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Combined Effect of *Senna occidentalis* (Fabaaceae) and *Khaya senegalensis* (Meliaceae) Leave Extracts on Stage II and IV Larvae of *Anopheles gambiae* Sensu Stricto Giles 1902

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Abstract: Objectives: As part of the research for effective biological control methods against malaria vectors, the larvicidal activity of combined leave extracts of *Senna occidentalis* and *Khaya senegalensis* collected in Maroua, Far North Cameroon was evaluated on stage II and IV larvae of *Anopheles gambiae* s.s. from January to February 2020. Methodology and Results: Biological tests performed according to the standard protocol of the World Health Organization (WHO, 2005). The results revealed that the combination P1 25% +P2 75% of extracts from both plants tested on stage II larvae and P1 50% +P2 50% tested on stage IV larvae were the most effective, with respective LC_{50} of 290 ppm and 320 ppm. The corresponding LH_{50} are 3 hr 42 min 55 sec and 2 hr 11 min 15. At 250 ppm, the extracts of *Senna occidentalis* and *Khaya senegalensis* induced 100% mortality in stage II larvae after 6 hours of exposure. In stage IV larvae, the same concentration induced 100% mortality after 12 hours of exposure. The efficacy was highest with the combination of *Senna occidentalis* at 75% and *Khaya senegalensis* at 25%. Conclusion and Application: In sum, due to their high extraction yields and proven insecticidal properties against *Anopheles gambiae* s.s, the combination of *Senna occidentalis* and *Khaya senegalensis* leave extracts should be highly recommended for biocide development.

Keywords: Anopheles *Gambiae* s.s, Extracts, *Senna occidentalis*, *Khaya senegalensis*, Malaria Control

1. Introduction

According to WHO [1], malaria remains one of the most worrying parasitic diseases in the intertropical world, and particularly in sub-Saharan Africa. Data estimate that 216 million malaria episodes are recorded each year, 81% of which are in Africa WHO [2]. Children under 5 years of age and pregnant women are the most vulnerable population group [2]. In Cameroon, malaria remains a major endemic disease. According to the Cameroonian Ministry of Public Health 2019, malaria (MINSANTE) accounts for 40-50% of medical consultations, 23% of hospitalizations and consumes about 40% of the annual household health budget [3]. The World Health Organization and the scientific community have always led the fight against malaria both curative and preventive PNLP 2019 [4]. Despite the progress made in the fight against malaria, there are still many limitations to the fight against malaria, namely: the resurgence of *Plasmodium* resistance to antimalarial drugs used for curative action; the resistance of the mosquito to insecticides; economic problems due to the high cost of new antimalarial drugs and insecticides; the very important side effects of synthetic insecticides used in vector control; Chandre *et al* [5], Hargreaves [6]. Faced with malaria, which has become a public health problem, the World Health Organization recommends the implementation of new strategies to

overcome this scourge. One of the methods that has proven to be effective and to which we adhere is the control of vector populations, especially larvae and adults, using natural insecticides. Hence the urgency to look for natural insecticides that are as active, biodegradable and well known by local communities Njan Nloga *et al* [7]. Previous studies and real experiences have shown that some plants contain substances in their structures (leaves, bark, roots and fruits) that have insecticidal, insect repellent, bactericidal and fungicidal properties Pasma *et al* [8]; Saotoing *et al* [9]. The use of natural substances extracted from plants with proven insecticidal properties is further encouraged. The choice of *Senna occidentalis* and *Khaya senegalensis* to study the insecticidal/repellent effect on insects is based on their odor and their use by some local populations to repel mosquitoes and flies. In African tradition, the use of plants for insecticidal purposes has long been known and used as an insect control agent. The general objective of this work is to contribute to the fight against malaria by reducing the Anopheles population.

2. Materials and Methods

2.1. Rearing of the Anopheles gambiae ss Strain

Anopheles gambiae ss eggs were provided by the Organization for the Coordination of Endemic Diseases in Central Africa (OCEAC, Yaoundé-Cameroon) to be maintained in rearing to obtain the bioassay populations. The rearing was carried out in the entomology laboratory of the University of Ngaoundéré. The eggs were soaked in plastic trays containing untreated natural well water. A few hours (18-24 h) after the eggs were soaked, they hatched into stage I larvae that were visibly 2 mm long. The stage I larvae developed after 48 h into stage II larvae. After 4 days, the larvae had reached stage IV. The larvae were fed according to the methods of Desfontaine *et al* [10]. The average temperature inside the insectarium, kept constant by a continuously operating heater, was 28.2°C ± 0.9°C, with a relative humidity of 80%. Biological tests were performed on stage II and IV larvae only.

2.2. Collection of Plant Leaves and Extraction Process of Essences

The plant material was collected on June 15, 2019 between 6:00 and 9:00 am in the Pitoaré area. The collected specimens were then identified at the Yaoundé National Herbarium under the following herbarium numbers: *Senna occidentalis* (42060/HNC); *Khaya senegalensis* (18628/SRF/Cam). The collected leaves were shade dried for 7 days and powdered by grinding and sieving with a 0.4 mesh sieve. 500 g of powder of each plant was mixed with 2 L of pure methanol; the pastes obtained from the mixture of the powder and pure methanol were left to rest for a period of 24 hours and then homogenized by manual shaking. The purpose of this process was to release the active molecules of the plant. Once the maceration time was over, the obtained pastes were filtered using a filter paper introduced in a funnel. The filtrates obtained were kept with it to bring them to concentration in the Rotavapor (Clevenger apparatus). The residual pastes were again mixed with ethanol and stored for later use. Once started, the Rotavapor evaporates the methanol as a vapor which is conducted to a condenser and then collected in a second flask as pure methanol. In the water bath flask, only the plant extract remains, this will serve as the active ingredient for the tests.

2.3. Larvicidal Test

The larvicidal test was performed according to the WHO protocol [1] using the Petri dish containing thirty larvae into which the solutions of the different extracts were introduced. A stock solution with a volume (Vm) of 5625 mg/mL was prepared from the different concentration ranges (125 ppm; 250 ppm; 500 ppm and 1000 ppm). The sampled volumes of the stock solution were 2.2mL; 4.4mL; 8.8mL and 17 mL. 25%, 50% and 75% of the sampled volumes were used in turn to combine the extracts. Temephos (Abate) was used as a positive control and water as a negative control. Once the extract solution was introduced into the small plastic dishes containing water, the plant extracts were added. Everything was well homogenized and then 30 larvae were introduced in the different concentrations. A timer was started and the larvae were observed every 30 minutes during the first 6 hours of exposure, then every 1 hour until 12 hours. The device was abandoned for a final observation after 24 hours of exposure. After each observation, dead larvae were gently removed from the small plastic dishes with a micro forceps. A larva was considered dead when it remained immobilized at the bottom of the small plastic dishes and did not respond to touch with a needle. The experiment was repeated three times to minimize errors. Figure 1 shows the bioassay setup.

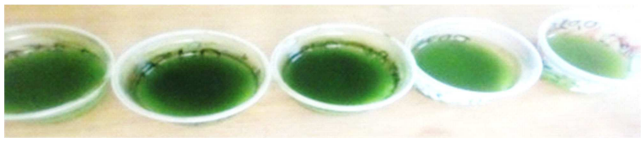

Figure 1. *Exposure of larvae to plant extracts in small plastic dishes.*

2.4. Data Analysis

The curves of evolution of larval mortality as a function of concentration and time were made with Graphpad software. Histograms, curves and regression lines (to determine LC_{50} and HL_{50}) were made with Microsoft Excel 2013 software. Then, the calculations of LC_{50} and HL_{50} were performed by Finney's logarithmic formula [11].

$$\mathrm{LC_{50}(LH_{50})} = log10^{-1}\left(\frac{y}{a} - \frac{b}{a}\right)$$

The mortalities obtained at different concentrations were compared using Schwartz's Z-test [12] with a threshold of 0.05%.

3. Results and Discussion

3.1. Yield of the Extract

From the total mass of the powders of each plant, the yields of the extracts were 14.6% and 17.8% for *Khaya senegalensis* and *Senna occidentalis* respectively. Information on the plants and yields are presented in Table 1.

Table 1. *Yield of plant extracts.*

Species	Parts	Powder Mass (g)	Place	Color	Mass of Extracts (g)	(%) Yield
Senna occidentalis	Sheets	500	Maroua	Green	89	17.8
Khaya senegalensis	Leaves	500	Maroua	Olive green	73	14.6

3.2. Phytochemical Analysis of Plant Essences

Figure 2 shows the flavonoid content of the two extracts. The flavonoid contents were 3.431 Eq.g Quercetin/100g and 2.488 Eq.g Quercetin/100g for *Khaya senegalensis* and *Senna occidentalis* respectively. The flavonoid content varied with each extract.

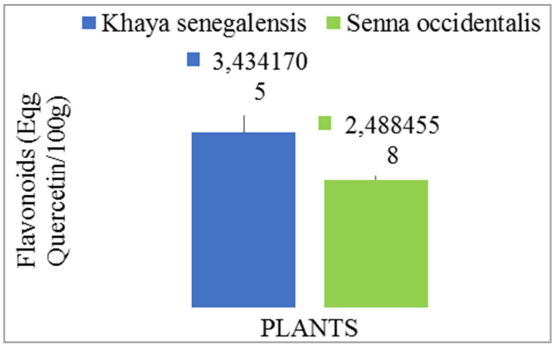

Figure 2. *Flavonoids content.*

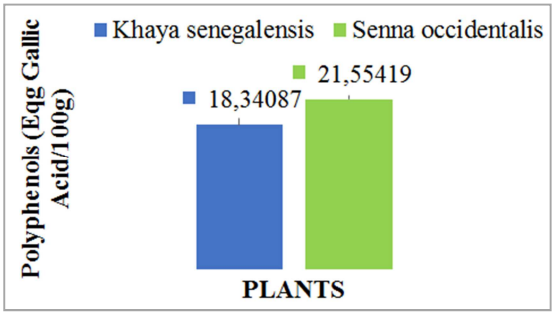

Figure 3. *Polyphenols content.*

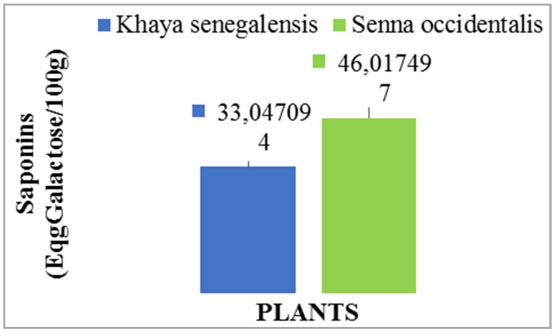

Figure 4. *Saponins content.*

Figure 3 shows the polyphenol content of the extracts. The polyphenol values were 21.554 Eq.g Gallic Acid /100g and 18.340 Eq.g Gallic Acid /100g for *Senna occidentalensis* and *Khaya senegalensis* respectively. The leave extracts of both plants contained more polyphenols than flavonoids. For saponin content (Figure 4), the values were 46.017 Eq. Galactose /100g and 33.047 Eq. Galactose /100g for *Senna occidentalensis* and *Khaya senegalensis* respectively, values higher than polyphenols and flavonoids.

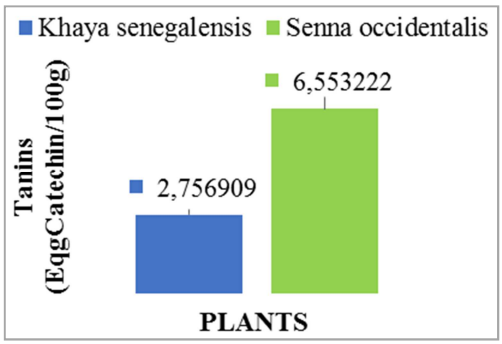

Figure 5. *Tanins content.*

The extracts of the two plants had the lowest tannin content: 6.553 Eq.g Catechin/100g and 2.756 Eq.g Catechin/100g respectively *Senna occidentalis* and *Khaya senegalensis*. It is evident from all figures 2, 3, 4 and 5 that with the exception of flavonoids (Figure 1), *Senna occidentalis* extracts had higher contents of polyphenols, tannins and saponins than *Khaya senegalensis* extracts. The variation of metabolites in the extracts of both plants is also reported in the work of Apiwat *et al* [13]. They had explained these variations by several factors such as climate, nature of the soil, condition of the plant.

3.3. Efficacy of Combined Extracts on Stage II Larvae

The combination of P1 25% + P2 75% extracts on stage II larvae (Figure 6) showed that all concentrations induced 100% mortality after 6 hours of exposure except the lowest concentration (125 ppm = 96%) while the positive control induced 100% mortality after 1 hour. Mortality rates varied with different concentrations and duration of exposure to the product.

For the P1 75% + P2 25% extract combination (Figure 7), all concentrations induced 100% mortality after 6 hours of exposure. Mortality rates varied with different concentrations and duration of exposure to the product. Plant 1 (*Senna occidentalis*) at 75% had a greater toxic effect than plant 2

(*Khaya senegalensis*).

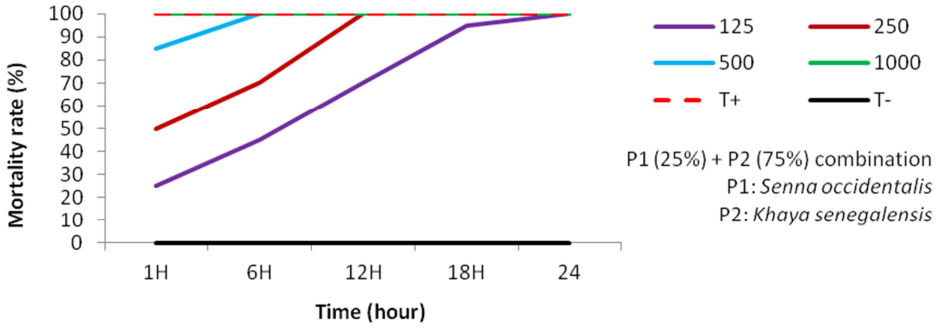

Figure 6. *Mortality of stage II larvae at the P1 25% + P2 75% combination.*

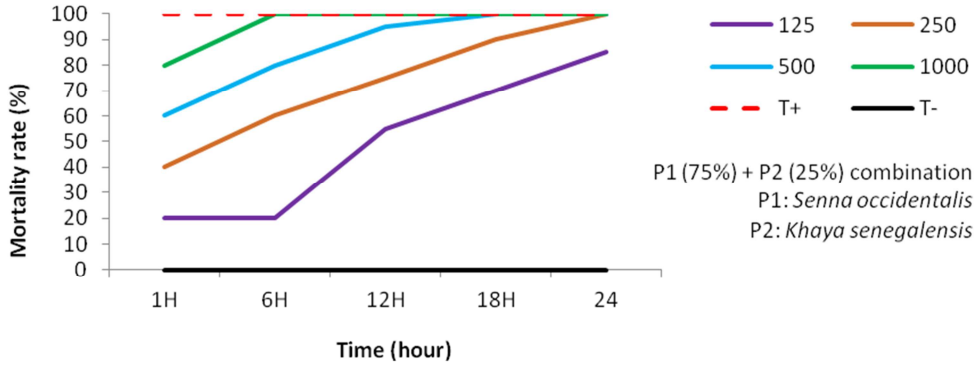

Figure 7. *Mortality of stage II larvae with the combination P1 75% + P2 25%.*

Combined at 50% (Figure 8), all concentrations also induced 100% mortality after 6 hours of exposure. However, after 1 hour of exposure, the lowest concentration induced low mortality (18% versus. 26%) compared to the P1 25% + P2 75% and P1 75% + P2 25% combinations. Mortality rates also varied with different concentrations and duration of exposure to the product.

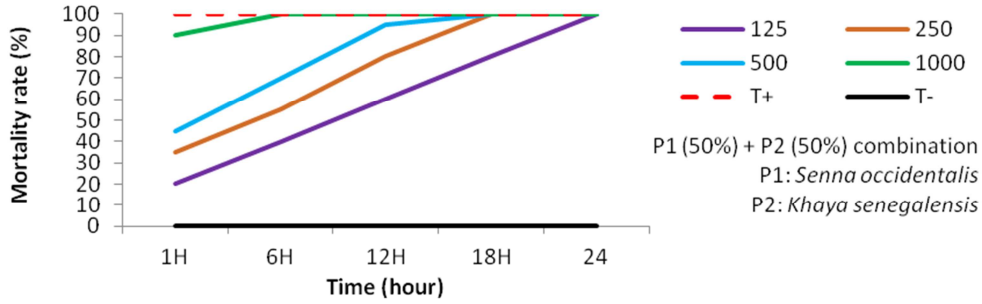

Figure 8. *Mortality of stage II larvae with the combination P1 50% + P2 50%.*

Of all the combinations on stage II larvae, the efficacy of the extracts increased with concentration and duration of exposure. Between hours of exposure, the difference was statistically significant between 6 hours and 24 hours for the 1000 ppm concentration. The evolution of mortality rates of mosquito larvae tested with plant leave extracts was also reported in the results of Diallo *et al.* [14]. Dichloromethane extracts of *Cussonia barteri* from Mali were toxic to *Anopheles* and *Aedes* larvae at the concentration of 500 mg/L and toxicity increased with increasing concentrations and duration of exposure. Tables 2 and 3 below show, respectively, the lethal concentrations (LC_{50}) and lethal hours of the combinations of plant essential oils tested on stage II larvae of *A.gambiae* ss. Compared with the first table, we can see that the combination P1 25% +P2 75% was more effective than combinations P1 75% +P2 25% and P1 50% +P2 50% with LC_{50} = 290 ppm, 330 ppm and 300ppm. The second table shows the effectiveness of these combinations in terms of time, and therefore the lethal hours are 3 hr 42 min 55 sec for combination P1 25% +P2 75%, 1hr 24min 45sec for combination P1 75% +P2 25% and 1hr 46min 41sec for combination P1 50% +P2 50%. This means that in the combinations, *Senna occidentalis* and *Khaya senegalensis* extracts showed an additive and synergistic effect on stage II *Anopheles gambiae* larvae. Synergistic effects of plant extracts are also reported by Akono *et al.* [15].

Table 2. *Lethal concentrations (LC_{50}) of the combinations of the plants studied on stage II larvae.*

Larval stage	Combinations	Regression equation	R	LC_{50}	
				(g/L)	(ppm)
Stage II	P1 25 % +P2 75%	y = 2,9507x - 0,8896	0,98**	0,29	290[a]
	P1 75 % + P2 25%	y = 2,0772x + 0,4713	0,96*	0,33	330[b]
	P1 50 % +P2 50%	y = 1,8165x + 1,339	0,95*	0,3	300[c]

R: correlation coefficient; **Very significant (p<0.01); *Significant (p<0.05). Values followed by different letters in the same column are significantly different at the 5% threshold.

Table 3. *Lethal hours (LH_{50}) of the combinations of the plants studied on stage II larvae.*

Larval stage	Combinations	Regression equation	R	LH_{50} (hour/min/sec)
Stage II	P1 25 % +P2 75%	y = 1,6807x + 4,0375	0,85*	3 hr 42 min 55 sec
	P1 75 % + P2 25%	y = 1,173x + 4,8134	0,92**	1 hr 24 min 45 sec
	P1 50 % +P2 50%	y = 1,8966x + 4,5184	0,83*	1 hr 46 min 41 sec

R: correlation coefficient; **Very significant (p<0.01); *Significant (p<0.05).

3.4. Efficacy of the Combined Extracts on Stage IV Larvae

The mortality curves of stage IV larvae tested with the combination of P1 25% + P2 75% extracts (Figure 9) show that all concentrations induce 100% mortality after 12 hours of exposure while the positive control had killed 100% of the larvae after 1 hour. The highest concentration (1000 ppm) induced 90% mortality after 1 hour.

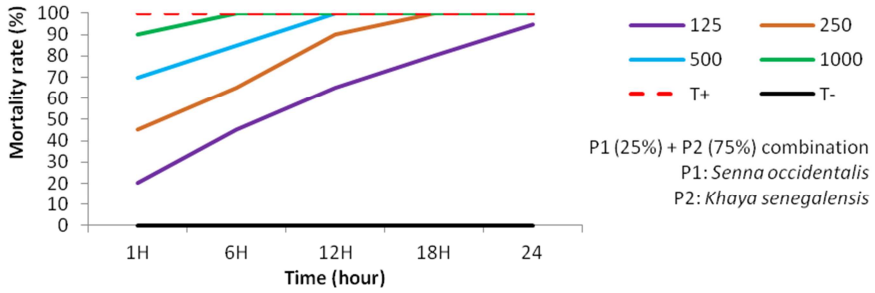

Figure 9. *Mortality of larvae at stage IV for the combination P1 25% + P2 75%.*

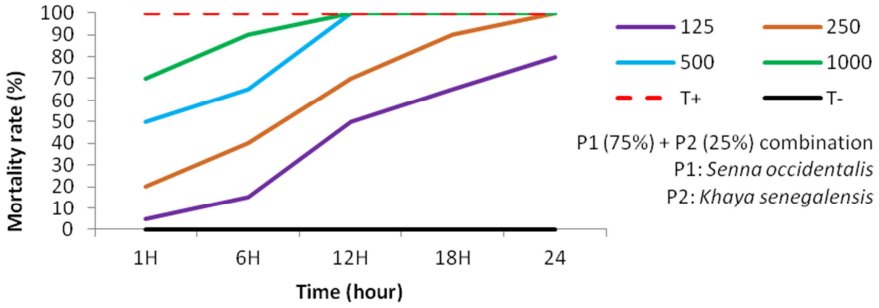

Figure 10. *Stage IV larval mortality at P1 75% + P2 25% combination.*

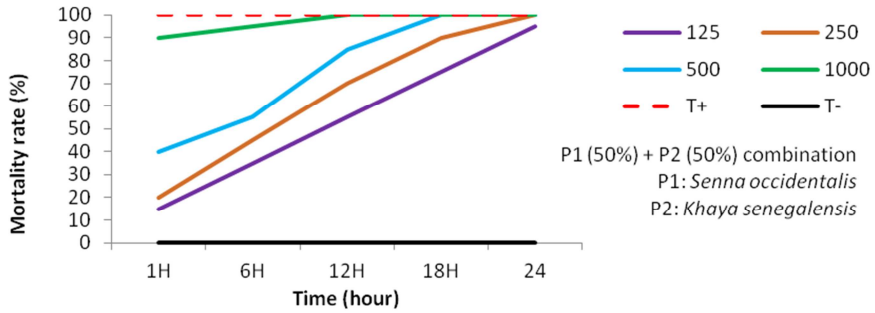

Figure 11. *Mortality of stage IV larvae at the P1 50% + P2 50% combination.*

For the combination P1 75% + P2 25% (Figure 10) 1000 ppm induced a mortality of 70% while 125 ppm (the lowest concentration) induced a mortality of 6.00%. The combination (Figure 11) P1 50% + P2 50% was less effective on stage IV larvae. The highest concentration (1000 ppm) induced 56% mortality; however, all concentrations induced 100% mortality after 6 hours.

The evolution of toxicity of the combined extracts *of Senna occidentalis* and *Khaya senegalensis* on stage IV larvae revealed that *Anopheles gambiae* ss stage IV larvae showed resistance to the extracts of *Senna occidentalis* and *Khaya senegalensis* compared to *Anopheles gambiae* ss stage II. Tables 4 and 5 summarize the efficacy of these essential oil combinations at lethal concentrations (LC_{50}) and lethal hours (LH_{50}). For table 4, the combination P1 50% +P2 50% was more effective than combinations P1 75% +P2 25% and P1 25% +P2 75% with LC_{50} = 320 ppm, 330 ppm and 940 ppm. For table 5, lethal hours were 2hr 11min 15sec for combination P1 50% +P2 50%, 2hr 34min 13sec for combination P1 75% +P2 25% and 1hr 19min 05sec for combination P1 25% +P2 75%.

Table 4. *Lethal concentrations (LC_{50}) of the combinations of the plants studied on stage IV larvae.*

Larval stage	Combinations	Regression equation	R	LC_{50}	
				(g/L)	(ppm)
Stage IV	P1 25 % +P2 75%	y = 1,8719x + 1,3583	0,99***	0,94	940[a]
	P1 75 % + P2 25%	y = 1,7616x + 1,1612	0,99***	0,33	330[b]
	P1 50 % +P2 50%	y = 1,8539x + 1,0663	0,93**	0,32	320[c]

R: correlation coefficient; ***Very highly significant (p<0.001); **Very significant (p<0.01); *Significant (p<0.05). Values followed by different letters in the same column are significantly different at the 5% threshold.

Table 5. *Lethal hours (LH_{50}) of the combinations of the plants studied on stage IV larvae.*

Larval stage	Combinations	Regression equation	R	LH_{50} (hour/min/sec)
Stage IV	P1 25 % +P2 75%	y = 1,738x + 4,7902	0,83*	1 hr 19 min 05 sec
	P1 75 % + P2 25%	y = 1,3946x + 4,4252	0,93**	2 hr 34 min 13 sec
	P1 50 % +P2 50%	y = 1,4688x + 4,4929	0,91**	2 hr 11 min 15 sec

**Very significant (p<0.01); *Significant (p<0.05)

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* [16] 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.

4. Conclusion

The results of quantification of *Senna occidentalis* and *Khaya senegalensis* extracts showed that *Senna occidentalis* leave extracts had high content of polyphenols, saponins and tannins compared to *Khaya senegalensis* leave extracts. The combinations of *Senna occidentalis* and *Khaya senegalensis* extracts produced remarkable larvicidal effects on *Anopheles gambiae* ss. Mortality rates were a function of the concentration of the extract and the duration of exposure of the larvae to the extracts. Given their proven insecticidal properties against *Anopheles gambiae* ss, the combination of *Senna occidentalis* and *Khaya senegalensis* leave extracts should be strongly recommended for the development of natural biocides.

Acknowledgments

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