Insectary maintenance of *Anopheles gambiae* (Dipteran, Culicidae): First instar (L_1) larvae depicts the accuracy of a pyrethroid insecticide, for malaria control

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Abstract

Malaria is a tropical disease responsible for the death of hundreds of children daily. Effort geared at eliminating the vector that transmit the causative agent are likely to reduce the incidence of malaria and consequent deaths. This study aims to identify the life cycle stage of *Anopheles gambiae* most susceptible to the insecticide, lambdacyhalothrin. Different life stages of *A. gambiae* were exposed to six different concentrations of the insecticide in an Insectary at the National Arbor-virus and Vectors Research Centre, Enugu, Southeastern Nigeria. A total of 650 each, of the different life stages and 350 adults selected from the insectary-bred strains were used for the study. The eggs mortality was monitored at 24 hours interval for 7days. Larvae, pupae and adult mortalities were measured after 6, 12 and 24 hours. Total survival and mortality rates were 6.17 and 0.83, respectively. Average developmental duration was 11.4 and 11.2 days for the parent and F1 generations, respectively. Lambdacyhalothrin concentration higher than 0.001mg/l was highly toxic to the different life stages of *A. gambiae* compared to the control. The first instar larvae (L_1) stage was the most susceptible to the insecticide and should be considered the most effective target when planning malaria control interventions.

Keywords: *Anopheles gambiae;* insectary; toxicity; first instar larva; malaria control.

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Introduction

Malaria is caused by a haemosporidan parasite of the genus *Plasmodium*, transmitted from human to human by infected dipteran female *Anopheles* mosquitoes. Different species of *Anopheles* mosquitos transmit *Plasmodium* in different parts of the world. In Sub-Saharan Africa, female *Anopheles* is the malaria parasite vector.

Between the years 2000 and 2014, the number of malaria-related deaths fell by 40% worldwide, from an estimated 743,000 to 446,000. However, in recent years, progress has slowly stopped. According to World Health Organization's (WHO) World Malaria Report (2019), there were no global gains in reducing new infections over the period, 2014 to 2018. In addition, nearly as many people died from malaria in 2018 as the year before. An estimated 228 million cases of malaria occurred worldwide in 2018, compared with 251 million cases in 2010 and 93% of these cases were recorded in WHO African Regions (World Malaria Report 2019). Many researchers have sought different ways to eradicate malaria but more need to be done. According to Malariasite (2019) malaria control began with swamp draining- a technique aimed to reduce mosquito breeding sites. This was followed by the prophylactic approach to malaria control, which included the use of insecticides. The discovery of the insecticide, dichlorodiphenyltrichloroethane (DDT) in 1942 made the idea of global control of mosquito seemed possible (Mulliken *et al* 2005**)**. It was very effective when sprayed inside houses and at breeding sites of mosquitoes. However, it was discovered that DDT was not easily degraded in the ecosystem as it could enter the food chain and adversely affect man and other animals. It was found to be toxic to humans (Munyinda *et al* 2015). In addition, mosquitoes have developed resistance to DDT and many other insecticides (World Malaria Report 2019). Subsequently, malaria control was augmented with chemotherapeutic measures. Drugs such as quinine, chloroquine, pyrimethamine and artemisin based combination therapies were used to treat infected persons.

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Unfortunately, strains of malaria parasite resistant to malaria drugs emerged, especially in Africa and the people are still challenged by malaria disease. Recently, the use of Insecticide Treated Nets (ITN) and Long Lasting Insecticide Treated Net (LLITNs) were introduced. These worked very effectively (Yang *et al* 2018). However, with time, people started withdrawing from using the net due to poverty, acceptability and compliance. People could no longer afford to buy the net. Those who were able to buy complained of poor ventilation, a situation that makes sleeping uncomfortable, especially during the hot season. Consequently, this led to a decline in the number of users (Atkinson *et al* 2009; Adeneye *et al* 2014).

Some recent malaria control programmes aim at vector elimination, such methods target the mosquitos' breeding pattern and life cycle stages, developmental and growth duration. For such control measures to be successful adequate and in-depth knowledge of the vectors' developmental duration and life cycle stages are essential. As this will identify the most vulnerable developmental satge of the mosquito and target the same with minimal impact on the environment. The present study was designed to identify the life stage of *A. gambiae* most vulnerable to lambdacyhalothrin insecticide in order to develop an effective vector control programme for sustainable elimination of malaria disease.

Materials and methods

Study site

The samples for this study were collected from Alulu, a village in Nike District Area, with headquarters at Nkwo Nike, a town within the Enugu East Local Government Area (LGA), Enugu State, Nigeria. Enugu East LGA has an area of 388.395km² and a population of 277,119 according to the 2006 national population census (National Population Commission 2006). It lies between longitudes 7°30'2″ E and latitudes 6°27'33″ N (Figure 1). The people of Enugu East LGA are mostly farmers and traders. Alulu village is sparsely populated but has huge land mass, covered by bushes, streams and stagnant water. These features provide suitable habitats for insect breeding, especially mosquitoes. In Alulu village, most compounds are not fenced and hence each compound extends into an adjoining farmland. It is difficult to demarcate farmland from the main compound. Furthermore, the inhabitants litter their compounds with dried palm fronds, logs of wood, leaves, animal dung, empty cans, bottles and tins, which when filled with water during the rainy season serve as breeding sites for mosquitoes. In addition to the adjoining farmlands, thick vegetations surround most houses, the drainage system is inadequate and the village roads are not tarred. Hence, there were stagnant water bodies both along the roads and within most compounds.

Sample collection

The larvae and pupae used in this study were collected from the wild, at various habitats including puddles, poorly drained cultivated ranch, tyre tracks and potholes along untarred roads in different areas of Alulu village. They were collected into a small basin using a ladle, and later sieved and washed into clean de-chlorinated water from the municipality. Tap water was dechlorinated by leaving it outside, in an open container under the sun for 24 hours according to Chopra (2017). It was done at the site, a day prior to sample collection. The samples were taken to the insectary, at the National Arbo-virus and Vectors Research Center, Enugu, in a plastic container with a dimension of 8.5×8×20cm

Insectary maintenance of *A. gambiae*

In the insectary, samples were sorted into larvae and pupae. The larvae were reared to pupate and pupae reared to adults. This made the identification and collection of adults easy. All the adults were identified using standard morphological identification guides (Erlank *et al* 2018; Obembe *et al* 2018; Coetzee 2020). They were all identified as *A. gambiae*.

Over 1000 adults of *A. gambiae* that emerged from the larvae and pupae collected from wild, were maintained with 10% sucrose solution in two big mosquito cages (50×50×70cm) tagged 'Y' and 'Z'. In addition, they were fed on human blood. The blood-fed adult female mosquitoes were further divided into six experimental groups; A, B, C, D, E and F, and maintained in cages. They were reared through two cycles. The first cycle was reared using the blood-fed female adults raised from the larvae and pupae collected from wild. Their progeny, first filial generation (F_1) were used to study developmental duration and survival rates of the life cycle stages. The second cycle was reared using blood-fed adult females raised from these (F_1) progeny. The progeny from the second cycle ($F_{2)}$ were used for lambdacyhalothrin toxicity tests. Both cycles were reared following the methods of Eukubay *et al*(2017) and Nepomichene *et al* (2017)**:**

Day 1: The emerged adults were fed *ad libitum* on 10% sucrose (glucose D) solution soaked in cotton wool placed on elevation inside the cages. Every two days, the soaked cotton was replaced with new ones to avoid microbial growth.

Day 3-4: Four (4) days from the day of emergence, adult females were fed human blood to lay eggs as stated below

Blood meal preparation and feeding

Blood group 'O' and positive Rhesus factor (O^+) collected from a human donor, was centrifuged at 3000 revolution per minute (rpm) for 10 minutes and the supernatant (serum) was collected and stored at 4°C. The packed red cell was washed three times with 0.9% normal saline. Each time, the supernatant was discarded. It was stored also at 4°C. An artificial membrane 30cm in length was soaked overnight in 300ml 0.1M phosphate buffer for purification. The next day, the red cell was rewashed two times and the supernatant discarded each time. The O^+ serum was heated in a water bath for 30 minutes at 37°C to inactivate all possible enzymes. At the end of 30

minutes, it was brought out and allowed to cool. It was scooped out with a spatula, into a laboratory mortar and macerated with 10ml of 0.9% normal saline. This was poured into an Eppendorf tube and centrifuged at 3000rpm for 10minutes and the supernatant collected. Equal volumes of the supernatant (O^+ serum) were measured and mixed with the volume of packed red cell. The purified membrane was tied at one end with a strip of thread; the blood was poured into it through a funnel. The membrane containing blood was placed on top of the cages in turn and mosquitoes were allowed to feed on the blood. Feeding lasted for 60 minutes each time (Eukubay *et al* 2017).

After each blood meal, all the blood-fed females, which were recognized by their distended reddish abdomen, were transferred using an aspirator, to smaller cages tagged A, B, C, D, E and F such that, each of the cages contained 100 females. Egg dishes with ovulation substrates (small piece of white cloth) were placed inside the different cages, for oviposition, by the blood-engorged females.

Day 7: The females did not lay eggs until the 3rd day after blood meal. The egg dishes with ovulation substrates were brought out the next day (four days following blood meal). The eggs were observed as black dots, spotted singly on the surface of the ovulation substrates. Eggs were counted, and washed with 30ml of 0.01% formaldehyde solution for 5 minutes, filtered on the ovulation substrate and stored in a humid container to avoid microsporidian infection. Like all *Anopheles spp, A. gambiae* eggs did not withstand extended dry condition (Mazigo *et al* 2019).

Day 8: The ovulation substrates containing the eggs were later soaked in four different plastic bowls containing de-chlorinated water, just enough to completely cover the bottom of the bowls. This prevented eggs from adhering to the sides of the bowl above the water level, where they would dry and not hatch. It also prevented larval mortality. More water was later added gently and pinch of food (pelletized broiler finisher feed) added. They were allowed to hatch into larvae.

Day 10-16: Eggs hatched to larvae the second day after they were soaked. A day after hatching, the larvae (which at this stage are called first instar larvae, L_1), from the four bowls were mixed together and then distributed with dropper into smaller bowls, each containing 250ml de-chlorinated water and 300 L_1. They were reared and monitored daily as they changed from one larval instar to another. The larvae were fed on pelletized broiler finisher feed. The composition of the feed (according to information on label) was as follows: Crude protein: 19.00%, Fat: 8.60%, Crude fiber: 5.40%, Calcium: 1.20% and Phosphorus: 0.41%. Each bowl was cleaned every two days and water replaced to prevent scum formation and accumulation of metabolites that may be toxic to the larvae (Eukubay *et al* 2017; Nepomichene *et al* 2017)). By the 7th day following egg hatch, pupae started developing from L_4 stage.

Day 17: Two hunded pupae were transferred into 250ml de-chlorinated water. The pupae were coma shaped, very active and did not feed at this stage. They were observed to have a light ash colour during early pupation but became black (or dark ash) as the days went by.

Day 19: Adults started to emerge from pupae. The bowls containing pupae were placed inside a new cage to avoid escape of adults on emergence. It was observed that adults emerged from pupae in the late evenings between 6.30pm and 8.00pm. Each time, the newly emerged adults fed on 10% sucrose (glucose D) solution. The insectary was maintained at average temperature of 27°C with 75% relative humidity, measured with Indoor-Outdoor Thermohygrometer.

Toxicity Study

The toxicity test was carried out at the National Arbo-Virus and Vectors Research Center, Enugu using different concentrations of lambdacyhalothrin (0.001mg/l and up to 0.025mg/l). Treatment with each concentration has a control prepared with de-chlorinated water. The testing was according to WHO larval and adult bioassay (WHO 2005). It was done under 25±2°C and 80±10% relative humidity laboratory conditions.

Six hundred and fifty each, of the life cycle stages of *A. gambiae* - eggs, larvae (1st, 2nd, 3rd and 4th instars), pupae and 350 of adults were selected from the insectary bred strains and used for lambdacyhalothrin toxicity study. A stock solution of lambdacyhalothrin (pyrethroid) insecticide was prepared in water. Six different concentrations (0.001, 0.005, 0.010, 0.015, 0.020 and 0.025mg/l) were prepared from the stock according to WHO larval and adult bioassay (WHO 2005). Treatment with each concentration has a control prepared with de-chlorinated water. Experimental animals were transferred into six small plastic bowls, each containing 200ml of each of the test concentrations. The control was equally placed in six different small plasic bowls, each containing 200ml de-chlorinated water. Adults were transferred into seven small cages using an aspirator. Whatman filter papers (40×12.5cm) were impregnated with the test concentrations and introduced inside six of the cages. Another filter paper was impregnated with dechlorinated water and introduced inside the 7th cage used as control. All the control animals were each time kept away from the test room. Eggs mortality was monitored after 24, 48, 72 hours and up to 7days exposure. Larvae, pupae and adult mortalities were measured 6, 12 and 24 hours during the test period and data recorded.

Data collection during rearing

During rearing, data were collected on (i) number of eggs produced (fecundity), (ii) percentage of eggs hatched to larvae (fertility), (iii) developmental duration and rate from one life stage to another**:** developmental rate from L_1 to L_2 and mortality rate of L_1, developmental rate from L_2 to L_3 and mortality rate of L_2, developmental rate from L_3 to L_4 and mortality rate of L_3, developmental rate from L_4

to pupae and mortality rate of L_4, developmental rate from pupae to adult and mortality rate of pupae. Mortality rate of the adults were also monitored.

Fecundity study: The number of eggs laid by the blood fed-females in each of the six cages were counted and recorded daily until no further eggs were laid over a period of seven days for the two cycles (Tables 1 and 2).

Fertility study: Eggs already washed with 0.01% formaldehyde solution were counted and then soaked into plastic bowls with de-chlorinated water just enough to cover the bottom of the bowls. More water was later added. Records of all the eggs hatched were taken (egg hatchability). Eggs, which could not hatch, were counted and recorded in each case as egg mortality. The number of larvae (L_1), which hatched out of eggs, were recorded and used to study developmental rate from one life stage to another and mortality rate at every life stage. Percentage adult emergence from pupae was also recorded.

Statistical analysis

The data was analysed using probit analysis, multiple regression, Analysis of Variance and Least Significant Difference. Data on developmental rate from one life cycle stage to another of *A. gambiae* were analysed using stage dependent mortality schedules analysis.

Mortality rate of individual life stage was calculated using the formula: $Q_x = D_x/L_x$

Where D_x = number of death, L_x = initial number alive in each life stages

Survivorship was computed using the formula: $S = 1\text{-}Q_x$, where Q_x = mortality rate

The natural loarithm of survival rate (K) was calculated as K= (In(S)). (K- value is another measure of mortality).

Results

Table 1 shows that Group D laid the highest number of eggs (2,732) and Group A laid the least number of eggs (2,357), a total of 15,156 eggs were laid. An average of 360.9 eggs were laid per day for seven days by each group of 100 *A. gambiae* (Table 1), therefore, one parent female *A. gambiae* laid a minimum of 3.6 eggs per day.

Table 2 shows that in the F1 generation, the highest number eggs (2,956) was laid by Group E while Group C laid the least number of eggs (1,902) and the total number eggs laid by the F1 generation was 15,639. The average number of eggs laid by each group of 100 *A. gambiae* in the F1 generation per day was 372.4, which gives an average of 3.72 eggs per mosquito. In all case, it took three days for the Adult *A. gambiae* to lay eggs and an average of 2.4±0.5 days for the eggs to hatch (Table 3). On the average, it took 11.4±0.5 and 11.2±0.5 for the eggs laid by the wild *A. gambaie* and F_1 generation, respectively to develop to adult (Table 3).

Table 1: Number of eggs laid per day, for seven days by different groups of blood-fed females of *A. gambiae* raised from larvae and pupae collected from wild

Cages	No of adult females	Number of eggs laidf per day for seven days							Total	Mean	p-value
		1	2	3	4	5	6	7			
A	100	1571	450	0	0	336	0	0	2357	336.7	0.589
B	100	2244	0	0	0	312	0	0	2556	365.1	0.256
C	100	1498	578	0	0	496	0	0	2572	367.4	0.488
D	100	2276	231	0	0	225	0	0	2732	390.3	0.146
E	100	1990	252	0	0	123	0	0	2365	337.9	0.204
F	100	1925	94	0	0	555	0	0	2574	367.7	0.314
Total									**15156**	**360.9**	

Table 2: Number of eggs laid per day for seven days by different groups of blood-fed Female *A. gambiae* raised from the progeny (F_1) of larvae and pupae collected from wild

Cages	No of adult females	Number of eggs laid per day for seven days							Total	Mean	p-value
		1	2	3	4	5	6	7			
A	100	1109	847	881	0	0	0	0	2837	405.3	0.334
B	100	2206	300	92	0	118	0	0	2716	388	0.212
C	100	52	28	109	0	1713	0	0	1902	271.7	0.106
D	100	1023	98	0	0	1434	0	0	2555	365	0.25
E	100	2094	446	0	0	416	0	0	2956	422.3	0.376
F	100	1019	902	0	0	752	0	0	2673	381.9	0.334
	Total								**15639**	**372.4**	

Table 3: The number of days required to attain different developmetal stages in *A. gambiae*

		No. days required to attain developmental stages							
Generation	Stages/Groups	A	B	C	D	E	F	mean	std
Parent	Laying of eggs	3	3	3	3	3	3	3	0
	Hatchig of egg	3	2	2	3	2	2	2.4	0.5
	L1	4	3	3	4	3	3	3.4	0.5
	L2	5	4	4	5	4	4	4.4	0.5
	L3	8	7	7	8	7	7	7.4	0.5
	L4	9	8	8	9	8	8	8.4	0.5
	Pupa	10	9	9	10	9	9	9.4	0.5
	Adult	12	11	11	12	11	11	11.4	0.5
F1	Laying of eggs	3	3	3	3	3	3	3	0
	Hatching of egg	2	2	2	2	3	3	2.2	0.5
	L1	3	3	3	3	4	4	3.2	0.5
	L2	4	4	4	4	5	5	4.2	0.5
	L3	7	7	7	7	8	8	7.2	0.5
	L4	8	8	8	8	9	9	8.2	0.5
	Pupa	9	9	9	9	10	10	9.2	0.5
	Adult	11	11	11	11	12	12	11.2	0.5

Table 4: Stage dependent mortality and survivorship of *A. gambiae*

Stages	(L_X)	(D_X)	(Q_X)	S	K
Egg	15156	4436	0.29	0.71	0.3425
EH	10720	658	0.06	0.94	0.0619
L_1	10062	745	0.07	0.93	0.0726
L_2	9317	1174	0.12	0.88	0.1278
L_3	8143	1042	0.13	0.87	0.1393
L_4	7101	632	0.09	0.91	0.0943
Pupae	6469	427	0.07	0.93	0.0726
Adult	6042				
Mean		1302	0.12	0.88	0.2057

EH=No of eggs hatched, Lx=Initial No., Dx=No. of deaths, S= Survivorship rate, K = 1-In(S)

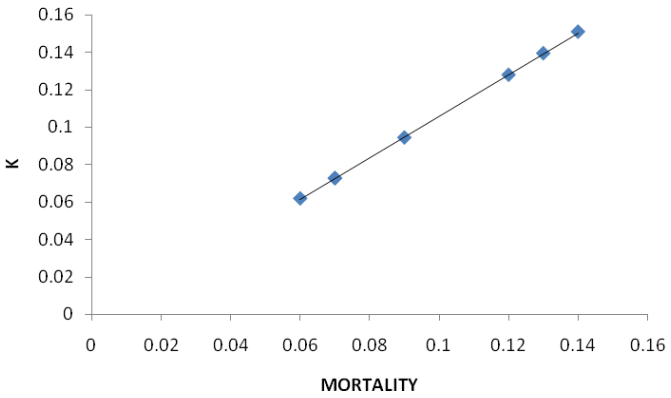

Figure 1. Relationship between mortality and k-value

All the larval instars were observed to remain active throughout the period. They jerked backwards, and lay parallel on the water surface. They dived to the bottom to feed and then quickly came up to the surface again. They clustered more by the edge of the bowels. They were earth yellow/light brown coloured. Survivorship rate varied from 0.71 (egg fertility) and 0.94 (egg hatchability) as shown in Table 4. The mortality rate of the life stages is summarized in K graph (Figure 1). The graph shows that as mortality increases as K- value increases.

The results of the toxicity test showed that the concentrations of lambdacyhalothrin (0.001 mg/l-0.025 mg/l) had very high significant ($p<0.05$) toxic effect on different life stages of *Anopheles* compared to the control group in de-chlorinated water. The results showed that percentage mortality of *A. gambiae* eggs exposed to 0.001mg/l, 0.005mg/l, 0.010mg/l, 0.015mg/l, 0.020mg/l and 0.025 mg/l concentrations of lambdacyhalothrin was 100%. None of the eggs expose to lambdacyhalothrin hatched after 7 days exposure, whereas in the control group, 94% of the eggs hatched to larvae within 48 hours of soaking (Table 5). The mortality of different stages of *A. gambiae* exposed to lambdcyhalothrin was significantly higher than the control group ($p<0.05$). The LC_{50} and LC_{90} values for the test eggs within 48 hours as summarized in Table 6 were 0.018mg/l and 0.029mg/l, respectively.

The results equally showed 100% mortality of all the different larval instars (L_1, L_2, L_3, and L_4) exposed to different concentrations of lambdacyhalothrin within 24 hours. In 0.020mg/l and 0.025mg/l concentrations, 100% mortality was observed within 12 hours. In the 0.001mg/l to 0.015mg, 100% mortality of larval instars occurred within 24 hours of test period. In the control group, only

6% and 14% mortality occurred in the L_1 and L_2, respectively within and none of the L_3 and L_4 larval instars died (Table 5). There was significant difference between mortality of experimental larval stage and their control ($p<0.05$). The LC_{50} and LC_{90} values for the larval instars at 6, 12 and 24 hours respectively, were surmarised in Table 6. Pupal mortality in 0.005mg/l, 0.010mg/l, 0.015mg/l, 0.020mg/l and 0.025 mg/l concentrations was 100% and 96% in 0.001mg at the end of 24 hours test period, whereas, 0% mortality was recorded in the control group.

The results showed that 100% mortality of adult *A. gambiae* was achieved within 24 hour of exposure to 0.020 mg/l and 0.025 mg/l concentrations. In 0.001mg/l, 76% mortality was recorded, 82% mortality was recorded in 0.005 mg/l and 0.010 mg/l and 86% in 0.015 mg/l test concentrations within the same period.

Analyses of the toxic effect of lambdacyhalothrin on the life cycle stages of *A. gambiae* using multiple regressions showed that lambdacyhalothrin was most toxic to L_1 stage at 6 hours. Further analyses using step wise method, revealed that L_1 mortality at 6 hours is a good predictor variable. It showed that L_1 mortality at 6 hours had a high Beta value of 0.982 and an Adjusted R. square value of 0.957, $F1,5 = 135.659$; $P<0.05$ (Figure 2).

Discussion

Anopheles gambiae demonstrated complete adaptation to insectary conditions. Optimum temperature, pH and dissolved oxygen might have provided conducive environment for the survival and breeding activity of *A. gambiae*. Das *et al.* (2007) reported that under laboratory conditions, *A. gambiae* carried out normal development even when the pH varied as much as from 4.0 to 7.8, as long as there was enough food to consume. Developmental duration from egg to adult emergence of 11.4 days was recorded in this study. This was in line with 11.04 days reported by Olayemi and Ande (2009) but shorter than 12 to 14 days reported by WHO (1975). This short developmental duration, in addition to low mortality rate of the different life cycle stages recorded in this study for *A. gambiae*, were indications of rapid population turnover and potential risk for malaria transmission.

The results showed that none of the eggs soaked in the different test concentrations hatched within the 7-days test period. This is in line with Mohapatra *et al* (1999) report that the pyrethroid insecticides, cyfluthrin and fenfluthrin significantly ($p<0.05$) reduced the fertility rates of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasicatus* and Loh and Yap (1989) reported that pyriroxyfen reduced egg hatchability in *A. aegypti* by 36.8%. Similarly, all the larval instars (L_1, L_2, L_3 and L_4) exposed to the insecticide died within 24 hours, which implies that lambdacyhalothrin was effective and had significant impact in inhibiting the development of *Anopheles* life cycle stages and hence a good *Anopheles* (vector) population modulator. This will ultimately help in possible total mosquito control and eradicate malaria transmission.

The bending of head to body movement observed in larval life stage and the confused running around and somersaulting movement in the test bowls by the pupae

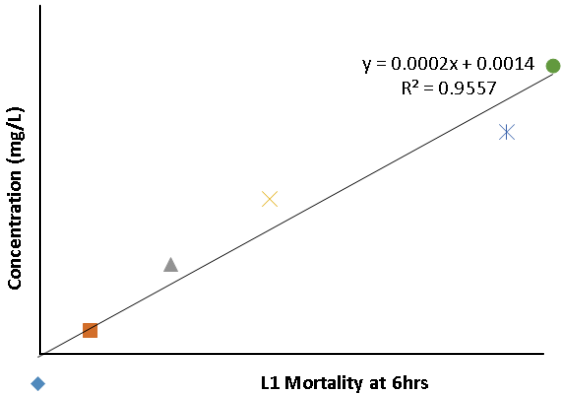

Figure 2. Relationship between concentration and mortality of L_1 life cycle stage of *A. gambiae*

Table 5: Percentage mortality of different *A. gambiae* life stages *exposed to* lambdacyhalothrin insecticide

		Concentrations of lambdacyhalothrin (µg/l)						
Stage	Days	0 (control)	1	5	10	15	20	25
EH	6	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0
	24	84	0	0	0	0	0	0
	48	10	0	0	0	0	0	0
L1	6	0	7	16	30	47	88	96
	12	4	20	36	69	27	12	4
	24	2	73	48	1	26	0	0
L2	6	0	7	9	8	20	48	51
	12	9	18	22	48	50	52	49
	24	5	75	69	44	30	0	0
L3	6	0	0	0	9	11	72	96
	12	0	20	23	47	51	28	4
	24	0	80	77	44	38	0	0
L4	6	0	0	0	7	7	53	91
	12	0	9	13	17	25	47	9
	24	0	91	87	76	68	0	0
Pupa	6	0	0	0	0	7	19	51
	12	0	56	80	88	90	81	49
	24	0	40	20	12	3	0	0
Adult	6	0	0	4	8	18	22	52
	12	0	18	24	20	28	34	40
	24	0	58	54	54	40	44	8

EH= Egg hatchability

Table 6: LC_{50} and LC_{90} values (µg/l) of different concentrations of lambdacyhalothrin on life cycle stages of *A. gambiae*

Life cycle stage	Exposure Duration	LC_{50} (µg/l)	LC_{90} (µg/l)
Egg	48 hours	18	29
L_1	6 hours	11	38
L_1	12 hours	0	0
L_1	24 hours	3	1
L_2	6 hours	39	387
L_2	12 hours	20	863
L_2	24 hours	5	1
L_3	6 hours	17	25
L_3	12 hours	0	0
L_3	24 hours	6	1
L_4	6 hours	19	3
L_4	12 hours	49	145
L_4	24 hours	11	3
Pupae	6 hours	31	68
Pupae	12 hours	1	7
Pupae	24 hours	1	0
Adult	6 hours	35	151
Adult	12 hours	0	0
Adult	24 hours	6	0

life stage could be due to neuro-excitatory effects of the lambdacyhalothrin. Riegart and Roberts (1999) and Mueller (1990) recorded that a few minutes after absorption by an insect, pyrethroid impairs ion transport through the membrane of nerve axons. This is as a result of the action of Gama Amino Butyric Acid (GABA). The Release of GABA by presynaptic nerve terminals activates a chloride channel on the postsynaptic membrane, leading to hyper-polarization of the postsynaptic nerve terminal and thus enhancing its excitatory threshold. This results in indirect neuro-excitatory effect, which caused the experimental larval and pupal stages to begin to have rapid twitching of voluntary muscle (bending) and uncoordinated movement (somersaulting) (George 1999; Amy 2006,), tremor (the convulsive movement), prostration and finally, death (Coat 1982; Bloomquist 1993). Eassam *et al* (2005) studied the effect of sub-lethal concentrations of lambdacyhalothrin, penitrothrin and *Callitris glamcophylla* extracts on the development of *A. aegypti* and reported that the LC_{50} and LC_{75} doses of lambdacyhalothrin significantly reduced larval survival and adult emergence ($p<0.05$) and that total mortality was high at all doses.

In this study, the first instar larvae (L_1) with 100% mortality after 6 hours exposure was established as the life cycle stage that best depict the toxic effect of different the test concentrations of lambdacyhalothrin. L_1 mortality at 6 hours had a high Beta value of 0.982, which means receiving greater impact of the test concentrations, showing high relationship between L_1 mortality at 6 hours (predictor variable) and the concentrations of the insecticides used (dependent variable). Having an Adjusted R square value of 95.7% means that the model accounted for 95.7% of mortality that occurred due to the use of the different concentrations (0.001 and up to 0.025mg/l) of the insecticide. This suggests that any *A. gambiae* population control using lambdacyhalothin should target the first instar larval stage for effective result. This would thus go down the line to reduce the population of the preceding life stages and ultimate possible total mosquito control. This further confirmed pyrethroid insecticides especially lambdacyhalothrin as promising in providing efficient malaria control and environmentally safety. The LC_{50} values ranged from 0.000mg/l to 0.049mg/l and 0.000mg/l to 0.863mg/l for the LC_{90}. This indicates that, even less than 0.001mg/l concentration of lambdacyhalothrin would cause mortality in 50-90% of *A. gambiae* life cycle stages population under the stated experimental conditions (Kenneth 1990). This confirmed the report by Smith and Strathon (1986), that the fourth generation pyrethroid lambdacyhalothrin was noted for its efficacy against insects at lower dosages than other generations or counterpart. This agreed with the report of USEPA (1995) and Royal Society of Chemistry (1991) that lambdacyhalothrin has proved excellent in the control of many insect pests and there has not been any failure when it was used.

Conclusion

Lambdacyhalothrin (pyrethroid insecticide) from every indication was a mosquito agent with high potency and easy to administer. The insecticide caused both local and systemic effect. It killed the different life cycle stages. This therefore ensures confidence to achieve tremendous result whenever lambdacyhalothrin is used for mosquito/malaria control. Government and donor agencies are advised to produce lambdacyhalothrin based insecticides in large quantity and make them accessible and affordable to the populace. It will go a long way in eliminatng mosquitoes, thereby reducing their constant bites and prevalence of malaria in Nigerian and African.

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