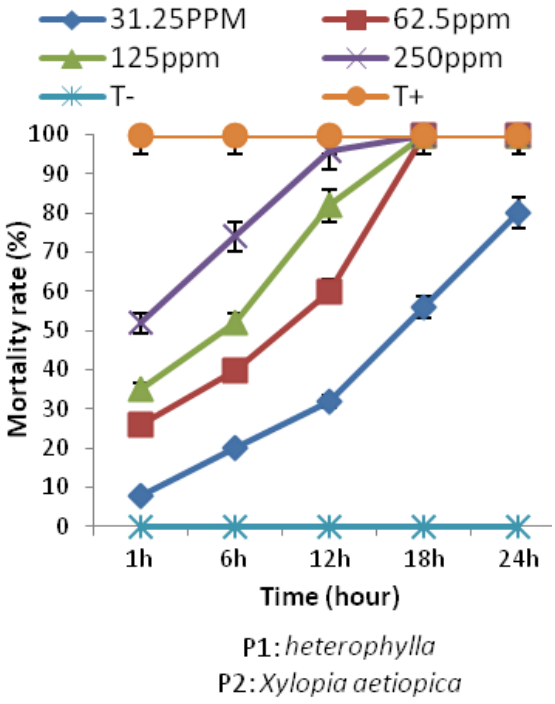

Figure 11. *Stage II larval mortality at P1 50% + P2 50% combination.*

Determination of the lethal concentration 50 (LC_{50}) and lethal hours 50 (LH_{50}) of the combination of essential oils of *V. heterophylla* and *X. aetiopica* on stage II larvae of *A. gambiae* ss was also carried out according to the method of Finney (1971) [16] from regression lines obtained by transforming percent mortality into probit after 24 hours of exposure as a function of the decimal logarithm of concentrations and hours. Table 5 shows the regression equations, coefficients of determinations, and the different lethal concentration 50 (LC_{50}) and corresponding lethal hours 50 (LH_{50}) of the essential oils of the studied plants.

This table shows that the combination P1: 50% + P2: 50% has a low LC_{50} (42.62ppm), followed by the combination P1: 25% + P2: 75% (44.24ppm) and that of P1: 75% + P2: 25% (53.66ppm). The Kruskal-wallis test showed a non-significant difference at α=5% (χ^2 = 0.18398, df = 2, p-value = 0.9121). The lethal times obtained were 3hr23min27sec for the combination P1: 50% + P2: 50%, 3hr31min21sec for the combination P1: 25% + P2: 75% the extract and 5hr07min59sec for the combination P1: 75% + P2: 25% (53.66ppm). Pearson's R correlation test showed a relationship between LC_{50} and LC_{50} (t = 3.5, df = 2, p-value = 0.07283). Our results corroborate those of Bouba *et al* [26] in their work on the evaluation of the larvicidal and pupecidal potential on *Anopheles gambiae* sl of methanolic extracts of *Cyperus rotundus* (Cyperaceae) and *Leucas martinicensis* (Lamiaceae). The find that the LC_{50} values of *Cyperus rotundus* were 61.27ppm, 70.84ppm, 85.86ppm, 104.44ppm for I to IV instars and 339.18ppm for the pupal instar, respectively [26]. The corresponding lethal hours (LH_{50}) were 10h19mn13s, 11h31mn55s, 13h31mn49s,

16h04mn13s, 36h58mn27s for first, second, third, fourth instar larvae and pupae, respectively [26]. The toxic effect of the extracts of *Leucas martinicensis* was also demonstrated with LC_{50} values of 28.24ppm, 39.1ppm, 54.79ppm, 80.78ppm for I to IV instars and 244.3ppm for the pupal instar, respectively. The corresponding lethal hours (LH_{50}) were are 06h29mn37s, 08h02mn45s, 06h24mn47s, 13h29mn45s, 27h05mn50s respectively for the same larval instars [26].

Table 5. *Regression equations, correlation coefficient, LC50 and LH50 of the essential oils of the studied plants.*

Proportion	Regression equation	r	LC_{50} (ppm)	Kruskal-test	Regression equation	r	LH_{50} (hr/min/sec)
P1: 25% + P2: 75%	y = 1.36x + 2.7616	0.99	44.24[a]		y = 2.1894x + 3.8027	0.81	03hr31min21sec
P1: 75% + P2: 25%	y = 1.1133x + 3.0801	0.99	53.66[a]	χ^2	y = 1.3936x + 4.0661	0.86	05hr07min59sec
P1: 50% + P2: 50%	y = 1.3567x + 2.8006	0.97	42.62[a]		y = 1.4849x + 4.2125	0.9	03hr23min27sec

P1: *Vepris heterophylla*; P2: *Xylopia aetiopica*; r: correlation coefficient. Values followed by same letters in the same column are not significantly different at α=5% (χ^2 = 0.18398, df = 2, p-value = 0.9121).

3.4. Combined Effect of Essential Oils on Stage IV Larvae

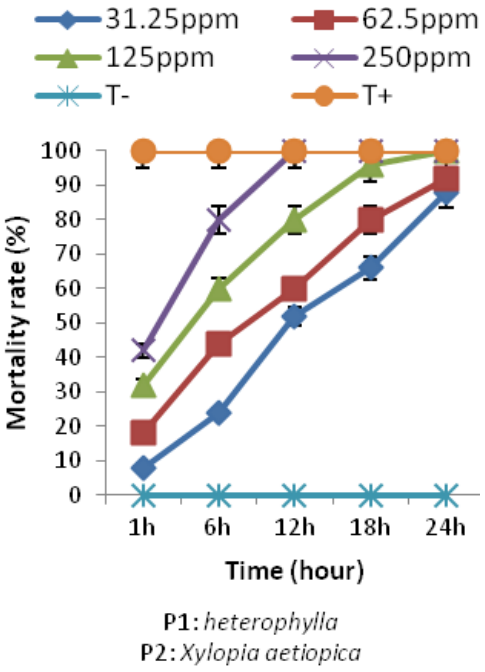

Figure 12. *Stage IV larval mortality at P1 25% + P2 75% combination.*

The evolution curves of mortality rates of stage IV larvae tested with the combination of P1 25% + P2 75% extracts (Figure 12) show that the highest concentrations 125ppm and 250ppm induced 100% mortality of larvae after 12 h and 18h of exposure, respectively. The smallest concentrations 31.25ppm and 62.5ppm induced 100% mortality after 24h of exposure. For the combination P1 75% + P2 25% (Figure 13) the highest concentration killed 100% of the larvae after 18h of exposure while the concentrations 31.25ppm, 62.5ppm and 125ppm induced 100% mortality after 24h exposure. For the combination P1 50% + P2 50% (Figure 14), the highest concentration induced 100% mortality of stage IV larvae after 12 hours of exposure. All other concentrations killed 100% of the larvae after 18h of exposure. The combination P1 50% +P2 50% was more effective than combinations P1 75% +P2 25% and P1 25% +P2 75%. A non-significant difference was also noted between these observed mortality rates (χ^2 = 2.8494, df = 2, p-value = 0.2406). This means that in the combinations, *V. heterophylla* and *X. aetiopica* essential oils showed an additive and synergistic effect on stage II *Anopheles gambiae* ss larvae. Synergistic effects of plant extracts are also reported by Akono *et al*. [25].

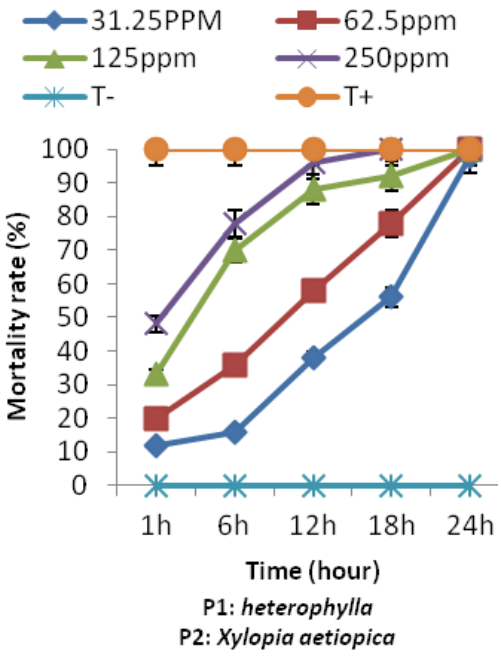

Figure 13. *Stage IV larval mortality at P1 75% + P2 25% combination.*

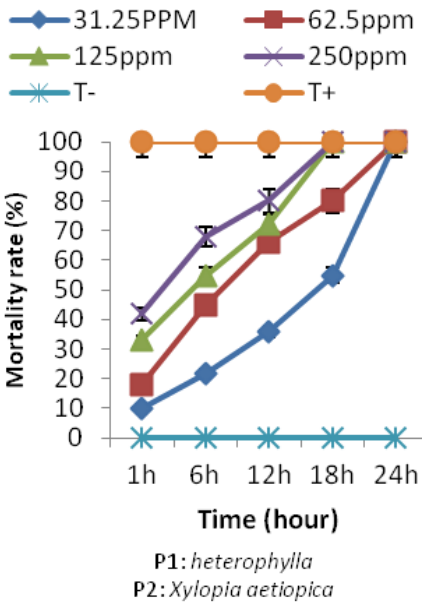

Figure 14. *Stage IV larval mortality at P1 50% + P2 50% combination.*

Determination of the lethal concentration 50 (LC_{50}) and lethal hours 50 (LH_{50}) of the combination of essential oils of *V. heterophylla* and *X. aetiopica* on stage IV larvae of *A. gambiae* ss was also carried out according to the method of Finney (1971) [16] from regression lines obtained by transforming percent mortality into probit after 24 hours of exposure as a function of the decimal logarithm of concentrations and hours.

Table 6 shows the regression equations, coefficients of determinations, and the different lethal concentration 50 (LC_{50}) and corresponding lethal hours 50 (LH_{50}) of the essential oils of the studied plants.

The analysis of Table 6 leads to the following observations: the combination P1: 50% + P2: 50% has a low LC_{50} (37.77ppm) compared to the combination P1: 25% + P2: 75% (LC_{50} = 38.32ppm) and the combination P1: 75% + P2: 25% (LC_{50} = 40.42ppm). The Kruskal-wallis test showed a non-significant difference at α=5% (χ^2 = 0.068021, df = 2, p-value = 0.9666). The lethal hours obtained are 3hr29min47sec for the combination P1: 50% + P2: 50%, 3hr31min26sec for the combination P1: 25% + P2: 75% and 3hr11min01sec for the combination P1: 50% + P2: 50%. In view of this observation, there would be a correlation between LC_{50} of the essential oils and their LH_{50} (t = 5.1962, df = 1, p-value = 0.121). Generally, an extract with a high efficiency (LC_{50} low) also has a low LH_{50} [26]. Our results corroborate those of Njan Nl ôga *et al.* [27] who find in their work that the essential oil of *Ocimum canum* is very effective (LC_{50}=11.95mg.m-2) and has a very low LH_{50} (6h36mn36s) compared to other plants. A study carried out by Saotoing *et al.* [28] based on acetone extracts from the leaves of *Calotropis procera* and *Boswellia dalzielii* revealed that these two plants show remarkable efficacy with an LC_{50} of 3.03g.m^{-2} and an LH_{50} of 7h31mn08s for *Calotropis procera* and an LC_{50} of 3.94g.m^{-2} and an LH_{50} of 9h52mn25s for *Boswellia dalzielii*.

Table 6. *Regression equations, correlation coefficient, LC_{50} and LH_{50} of the essential oils of the studied plants.*

Proportion	Regression equation	R	LC_{50} (ppm)	Kruskal-test	Regression equation	R	LH_{50} (hr/min/sec)
P1: 25% + P2: 75%	y = 1.22x + 3.0682	0.99	38.32[a]		y = 1.5507x + 4.1517	0.95	03hr31min26sec
P1: 75% + P2: 25%	y = 1.32x + 2.8792	0.99	40.42[a]	χ^2	y = 2.0202x + 3.984	0.78	03hr11min01sec
P1: 50% + P2: 50%	y = 1.0333x + 3.3703	0.98	37.77[a]		y = 2.0894x + 3.8641	0.78	03hr29min47sec

P1: Vepris heterophylla; P2: Xylopia aetiopica; R: correlation coefficient. Values followed by same letters in the same column are not significantly different at α=5% (χ^2= 0.068021, df = 2, p-value = 0.9666)

4. Conclusion

The present work showed that the essential oils of *V. heterophylla* and *X. aetiopica* possess remarkable larvicidal activities on *A. gambiae* ss. The combinations *V. heterophylla* 50% and *X. aetiopica* 50% were the most effective in terms of concentration and time for stage II (LC_{50}=42.62; LH_{50}=03hr23min27sec) and IV (LC_{50} = 37.77; LH_{50}= 03hr29min47sec) larvae. In view of these indices, these two plant species could be the subject of an intermediate solution in the search for new biocides. The present study will be quite helpful in developing plant based anti- malarial vector and others borne diseases.

Acknowledgments

The authors of this article are grateful to the Organization for Cooperation on Endemic Diseases in Central Africa (OCEAC) for the provision of *A. gambiae* ss eggs. They also thank the Head of the Biology Department of the Faculty of Science of the University of Ngaound é r é for accepting the

manipulation of biological tests in the Entomology laboratory. Finally, the authors would like to express their gratitude to Drs Younoussa Lamé and Oumarou Mallam Kary for their help in carrying out the biological tests.

Data Availability Statement

The data used in this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] WHO, 2022. Geneva: World malaria report. Licence: CC BY-NC-SA 3.0 IGO. ISBN 978-92-4-006489-8 (electronic version), ISBN 978-92-4-006490-4 (print version). 131 pages. https://www.who.int/teams/global-malaria-programme

[2] Programme National de Lutte contre le Paludisme (PNLP), 2020. Rapport d'activité 2019 du Programme Nationale de Lutte contre le Paludisme. [Activity report of the National Malaria Program.] 60 p.

[3] Minsanté, 2019. 4ème Campagne de Chimioprévention du paludisme saisonnier (CPS) chez les enfants de 03 à 59 mois dans les régions du Nord et de l'Extrême-Nord du Cameroun [4th Seasonal Malaria Prevention Chemotherapy Campaign (CPS) in children aged 3 to 59 months in the North and Far North regions of Cameroon.]

[4] Bouba, 2022. Effets larvicides des extraits de quatre essences végétales sur *Anopheles arabiensis* à Maroua (Extrême-Nord, Cameroun) [Larvicidal effects of extracts of four plant species on *Anopheles arabiensis* in Maroua (Far North, Cameroon).] Thèse de Doctorat/Ph. D, Université de Ngaoundéré Cameroun, 182p.

[5] Djogbénou, 2009. Lutte antivectorielle contre le paludisme et résistance des vecteurs aux insecticides en Afrique [Vector control against malaria and vector resistance to insecticides in Africa.] Institut Régional de Santé Publique (IRSP), Ouidah, Bénin, Médecine

[6] Tchoumbougnang F., Dongmo P. M., Sameza M. L., Nkouaya E. G., Fotso G. B., Zollo P. H. et Menut C., 2009. Activité larvicide sur *Anopheles gambiae* Giles 1902 et composition chimique des huiles essentielles extraites de quatre plantes cultivées au Cameroun [Larvicidal activity on *Anopheles gambiae* Giles 1902 and chemical composition of essential oils extracted from four plants cultivated in Cameroon.]. Biotechnologie, Agronomie, Société et Environnement, 1: 77-84.

[7] WHO, 2014. Entomologie du paludisme et lutte antivectorielle. Guide du participant [Malaria entomology and vector control. Participant's guide.] 187p.

[8] Chandre F, Darrier F, Manga L. Status of pyrethroid resistance in *Anopheles gambiae* sensu lato, 2000. Bull World Health Organization. 1999; 77: 230-234.

[9] Saotoing, 2017. Faune culicidienne, transmission de Plasmodium et essais de lutte contre *Anopheles gambiae* par l'utilisation des extraits de plantes locales à Maroua (Cameroun) [Culicidian fauna, transmission of Plasmodium and trials to combat *Anopheles gambiae* using local plant extracts in Maroua (Cameroon).] Thèse de Doctorat/Ph. D, Université de Ngaoundéré Cameroun, 141.

[10] Diksha N. K. G., Kumar R. and Dhar K. L., 2012. Study on contents and chemical constituents of the essential oil of the leaves of *Eucalyptus globulus* Labill. International Journal of Universal Pharmacy and Life Science, 2(4): 264-269.

[11] Kraiffi Fella, Boualam Khaoula, 2021. Extraction et caractérisation de quelques huiles essentielles des plantes utilisées dans la thérapie grippale (*Tymus lanceolatus*, *Eucaluptus globulus*) [Extraction and characterization of some essential oils from plants used in influenza therapy (*Tymus lanceolatus*, *Eucaluptus globulus*).] Mémoire de Master, Université de Biskra Algérie, 50p.

[12] Desfontaine M, Tchikangwa I, Le Goff G, Robert V, Carnevale P, 1991. Influence de l'alimentation des larves de Anopheles gambiae (Diptera: Culicidae) sur le développement préimaginal en insectarium [Influence of the diet of larvae of *Anopheles gambiae* (Diptera: Culicidae) on preimaginal development in the insectary.] Bulletin de liaison et de documentation de l'OCEAC 98: 12-14.

[13] OMS, 2005. Roll Back Malaria Project: Resources Support Network for Prevention and control of Malaria Epidemics., 88 p.

[14] Bezzaoui Ouarda, 2013. Comparaison de l'efficacité des extraits aqueux et des huiles essentielles de *Rosmarinus officinalis* (la sauge) et de *Salvia officinalis* avec un insecticide chimique la Cyperméthrine sur les larves de *Culex pipiens* en conditions controlées [Comparison of the effectiveness of aqueous extracts and essential oils of *Rosmarinus officinalis* (sage) and *Salvia officinalis* with a chemical insecticide Cypermethrin on *Culex pipiens* larvae under controlled conditions.] Mémoire de Master, Université de Blida, 49p.

[15] Dongmo Jazet P. M., Tatsadjieu L. N., Tchinda Sonwa1E., Kuate J., Amvam Zollo P. H. and Menut C., 2009. Essential oils of *Citrus aurantifolia* from Cameroon and their antifungal activity against *Phaeoramularia angolensis*. African Journal of Agricultural Research Vol. 4 (4), pp. 354-358, April 2009 Available online at http://www.academicjournals.org/AJAR ISSN 1991-637X © 2009 Academic Journals.

[16] Finney, 1971. Probit analysis. 3rd Ed. Cambridge University Press, 333 p.

[17] Derradhi Heffaf F., 2013. Composition chimique et activité insecticide de trois extraits végétaux à l'égard de *Sitophylus orizae* L. (Coleoptera: Curculionidae) [Chemical composition and insecticidal activity of three plant extracts against *Sitophylus orizae* L. (Coleoptera: Curculionidae)]. Thèse de Doctorat, ENSA, Algérie, 89p.

[18] Chemat, F., Abert-Vian, M., Fabiano-Tixier, A. S., Strube, J., Uhlenbrock, L., Gunjevic, V. & Cravotto, G., 2019. Green extraction of natural products. Origins, current status, and future challenges. Trends in Analytical Chemistry, 118, 248-263.

[19] Matu, E. N., 2011. *Vepris heterophylla* (Engl.) Letouzey. In: Schmelzer, G. H. & Gurib Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa.

[20] Aouinty B, Saadia O, Fouad Mr. and Saadia Mr., 2006. Preliminary assessment of the larvicide activity of the aqueous extracts of the sheets of the ricinus (*Ricinus communis* L.) and of the wood of thuja (*Tetraclinis articulata* (Vahl) Mast.) on the larvae of the four culicidés mosquitos: *Culex pipiens* (Linneus), *Aedes caspius* (Pallas), Culiseta longiareolata (Aitken) and *Anopheles maculipennis* (Meigen)". Biothchnol. Agron. Ploughshare. Approximately. 10 (2), 67-71.

[21] Sidibe, L., Chalchat, J. C., Garry, R. P. & Harama, M., 2001. Aromatic plants of Mali (VI): chemical composition of essential oil of *Vepris heterophylla* (Engl.). Journal of Essential Oil Research 13(3): 183–184.

[22] Koffi Senam Etsè, Roger H. Ch. N'dbi é, Kodjo Eloh, Kokou Agbekonyi Agbodan, Kokouvi Dotse, Honoré Kossi Koumaglo, 2012. Chemical composition and insecticidal activity of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) essential oil on *Callosobruchus maculatus*. J. Soc. Ouest-Afr. Chim. (2012) 034; 71-77.

[23] Saotoing P., Tchuenguem Fohouo F. N., Febal H., Amba A. H. B., Amadou H., Nawe T. U., 2014. Evaluation of insecticidal effect of vegetable oils Extracted from the ripe seeds of *Azadirachta indica* and *Khaya senegalensis* in adult *Anopheles gambiae* s. l. In Maroua, Far north region Cameroon. GJRMI 3(8) 312-322.

[24] Diallo D., Marston A. Terreaux C., Touré Y., Paulsen B., Smestad et Hostettman K., 2001. Screening of Malian Medicinal Plants for Antifungal, Larvicidal, Molluscicidal, Antioxydant and Radical Scavenging Activities". Phytotherapy Research, 15, 401–406.

[25] Akono Ntonga P., Philippe Belong, Fran çois Tchoumbougnang, Eric Mo ïse Bakwo Wire, Henri Fankem, 2012. Chemical composition and insecticidal effects of essential oils of the fresh sheets of *Ocimum canum* Sims and *Ocimum basilicum* L on the adults of *Anopheles funestus* ss, vector of malaria in Cameroun: ISSN 1997-5902.

[26] Bouba Theophile, Saotoing Pierre and Ndjonka Dieudonne, 2020. Evaluation of the larvicidal and pupecidal potential on *Anopheles gambiae* sl of methanolic extracts of *Cyperus rotundus* (Cyperaceae) and Leucas martinicensis (Lamiaceae). International Journal of Mosquito Research 2020; 7(2): 01-07. www.dipterajournal.com

[27] Njan Nloga A. M, Saotoing P, Tchouankeu J. C and Messi J 2007- Effect of Essential Oils of Six Room Insecticidal Used Seedlings one Adults of *Anopheles gambiae* Giles 1902 Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Cameroun Newspaper of Entomology 4 (6): 444-450.

[28] Saotoing P, Tchuenguem Fohouo FN, Amadou Dawe, Ngatarang C. Evaluation of the insecticidal effect of the acetone extract of the dried leaves of *Calotropis procera* Ait. (Asclepiadaceae) in adults of *Anopheles gambiae*, Maroua (Cameroon). International Journal of Biological and Chemical Sciences. 2014; 8(4): 1438-1444.