Biochemical effects of lambdacyhalothrin on activities of some detoxifying enzymes of *Anopheles gambiae* life cycle stages

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Abstract

Recent decrease in global malaria burden was achieved through vector control interventions with pyrethroid insecticides. This study was carried out to determine the influence of lambdacyhalothrin on the physiology and activities of some detoxifying enzymes and to establish the systemic effects on *Anopheles gambiae*. The life cycle stages of the vector were exposed to six different concentrations of lambdacyhalothrin, in an insectary. After the toxicity tests, 325 each, of the dead eggs, larvae and pupae, and 175 of the dead adults from the toxicity test, were carefully selected and further used for enzyme study. Fifty (50) individuals were used for experimental study and 25 as control, for each of egg, larvae and pupae; 25 were used for experimental study and 25 as control for the adult. Each sample was homogenized in 10ml 0.1M phosphate buffer of pH 7.4, filtered and the filtrate used for enzymes study. Determination of the enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was done using standard biochemical methods. The results showed that 97% mortality of the life cycle stages was achieved following exposure to six concentrations of lambdacyhalothrin. Some concentrations (1 and 05µg/l) induced stimulatory effect on the enzyme activities while others (15 and 25µg/l) elicited inhibitory effect and some (10µg/l and 20µg/l) had no significant effect ($p>0.05$) on the enzyme activities. Alkaline phosphatase (ALP) was the most active in all the life cycle stages. Nevertheless, in older stages (pupae and adults), AST and ALT activities were significantly ($p<0.05$) elevated. This suggests that lambdacyhalothrin is promising in providing effective mosquito control. The results also suggest that the enzymes play a part in detoxification of lambdacyhalothrin and hence could be correlated with the pyrethroid tolerance status of *A. gambiae.*

Keywords: Liver enzymes, lambdacyhalothrin influence, malaria control, life cycle stages, *Anopheles gambiae*

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Introduction

Malaria is a mosquito-borne infectious disease that affects humans and other animals (Farah *et al* 2019). Malaria causes symptoms that typically include fever, tiredness, vomiting, and headaches. In severe cases, it can cause jaundice, seizures, coma or death (Basu and Sahi 2017). Malaria is caused by singled-celled microorganism of the *Plasmodium* group. Naturally, it is spread exclusively through bites of infected female *Anopheles* mosquitoes. Risk of the disease can be reduced by preventing mosquito bites through the use of mosquito nets and insect repellents or with mosquito-control measures such as spraying insecticides. Our primitive ancestors hug smoky fire or spread mud and dust over their skin to scare biting and tickling mosquitoes (Ware and Whitacre 2004) and they also built houses on dry and elevated land, there were swamp drainages too. This was followed by the prophylactic approach to malaria control, which included the use of insecticides. According to Alanwood (2007) different kinds of insecticides exist, made of unique number of matter with associated toxic activities.

Pyrethroid insecticides have been widely used for *Anopheles* mosquito control and it has always proved effective. It has been shown, not only to be an effective means of malaria control but also selective and sustainable, thereby fulfilling the criteria for inclusion in global malaria control strategy as defined at the Ministerial Conference in Amsterdam in 1992 (WHO 2005a). Lambdacyhalothrin is a pyrethroid insecticide that consists of a mixture of the two most active of the four isomers of cyhalothrin (Moretto 1991). It is a synthetic organic insecticide, which poses no hazard to living organisms and their environment and is well tolerated by humans (WHO 2005a; Okoh *et al* 2013, 2014).

Curtis and Mnzava (2000) reported that both spraying and impregnated netting intervention with lambdacyhalothrin reduced malaria vector and hence malaria incidence by about 60% in Kenya and United Republic of Tanzania. Miguel and Mazariego (2002) observed 100% effectiveness up to 12 months, after lambdacyhalothrin was sprayed on palm leaves against *Rhodnius prolixus* in the state of Chiapas, Mexico. Above 44.29% reduction in malaria infection and other morbidity indicators such as fever, hospital attendance, health seeking movement and school absenteeism was achieved in children of Oruku, Enugu State, Nigeria with

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lambdacyhalothrin-treated curtains, mats and blankets (Okoh *et al* 2013, 2014).

However, toxic activity of lambdacyhalothrin against malaria vector is a research area in insecticide toxicology that is less explored and understood. Verghese (2000) compared its efficacy with that of imidacloprid and azadirachtin on mango hopper and concluded that lambdacyhalothrin was more effective and was comparable with standard monocrotophos. Bostanian *et al* (2001) compared imidacloprid, deltamethrin and lambdacyhalothrin against *Hyaliodes vitripennis* and found adults to be more significantly susceptible than nymphs.

Lambdacyhalothrin, like all pyrethroids was toxic to mosquito and fish, but it degrades rapidly and has a low potential to contaminate ground water due to its low water solubility and high potential to bind to soil (Lawler *et al* 2003). Essam *et al* (2005) investigated the effect of sub-lethal concentrations of fenitrothrin, 0.0025mg/l (LC_{25}), 0.0044mg/l (LC_{50}) and 0.0062mg/l (LC_{75}); lambdacyhalothrin - 0.00004 (LC_{25}), 0.00015 (LC_{50}) and 0.00016mg/l (LC_{75}) and natural insecticide (Cupressaceae) extract on the developmental period of *Aedes aegypti* (yellow fever and dengue vector) and reported that lambdacyhalothrin at LC_{50} and LC_{75} doses, significantly reduced larval survival and adult emergence and total mortality was high at all doses.

Lambdacyhalothrins are neurotoxins (Beat *et al* 1997; Coppo *et al* 2002). They act on the axon and are believed to interfere with sodium channels and permeability of nerve cells, so affecting the transmission of nerve impulses. As *Anopheles* populations are continuously being subjected directly or indirectly to different insecticides including lambdacyhalothrin, their tissues are obviously physiologically harassed. Therefore, there is need to correlate the activities of certain detoxifying enzymes in relation to tolerance level by the insects. This study was designed to determine the influence of lambdacyhalothrin on the physiology of *Anopheles gambiae* life cycle stages by observations on the activities of some vital enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and to establish if it has systemic effect on the malaria vector.

Materials and methods

Preliminary toxicity test

A toxicity test of *A. gambiae* life cycle stages [egg, larvae (L_1, L_2, L_3, L_4,), pupa and adult] was carried out at the National Arbo-Virus and Vectors Research Centre (NAVRC), Enugu, Southeast, Nigeria, using a stock solution of lambdacyhalothrin insecticide which was prepared in water. Six different concentrations (1µg/l, 5µg/l, 10µg/l, 15µg/l, 20µg/l and 25µg/l) were prepared from the stock, following the World Health Organization larval and adult bioassay (WHO 2005a). A total of 4250 life cycle stages were used for lambdacyhalothrin toxicity test following World Health Organization larval and adult bioassay (WHO 2005b). A total of 100 individuals of each life cycle stage was stocked in each concentration and mortality observed for 7 days for the eggs and 24 hours for the other stages. Treatment with each concentration had a control (de-chlorinated water) and the control were kept away from the test room to avoid being affected by the insecticide. Both the test and control life stages were satisfactorily fed with chicken broiler feed to eliminate the effect of starvation. A total of 4112 (97%) died, out of which 2125(51.67%) were selected and used for enzyme study.

Enzyme study

At the end of the toxicity test, the 100 eggs soaked in each of the six test concentrations were filtered separately using filter paper (Whatman filter paper Cat No 1001 125). Among the dead eggs filtered out of each of the six-test concentration, 50 were selected randomly using wooden tooth pick, and used for enzyme study; fresh 25 eggs were selected from stock, and used as control for the egg enzyme study, totalling 325. Fifty of the larvae (L_1, L_2, L_3 and L_4) and pupae were carefully selected from each 100 tested in each of the six test concentrations. Those larvae, which had their body parts peeled out were excluded for the enzyme study; 25 each were selected from control, all totalling 1625. Twenty-five of the adults used for the toxicity test, were selected from each test concentration and 25 from the control, making a total of 175. The grand total used for the enzyme study was 2125 individuals of different life cycle stages (Table 1). These were homogenized separately in 10ml 0.1M phosphate buffer of pH 7.4. The homogenate for each was filtered using Whatman filter paper Cat No 1001 125. The filtrate was used for the enzyme assay in triplicate using colorimetric method of Teitz (1976) and Ochei and Kolhatkar (2000).

Table 1: Number of samples used for enzyme study, from each life cycle stage tested with the different test concentrations

Life cycle stages	Concentrations of lambdacyhalothrin (µg/l)							
	Control	1	5	10	15	20	25	Total
Egg	25	50	50	50	50	50	50	325
L_1	25	50	50	50	50	50	50	325
L_2	25	50	50	50	50	50	50	325
L_3	25	50	50	50	50	50	50	325
L_4	25	50	50	50	50	50	50	325
Pupa	25	50	50	50	50	50	50	325
Adult	25	25	25	25	25	25	25	175
Total								2125

Serum aspartate aminotransferase (AST) activity

This was evaluated using manufacturers protocol of Randox AST Kit based on the principle reported by Reitman and Frankel (1957). Then, 0.1ml of homogenate filtrate was mixed with 0.5ml of the phosphate buffer (100mmol/l, pH 7.4), L-aspartate (100mmol/l) and α-oxoglutarate (2.0mol/l). The mixture was incubated for 30 minutes at 37°C in a water bath, after which 0.5ml of 2,4-dinitrophenylhydrazine (2.0mol/l) was added to the reaction mixture and allowed to stand for another 20 minutes at room temperature. Then 5.0ml of NaOH (0.4mol/l) was added, mixed and allowed to stand for 5 minutes, then the absorbance was read at 540nm against

a blank. The activity of AST in the homogenate was obtained from extrapolation of absorbance value on AST standard curve.

Serum alanine aminotransferase (ALT) activity

This was carried out using manufacturers protocol of Randox ALT Kit based on the principle reported by Reitman and Frankel (1957). 0.5ml phosphate buffer (100mmol/l, pH 7.4), L-alanine (200mmol/l) and α-oxoglutarate (2.0mol/l) were added into a test tube containing 0.1ml of the homogenate, mixed and then incubated at 37°C for 30 minutes in a water bath. Then, 0.5ml of 2,4-dinitrophenylhydrazine (2.0mol/l) was added to the mixture, incubated again at 20°C for 20 minutes and 5.0ml of NaOH (0.4mol/l) was added. The mixture was allowed to stand for 5 minutes at room temperature and the absorbance was read at 540nm against a blank. The activity of ALT in the homogenate was obtained using ALT standard curve.

Serum alkaline phosphatase (ALP) activity

In ALP assay, 0.5ml of the homogenate filtrate was transferred into test tubes containing 2ml of working buffer substrate solution (1 mol/l, pH 9.8 diethanolamine buffer, 0.5mmol/l $MgCl_2$; substrate: 10mmol/l p-nitrophenolphosphate). They were mixed and incubated at 37°C for 5 minutes, then 0.8ml of sodium hydroxide was added followed by 1.2ml of sodium bicarbonate solution. Thereafter, 1ml of 0.6% 4-aminophenazone was added followed by 1ml of potassium ferricyanide, mixed and the absorbance was taken at 520nm against a reagent blank. The activity of ALP in the homogenate was obtained using ALP standard curve (Alexander *et al* 1993).

Statistical analysis

Data collected were analysed using the descriptive statistics. Data on the impact of the insecticide on the enzymes of both experimental life cycle stages and their control were analysed using Two-Way ANOVA. Differences between treatment means were detected using Least Significant Difference (LSD).

Results

Effect of lambdacyhalothrin on aspartate aminotransferase activity of *A. gambiae* life cycle stages

Table 2 shows the effects of the six different test concentrations of lambdacyhalothrin (1µg/l to 25µg/l) on AST activities of different life cycle stages of *A. gambiea*. The results showed that 1µg/l and 25µg/l concentrations significantly ($p<0.05$) inhibited AST activity of egg stage (14.18±8.74IU/l and 13.10±4.20IU/l, respectively); whereas 5 to 20µg/l showed no significant ($p>0.05$) effects on AST activity (18.61±11.71IU/l-26.06±18.64IU/l) when compared with the control (26.76 ±0.67IU/l). The highest (26.06±18.64IU/l) and lowest (13.10±4.20IU/l) AST activities were recorded in 15µg/l and 25µg/l, respectively.

The results also show that 1 to 25µg/l had significant inhibition effects ($p<0.05$) on AST activity (8.09±0.24IU/l-1.27±3.55IU/l) of L_1 life stage when compared with the control (30.90 ±2.34IU/l). The highest (11.27±3.55IU/l) and lowest (8.09±0.24IU/l) AST activities were recorded in 1µg/l and 10µg/l, respectively. Effects of all the test concentrations on AST of L_1 were significantly different ($p<0.05$). Likewise, 1µg/l to 25µg/l, had significant inhibition effects ($p<0.05$) on AST activity (9.11±0.55IU/l-40.78±0.14IU/l) of L_2 life cycle stage when compared with the control (61.36±0.85IU/l). The highest (40.78±0.14IU/l) and lowest (9.11±0.55IU/l) AST activities were recorded in 25µg/l and 10µg/l, respectively. Effects of all the test concentrations on AST activity of L_2 were significantly different ($p<0.05$).

On the other hand, 1 and 5µg/l had significant ($p<0.05$) stimulation effects on AST activity (52.09±0.96IU/l and 49.46±0.29IU/l, respectively) of L_3; while 10µg/l to 20µg/l showed significant ($p<0.05$) inhibition effects (18.55±0.21IU/l-26.10±0.19IU/l) and 25µg/l showed no significant ($p>0.05$) effect on the AST activity (30.49±1.70IU/l) of the L_3 when compared with the control (33.62±1.05IU/l). The highest (52.09±0.96IU/l) and lowest (18.28±0.33IU/l) AST activities were recorded in 1 and 20µg/l, respectively. Effects of 1 to 20µg/l concentrations were significantly different ($p<0.05$) while effects of 25µg/l were not significantly different ($p>0.05$). Similarly, 1, 5 and 25µg/l induced significant ($p<0.05$) stimulatory effect on AST activity of L_4 (59.98±1.09IU/l, 77.74±1.01IU/l and 41.72±0.18IU/l, respectively), 10µg/l showed significant ($p<0.05$) inhibition (19.49±0.20IU/l) while 15µg/l and 20µg/l had no significant ($p>0.05$) effects (29.00±1.09IU/l and 20.77±0.21IU/l, respectively) when compared with the control (29.83±0.98 IU/l). The highest (77.74±1.01 IU/l) and lowest (19.49±0.20IU/l) AST activities were recorded in 5 and 10µg/l, respectively (Table 2).

Concentrations of 1µg/l to 25µg/l had significant ($p<0.05$) stimulation effects on AST activity of pupae (67.35±0.22 IU/l-109.66±0.88 IU/l) when compared with the control (21.38 ±1. 39 IU/l). The highest (109.66±0.88 IU/l) and lowest (33.19±0.93 IU/l) AST activities were recorded in 1µg/l and 20µg/l, respectively. The effect of all the test concentrations on AST activity of pupae were significantly different ($p<0.05$). Effects of 1, 15 and 20µg/l on AST activity of the adult showed significant ($p<0.05$) stimulation effects (130.46±4.22IU/l, 174.69±1.37IU/l and 127.60±1.37IU/l, respectively) while the effects (108.59±9.72IU/l-114.37±7.04 IU/l) of the other concentrations were not significantly different ($p>0.05$) when compared with the control (107.74±2.97IU/l). The highest (174.69±1.37IU/l) and lowest (108.59±9.72IU/l) AST activities were recorded in 15µg/l and 5µg/l, respectively (Table 2).

Effects of lambdacyhalothrin on alanine aminotransferase activity of *A. gambiae* life cycle stages

Table 3 shows the effects of the six test concentrations of lambdacyhalothrin on ALT activities of life cycle stages

Table 2: Effects of lambdacyhalothrin on Aspartate Aminotransferase activity (IU/l) of the various *A. gambiae* life stages

Lambda. conc (mg/l	*Anopheles gambiae life cycle* stages					
	Egg	L_1	L_2	L_3	L_4	Pupae	Adult
Control	26.76±0.67^{c1}	30.90 ±2.34^{c1}	61.36±0.85^{b1}	33.62±1.05^{c2}	29.83± 0.98^{c4}	21.38 ±1. 39^{c5}	107.74 ±2.97^{a3}
1	14.18±8.74^{e2}	11.27±3.55^{e2}	33.18±4.62^{d3}	52.09±0.96^{c1}	59.98±1.09^{c2}	109.66±0.88^{b1}	130.46±.4.22^{a2}
5	18.61±11.71^{e1}	9.16± 0.56^{f2}	28.14±0.25^{e3}	49.46±0.29^{d1}	77.74±1.01^{b1}	67.35±0.22^{c2}	108.59±9.72^{a3}
10	25.06±6.98^{c1}	8.09±0.24^{d2}	9.11±0.55^{d4}	18.55±0.21^{c3}	19.49±0.20^{c5}	39.63±0.13^{b4}	114.37±7.04^{a3}
15	26.06+18.64^{c1}	10.24+0.84^{d2}	11.51+0.31^{d4}	26.10+0.19^{c23}	29.00+1.09^{c4}	41.22+0.32^{b3}	174.69+1.37^{a1}
20	23.40±10.43^{bc1}	10.58±0.16^{d2}	17.53±0.52$^{cd\ 4}$	18.28±0.33$^{c\ d\ 3}$	20.77±0.21^{cd4}	33.19±0.93^{b4}	127.60±1.37^{a23}
25	13.10±4.20^{d2}	9.99±2.09^{d2}	40.78±0.14^{b2}	30.49±1.70^{c2}	41.72±0.18^{b3}	50.15±0.99^{b3}	110.20±12.40^{a3}

Mean values in a column and row with different alphabets are significantly different (p<0.05)

of *A. gambiea*. The results showed that all the six concentrations significantly (p<0.05) inhibited ALT activity of the egg stage (21.38±2.8-37.92±8.31IU/l) compared with the control (79.40±1.86 IU/l). The highest (37.92±8.31IU/l) and lowest (21.38±2.82 IU/l) ALT activities were recorded in 5µg/l and 15µg/l, respectively. The effect of all the six test concentrations were significantly (p<0.05) different.

The Table also shows that ALT activity (3.19±0.33-5.26±4.59IU/l) in lambdacyhalothrin exposed L_1 life stage was significantly (p<0.05) inhibited when compared to the control (15.74 ±1.46 IU/l), likewise their effects on ALT activity (1.99±0.20 IU/l-8.47±0.40 IU/l) of L_2 life stage compared with the control (14.81 ±0. 96 IU/l). The highest (5.26±4.59IU/l) and lowest (3.19±0.33IU/l) ALT activities in L_1 were recorded in 1 and 25µg/l, respectively, while for L_2, the highest (8.47±0.40IU/l) and lowest (1.99±0.20IU/l) ALT activities were recorded in 01 and 5µg/l, respectively. Table 3 further shows that 1µg/l and 5µg/l induced significant (p<0.05) stimulation effects on ALT activity of L_3 life stage (24.45±1.68IU/l and 17.60±0.10IU/l, respectively), whereas 10 to 25µg/l significantly (p<0.05) inhibited the ALT activity (2.70±0.12-4.95±0.21IU/l) when compared with the control (11.20±0.32IU/l). The highest (24.45±1.68IU/l) and lowest (2.70±0.12 IU/l) ALT activities were observed in 1 and 10 mg/l, respectively. Concentrations of 1µg/l and 5µg/l induced significant (p<0.05) stimulation effects on ALT activity (57.97±1.31 and 47.59±0.84IU/l respectively) of L_4 life stage, while 10 to 25µg/l showed significant (p<0.05) inhibition effect (2.88±0.14-4.61±0.06IU/l) when compared with the control. The highest (57.97±1.31IU/l and lowest (2.88±0.14 IU/l) ALT activities on L_4 were observed in 1 and 10µg/l, respectively. The effect of all the test concentrations on ALT activity of L_4 life stage were significantly (p<0.05) different.

In the pupa, 1µg/l induced significant (p<0.05) stimulation on ALT activity (20.01±0.09IU/l). Effects of 5µg/l and 15µg/l on ALT activity (15.50±0.14 and 14.14±0.14IU/l, respectively) were not significant different (p>0.05). The concentrations 10, 0.020 and 25µg/l significantly (p<0.05) inhibited ALT activities (13.52±0.24IU/l, 9.58±0.17 IU/l and 9.60±2.12 IU/l, respectively) when compared with the control (13.92±0.22IU/l). The highest (20.01±0.09 IU/l) and lowest (9.58±0.17IU/l) ALT activities of pupae were recorded in 1µg/l and 20µg/l, respectively (Table 3).

In the adult 1µg/l showed significant inhibition effects on ALT activity (38.90±1.29IU/l); 15µg/l induced significant (p<0.05) stimulation effects (98.61±4.22IU/l), whereas the effects of 5µg/l, 10, 20 and 25µg/l were significantly not different (p>0.05) (69.92±4.12-77.05±5.42 IU/l) when compared with the control (75.13± 0.19IU/l). The highest (98.61±4.22IU/l) and lowest (38.90±1.29IU/l) ALT activities were observed in 15µg/l and 1µg/l respectively (Table 3).

Effects of lambdacyhalothrin on alkaline phosphatase activity of *A. gambiae* life cycle stages

Table 4 shows the effect of the six test concentrations of lambdacyhalothrin on ALP activities of life cycle stages of *A. gambiae.* The results showed that 1µg/l and 10µg/l induced significant (p<0.05) stimulatory effects on ALP activity (684.91±140.22 and 659.59±7.03IU/l, respectively) of the egg, whereas 5, 15, 20 and 25µg/l significantly (p<0.05) inhibited the ALP activity (429.08±20.75, 477.36±75.29, 423.17±95.67 and 420.79±28.70 IU/l, respectively) compared with the control (518.54±141.00IU/l). The highest

(684.91±140.22 IU/l) and lowest (420.79±28.70 IU/l) ALP activities in the egg were observed in 1µg/l and 25µg/l respectively. The effect of all the test concentrations on ALP activity of egg were significantly ($p<0.05$) different.

Table 4 further showed that all the test concentrations 1µg/l to 25µg/l significantly ($p<0.05$) inhibited ALP activity (38.10±3.73-75.97±3.23 IU/l) of L_1 life stage, when compared with the control (158.33±8.92IU/l).

Table 3: Effects of lambdacyhalothrin on Alamine Aminotransferase activity (IU/l) of the various *A. gambiae* life stages

Lambda. conc. (µg/l)	*Anopheles gambiae life cycle* stages						
	Egg	L_1	L_2	L_3	L_4	Pupae	Adult
control	79.40 ±1.86^{a1}	15.74 ±1.46^{b1}	14.81 ±0. 96^{b1}	11.20±0.32^{b23}	11.67 ±0.47^{b2}	13.92±0.22^{b12}	75.13± 0.19^{a2}
1	36.88±9.74^{b2}	5.26±4.59^{d2}	8.47±0.40^{d12}	24.45±1.68^{c1}	57.97±1.31^{a1}	20.01±0.09^{c1}	38.90±1.29^{b3}
5	37.92±8.31^{c2}	3.56±1.20^{e2}	7.46±2.19^{e2}	17.60±0.10^{d12}	47.59±0.84^{b12}	15.50±0.14^{d12}	72.12+1.83^{a2}
10	28.09±12.87^{b3}	4.21±0.18^{d2}	4.93±0.07^{d2}	2.70±0.12^{d4}	2.88±0.14^{d3}	13.52±0.24^{c2}	69.92±4.12^{a2}
15	21.38±2.82^{b3}	3.57±0.55^{d2}	1.99±0.20^{d2}	3.51±0.07^{d4}	3.18±0.09^{d3}	14.14±0.14^{c12}	98.61±4.22^{a1}
20	21.75±9.44^{b3}	3.53±0.33^{c2}	3.76±0.37^{c2}	4.95±0.21^{c34}	3.40±0.35^{c3}	9.58±0.17^{c2}	73.30±2.07^{a2}
25	28.15±3.15^{b3}	3.19±0.33^{c2}	3.63±0.33^{c2}	3.27±0.05^{c4}	4.61±0.06^{c3}	9.60±2.12^{c2}	77.05±5.42^{a2}

Mean values in a column and row with different alphabets are significantly different ($p<0.05$)

Likewise, all the test concentrations had significant ($p<0.05$) inhibition effect on ALP activity (22.28±2.83-57.07±3.33 IU/l) of L_2 compared with the control (200.94±8.57 IU/l). Highest (75.97±3.23 IU/l) and lowest (34.55±2.34 IU/l) ALP activities for L_1 were recorded in 15µg/l and 10µg/l, respectively, while for L_2, the highest (57.07±3.33 IU/l) and lowest (22.28±2.83 IU/l) activities were in 25µg/l and 1µg/l, respectively. All concentrations significantly ($p<0.05$) inhibited ALP activity of L_3 (13.53±0.44-87.46±12.86IU/l) compared with the control (144.37±5.57IU/l). The highest (87.46±12.86 IU/l) and lowest (13.53±0.44 U/l) ALP activities were recorded in 15 and 10µg/l, respectively.

Table 4: Effects of lambdacyhalothrin on Alkaline Phosphatase (ALP) activity (IU/l) of the various *A. gambiae* life stages

Lambda. conc (mg/l	*Anopheles gambiae life cycle* stages						
	Egg	L_1	L_2	L_3	L_4	Pupae	Adult
control	518.54 ±141.00^{a2}	158.33±8.92^{bc1}	200.94 ±18.57^{b1}	144.37±15.57^{bc12}	75.50±15.39^{de1}	116.44 ±17.03^{cd1}	24.61 ±18.87^{e3}
1	684.91±1140.22^{a1}	67.45±14.60^{b2}	22.28±12.83^{b2}	46.52±10.74^{b34}	14.32±14.97^{b1}	12.29±10.50^{b2}	68.67±111.42^{b23}
5	29.08±120.75^{a3}	42.07±13.80^{b2}	25.85±11.06^{b2}	42.92±14.68^{b34}	18.97±12.68^{b1}	19.93±14.50^{b2}	7.91±127.57^{b23}
10	659.59±17.03^{a1}	34.55±12.34^{b2}	43.47±13.70^{b2}	13.53±10.44^{b4}	17.54±10.74^{b1}	28.12±10.85^{b2}	50.25±117.91^{b23}
15	477.36±175.29^{a3}	75.97±13.23^{b2}	40.73±110.51^{b2}	87.46±112.86^{b23}	33.12±14.67^{b1}	31.36±15.56^{b2}	88.52±18.34^{b12}
20	423.17±195.67^{a3}	38.10±13.73^{c2}	27.03±14.06^{c2}	22.53±16.04^{c34}	19.61±10.00^{c1}	21.90±11.63^{c2}	104.54±122.52^{b12}
25	420.79±128.70^{a3}	53.49±12.61^{c2}	57.07±13.33^{c2}	62.11±11.54^{c34}	77.03±11.58^{c1}	69.91±12.98^{c12}	166.28±158.06^{b1}

Mean values in a column and row with different alphabets are significantly different ($p<0.05$)

The effects of all the test concentrations of lambdacyhalothrin on ALP activity of L_3 life stage were significantly different ($p<0.05$) compared with the control. Similarly, 1µg/l to 20µg/l inhibited ALP activity (14.32±4.97-33.12±4.67IU/l) of L_4; however, 25µg/l significantly induced ($p<0.05$) stimulatory effect on the ALP activity (77.03±1.58IU/l) when compared with the control (75.50±5.39IU/l) group. The highest (77.03±1.58IU/l) and lowest 14.32±4.97IU/l ALP activities were recorded in 25 and 1µg/l respectively. Effects of all the six test concentrations on ALP activity of L_4 were significantly not different ($p>0.05$) compared with the control (Table 4).

All the test concentrations significantly inhibited ($p<0.05$) ALP activity (12.29±0.50- 69.91±2.98IU/l) of pupae when compared with the control (116.44±7.03IU/l). The highest (69.91±2.98IU/l) and lowest (12.29±0.50IU/l) ALP activities were recorded in 25mg/l and 1µg/l, respectively. When compared with the control, effects of all the tested concentrations were significantly different ($p<0.05$). Furthermore, the results also showed that all the test concentrations significantly ($p<0.05$) induced stimulation effects on ALP activity (47.91±27.57-166.28±58.06IU/l) of adults compared with the control (24.61±8.87IU/l). The highest (166.28±58.06 IU/l) and lowest (47.91±27.57IU/l) ALP activities were recorded in 25µg/l and 5µg/l, respectively. Effects of all the tested concentrations on the adults were significantly different ($p<0.05$) (Table 4).

Discussion

This study revealed that some of the test concentrations of lambdacyhalothrin significantly ($p<0.05$) increased the activities of the liver enzymes studied (e.g. 1 and 5µg/l on the late larval stages - L_3 and L_4), some inhibited their activities (such as 1 to 25µg/l on AST and ALT of the larva and pupae), while some concentrations had no significant effect ($p>0.05$) on enzyme activities (e.g. 5 to 20µg/l on AST of the egg, 5, 10, 20 and 25µg/l on ALP of adults). The increased activities of these enzymes suggest that they play a role in detoxification of the insecticide and also could be correlated with the pyrethroid tolerance status of the malaria vector.

In response to poisons, insects normally break them down into soluble form before they excrete it. To do this, they rely on transferase enzymes among others (Brogdon and McAllister 1998). So, when exposed to such organic substances, these enzyme activities are often induced to higher level trying to detoxify the toxic effect of the insecticide. So, it is not surprising that enzyme activities increased up to 684.91±140.22IU/l for ALP in concentrations of 1µg/l and 174.69±1.37IU/l for AST in a concentration of 15µg/l. Rayan *et al* (2010) reported that Glutathione S-transferase enzyme increased significantly when *Aedes aegypti* larvae were exposed to 2.5µg/ml of α-Mangostin for 24hours, which suggest that the enzyme plays some role in its detoxification. Therefore, increase in the enzyme activity observed in this work seems to be a protective/defensive mechanism against the toxic lambdacyhalothrin, but the high potency of the insecticide overwhelmed the elevated enzymes and subdued them, inhibited further action at the point, hence the death of the life cycle stages which was so high (97%).

Enzyme inhibition means reduction in enzyme activity. Enzyme inhibitors are molecules that bind to enzymes and decrease their activities (Bogoyevitch *et al* 2005). This means blocking an enzyme's active site and hindering it from catalysing its reaction and consequence metabolic imbalance, the ultimate is death of an organism (Bogoyevitch *et al* 2005). In this study, all the test concentrations did inhibit the enzyme activities of the experimental life stages used for the study. The inhibition was most on the enzyme activities of L_1 life cycle stage. The inhibition of enzyme activities of *Anopheles gambiae* life cycle stages observed in this work showed that the test concentrations of lambdacyhalothrin used for this study did bind to the active site of AST, ALT and ALP of the life cycle stages and decreased their enzymatic activity. Consequently, the inhibition prevented detoxification leading to possible accumulation of hazardous lambdacyhalothrin causing death of the life cycle stages. This implies that lambdacyhalothrin is promising in providing effective mosquito/malaria control. This is attributed to high percentage mortality achieved during the toxicity test with concentrations of lambdacyhalothrin and confirms the report by Okoh *et al* (2021).

The heavy inhibition of L_1 enzyme activity may explain why L_1 was established as the life cycle stage, which best depict the specific effect of the lambdacyhalothrin, accounting for heavy mortality that occurred during the preliminary toxicity test (Okoh *et al* 2021). This explains why Henry *et al* (2005) was able to achieve 12% reduction in prevalence of asymptomatic malaria infection and estimated protected efficacy against malaria disease, of 56% in *Anopheles gambiae* pyrethroid resistance area, using nets retreated with lambdacyhalothrin. Similarly, Okoh *et al* (2013) and (2014) achieved above 44.29% reduction in malaria infection and other morbidity indicators such as fever, hospital attendance, health seeking movement and school absenteeism in children of Oruku, Enugu state, Nigeria, with lambdacyhalothrin treated curtains, mats and blankets. Curtis and Mnzava (2000) achieved 60% reduction in malaria incidence with both spraying and impregnated netting intervention with lambdacyhalothrin in Kenya and United Republic of Tanzania; Miguel and Mazariego (2002) reported 100% effectiveness up to 12months after lambdacyhalothrin was sprayed on palm leaves against *Rhodnius prolixus* in the state of Chiapas Mexico.

The stimulation effects among the enzymes (AST, ALT and ALP) of *A. gambiae* life stages observed in this study suggest that the malaria vector may develop resistance to lambdacyhalothrin. Research findings have revealed that *Anopheles gambiae* has developed phenotypic resistance to pyrethroids and DDT especially in Northwestern Tanzania (Kabula *et al* 2014). However, the scientific community are in constant monitoring of pyrethroid resistance in African anopheline mosquitoes (Ranson *et al* 2011; Protopopoff *et al* 2013; Strode *et al*

2014). Consequently, there should be an immediate proactive response by resistance management to avoid compromising these effective interventions approach.

There are several insecticides in the market, many creating apprehensions over their environmental adverse effects, but lambdacyhalothrin still remains an insecticide of choice because it is environmentally friendly – it poses no hazard to human and plant and degrades easily in the soil, unlike its counterpart. Djouaka *et al* (2018) observed that lambdacyhalothrin residues in lettuce and cabbage from farms and markets in Parakou and Cotonou are within the maximum residue level (MRL), and hence are relatively safe for consumption. It is easily excreted in urine and faeces after accidental oral administration (Djouaka *et al* 2018). It was well tolerated by humans; when impregnated in curtain, mat and blanket and deployed in living homes for mosquito/malaria control, the inhabitants did not complain of any adverse reaction in the course of using the treated items and manifestations of acute or residual poisoning were not found (Okoh et *al* 2014).

Results of the effect of lambdacyhalothrin on AST, ALT and ALP of individual life cycle stages compared with one another showed that all the test concentrations inhibited AST, ALT and ALP of L_1 life cycle stage more than those of egg, L_2, L_3, L_4, pupa and adult stages. The result further showed that all the test concentrations induced stimulatory effect more on ALP of egg life cycle stage than on ALP of other life cycle stages. Christen and Metzler (1984) stated that both AST and ALT are located in organelles of eukaryotes such as mitochondria and vacuole. Stress which can be defined as altered physiological conditions is generally known to increase the activities of mitochondrial density in an animal's muscle fiber and thus elevate aminotransferase activities (Natarajan 1985; Gurderley 1990).

In the present study, the activities of the three enzymes were observed to be independent of concentration gradient. The varied enzyme activities recorded across the different concentrations against different groups of the life cycle stages used in this study could be related to the insecticide pressure and the ability of the groups of the insect in a particular concentration to absorb stress of the insecticide. Alkaline phosphatase (ALP) activity was found to be high compared with AST and ALT activity especially in egg stage. The increase in ALP activity may be attributed to phosphorus level due to its requirement for bone (chitin) growth and metabolic energy of the cells.

The researchers of this study did not complain of any undue hazard after working with lambdacyhalothrin. This agrees with Rayman (2006) who reported that pyrethroid insecticides are extremely effective insecticide and pose minimal risks to users and also with United States Department of Agriculture (USDA) extension service and National Agricultural pesticide impact Assessment program, which described lambdacyhalothrin as one of the chemically synthesized pesticides which pose no hazard to living organism and their environment and is well tolerated by humans (Okoh *et al* 2014; WHO 2005a).

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