



Original article

ACUTE TOXICITY OF LAMBDA-CYHALOTHRIN (Karate) ON *Clarias gariepinus* (AFRICAN CATFISH, BURCHELL 1822)

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ABSTRACT

Acute toxicity assessment was conducted for 96hr exposure duration using synthetic insecticide Lambda-cyhalothrin (Karate 50g/L) on *Claris gariepinus* (juvenile). Fish with mean weight range of 27.2 - 29.7g and mean length 10.95 -15.5cm were exposed to the varying concentrations of the insecticide (0.0, 0.63, 1.25, 1.87, 2.5 and 3.12 mg/L) with 6-levels exposure concentrations in a Completely Randomized Design (CRD). Blood samples were collected for haematology analysis using standard methods. The four days lethal concentration (LC₅₀) value for 96hr (95% confidence limits) was found to be 1.169mg/L. The exposed fish developed mucus secretion, erratic swimming with irregular opercular movement, loss of reflex, increased air gulping and lost consciousness with the increasing concentration of the insecticide compared with the control fish. Haematological examination revealed no significant difference ($P < 0.05$) in haemoglobin (Hb), packed cell volume (PCV), Red blood Cells (RBCs), Monocytes and Lymphocytes counts with increase in the concentrations of the insecticide in a dose dependent manner when compared to control. However, significant increase ($P < 0.05$) relative to the control was recorded in the values of white blood cell (WBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentrations (MCHC), neutrophils and eosinophils. The study indicated that Lambda cyhalothrin exert toxic effect on the exposed *Clarias gariepinus* as it predisposes to behavioral and haematological alterations. It is therefore recommended that appropriate authorities should develop strategy on minimizing the indiscriminate use of synthetic insecticides due to their impact on aquatic biota such as fish in order to reduce its potential risk to other non-target organisms.

Keywords: Acute toxicity, Behavioural changes, *Clarias gariepinus* Haematological alterations, Lambda-cyhalothrin,

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INTRODUCTION

Pesticides are substances produced to control, mitigate and regulate the growth of harmful organisms [1]. They are employed globally in agricultural processes for reducing and preventing crops against pests with low labour and efforts [2]. Contamination of aquatic ecosystems by pesticides have recently received urgent attention by researchers [3, 4, 5, 6, 7, 8]. They have been reported to disrupt the food chain in the aquatic ecosystem by indirectly interrupting the fish food supply and habitat alteration [9]. Many findings indicates acute and chronic exposure of pesticides produced adverse effects in the exposed organisms [10, 8].

Lambda-cyhalothrin belongs to type II pyrethroid insecticides with broad spectrum application in the management of agricultural and household's pests [11]. Accidental spills, aerial drift or runoff from indiscriminate usage resulted in the presence of their residues in the aquatic ecosystem [8]. In aquatic biota, the residue bioaccumulate and biomagnify in food chain causing disruption in the normal function of sodium channels in the nervous system [8]. This effect causes synaptic upheaval and membrane depolarization with eventual hyper excitability examined in the exposed organisms. Lambda-cyhalothrin has been reported to intoxicate many fish species and other aquatic invertebrates [12]. Rakesh and Kumar (2019)[13] reported that lambda cyhalothrin causes alterations in behavior in catfish (*Clarias batrachus*) while Fetoui *et al.* (2010)[14] reported a severe cells infiltration in the kidney of fish exposed to lambda-cyhalothrin. Reuben *et al.* (2016) [15] reported that lambda-cyhalothrin induces

oxidative stress and altered biochemical parameters in the liver of fish.

Clarias gariepinus (African catfish) has gained commercial value in Nigeria through rapid expansion in aquaculture [16]. In many part of the country, the species is widely cultured in ponds [16]. The streamline bodies and reduced scales of catfish make them sensitive to water environment. The sensitive cells in fish (chemoreceptors) detect chemical intoxicants upon dermal contact. Rakesh and Kumar (2019) [13] observed certain behavioural changes due to exposure of aquatic biota to chemical substances. Acute toxicity due to pesticides exposure has been reported to affect aquatic biota's responses and physiology [17, 18]. Hundekari *et al.* (2013) [19] opined that formulation of the pesticides, sensitivity of the organisms and nature of water quality equally modify toxicity of the pesticide to the exposed organisms.

The use of haematological indices in fisheries for toxicological biomonitoring have grown rapidly in recent times [20]. Many researches were carried out on haematological alterations in the fish exposed to pesticides [3, 21, 22, 23, 7]. Haematological investigation provides an insight on the immunological status in fish during exposure to toxicants including pesticides [23]. Recently an increase in the use of lambda-cyhalothrin insecticides by farmers has been a common practice in Nigeria [24]. In view of the foregoing this research aimed at assessing the acute toxicity profile of lambda-cyhalothrin on behavioural and haematological indices in *C. gariepinus*.

MATERIALS AND METHODS

Sample Collection and Preparation for Bioassay

A total of 360 healthy juveniles' catfish were procured from Dalar Kifi Fish Farm,

Langel Town along Gwarzo Road, Kano State, Nigeria located between latitude 12° 40'-10° 30' and longitude 7° 40'- 9° 40'. The fish were maintained in a dark plastic container (100L), filled with clean borehole water and renewed daily. The health status of selected fish was determined based on the presence or absence of physical injuries and morphological deformations. They were acclimatized at 27°C and relative humidity (38%), for 14 days in aquarium at Biological Sciences Department, Bayero University, Kano as described by [4]. They were fed twice with pellet diet containing 42% crude proteins with 2mm in size produced by Vital fish feed Nigeria Plc. However, feeding was terminated 24h prior to toxicity test as adopted by Tasneem and Yasmeen (2018) [25].

Water Quality Parameters Measurement

Water quality parameters were measured before and during the acute toxicity test using multifunction water testing kit (Model no. EZ-9909-SP). Parameters such as pH and Dissolved oxygen, Electrical Conductivity, water temperature and Total Dissolved Solids were determined as described by APHA (2005)[26].

Source of Test Chemical

Lambda-cyhalothrin (Karate) was procured from registered store at Sabongari market, Kano with 50g/L active ingredient, Batch No: L1012434 and NAFDAC Reg: A5-220 manufactured by Syngenta Crop Protection AG, Basale, Switzerland, marketed by Syngenta Nigeria Ltd, Ikeja GRA, Lagos, Nigeria.

Experimental Design

The fish samples regardless of their sex was subjected to completely randomized design (CRD) using GenStat version 2.4. Six (6) levels exposure concentrations

was prepared as adopted by Akinrotimi *et al.* (2013)[6]. A set of ten fishes with six treatments and three replications were randomly exposed to lambda-cyhalothrin making a total of eighteen experimental units. One treatment with a set of 10 fishes was maintained in water, without test chemicals, which served as a control.

Range Finding Test and Acute Toxicity Test

The acute toxicity test to determine the 96h LC₅₀ values for lambda-cyhalothrin was conducted in static renewal system (complete replacement of test water after every 24 hours) using 50 x 80 x 50 cm plastic containers (100L) habouring 40L of borehole water as described by Nwani *et al.* (2013)[5]. The set of experiment was carried in Aquarium at Biological Sciences Department, Bayero University Kano using the procedure described by OECD (2013)[27]. Lambda-cyhalothrin concentration at 0.0 (control) 0.5, 1.0, 1.5, 2.0 and 2.5ml/L was converted to 0.0, 0.63, 1.25, 1.87, 2.5 and 3.12 mg/L. This aimed at determining the definitive concentration of the insecticide as described by Adekunle (2015)[28]. The conversion and dilution for +range finding test was carried out using formula described by Fayinminmu *et al.* (2017)[29] and Ezenwosu *et al.* (2020)[24]. The formula is illustrated as follows:

$$C_1V_1 = C_2V_2$$

Where C₁ = initial concentration of stock solution (g/L)

V₁ = volume of stock used (ml)

C₂ = desired concentration (g/L)

V₂ = required volume of water for dilution (ml)

Therefore, forty liters (40L) of dechlorinated water was poured into each container, while 0.5, 1.0, 1.5, 2.0 and 2.5ml of water was removed and replaced

with equal volume of lambda-cyhalothrin from the stock solution. The fish was starved for 24 hours prior to acute toxicity test. The rate of mortality was assessed and recorded to avoid possible deterioration of the water quality.

Observation of Behavioural Responses

The visual observation was carried out after each exposure for the period of thirty (30) minutes to record response shown by the specimens according to Nwani *et al.* (2013)[5], Ullah *et al.* (2014)[30] and Rakesh and Kumar (2019)[13]. Fish was considered dead when there is no sign of opercular movement or no response to gentle prodding.

Haematological Studies

Haematological examination was carried out after 96hr exposure duration according to the procedure described by Dahunsi and Oranusi (2013) [31]. Blood samples was collected from experimental and control fish from the liver, behind the anal fins with heparinized plastic syringe, fitted with 21gauge hypodermic needle of plastic syringe and stored in labeled ethylene diamine tetra-acetic acid (EDTA) bottles. The blood samples was analyzed for PCV, Hb, lymphocytes, monocytes, eosinophils, neutrophils, RBCs and WBCs. Further, red blood indices such as mean corpuscular haemoglobin concentration (MCHC), Mean Cell Volume (MCV) and mean corpuscular haemoglobin (MCH) was calculated using the formulae described by Dahunsi and Oranusi (2013)[31].

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)} \times 100}{\text{Pcv (\%)}}$$

$$\text{MCH (pg/cell)} = \frac{\text{Hb (G/dl)} \times 10}{\text{RBC count in million mm}^{-6}}$$

$$\text{MCV (fl/cell)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC count in million mm}^{-6}}$$

Data Analyses

Probit analysis was used to determine the mortality profile (LC₅₀) of the experimental fish using SPSS version 20.0. One-way Analysis of Variance (ANOVA) was used to determine the effect of varying pesticide concentrations on the mean haematological variables in *C. gariepinus*. Duncan Multiple Range Test at 5% probability level was used to compare the means.

RESULTS

Table 1, indicates the mean and range of physicochemical parameters obtained before and after 96hr exposure to Lambda-cyhalothrin. DO, TDS, pH, EC, turbidity and temperature before exposure ranged from 4.42-5.78, 263-284mg/L, 6.70-7.50, 273-324μS/cm, 22-34NTU and 28.5-31.7°C. Moreover, after 96hr exposure duration, DO, TDS, pH, EC, turbidity and temperature ranged from 3.40-5.24, 297-344mg/L, 8.52-9.54, 283-297μS/cm, 25-41NTU and 33.1-34.6°C respectively.

Table 1: Physicochemical Parameters of Water Before and After 96hr exposure to Lambda-cyhalothrin (Karate)

Parameters	Range	Before	After	Mean (\pm SD)	Standard limits
		Mean (\pm SD)	Range		
Water temperature (°C)	28.5- 31.8	29.4 \pm 0.31	33.1-34.6	34.0 \pm 0.54	<40°Cmg/L*
DO (mg/L)	4.42- 5.78	5.10 \pm 0.13	3.40-5.24	4.51 \pm 0.10	5.0-9.0mg/L**
TDS (mg/L)	263-285	278 \pm 2.17	297-344	314 \pm 4.10	500mg/L**
E.C (μ S/cm)	273-324	289 \pm 3.01	283-297	286 \pm 1.78	1000 μ S/cm**
Turbidity (NTU)	22-34	26.8 \pm 1.13	25-41	35.8 \pm 0.19	25 NTU**
pH	6.70- 7.50	6.80 \pm 0.71	8.52-9.54	8.93 \pm 0.51	6.0-9.0*

*FME (2001), **FAO/WHO (2018)

Behavioural Changes in *Clarias gariepinus* Exposed to Lambda-cyhalothrin

Table 2 illustrates the behavioral changes observed to various concentrations in the exposed fish as well as in the control treatments. Normal swimming behaviour was observed in the control concentrations. Natural colour in the control fishes was examined throughout the exposure period and in the lowest (2.5mg/L) concentration at 24 hour exposure period. In containers with higher concentration of 2.50 and 3.12mg/L, the fish swam erratically with mucus secretion and faster opercular movement were observed. With an increase in the exposure duration 48-72 hours, swimming rate and body movements reduced greatly. The abnormal behaviour was more pronounced in the fish exposed to lambda-cyhalothrin than in glyphosate. In the highest concentrations of 2.50 and 3.12mg/L, all the experimental fish converged at the corner of the container with an increase in air gulping. By 72-96hour, the fish were motionless, decreased opercular movement, lost reflex and consciousness and finally

settled down at the bottom of the tank with the operculum wide opened and eventually died.

Acute Toxicity (96-hour LC₅₀) for Lambda-cyhalothrin

The concentrations of pesticides that caused 50% mortality of the test organism at different time interval is referred to as LC₅₀. Fish mortality was examined in every concentration of pesticides. Mortality profile indicates an increase with increasing concentrations and exposure duration and the highest mortality was recorded in the highest concentrations. The mortality corresponding to each concentration and the percentage survival were illustrated in Figure 1, Tables 3. The 96hr LC₅₀ values Lambda -cyhalothrin (Karate) was determined based on the transformed probit analysis was 1.169mg/L. Linear relationship between the probit mortality and the concentration of lambda-cyhalothrin indicated a positive correlation of $r^2 = 0.9699$. This revealed that mortality rate of exposed fish increased as the concentration of the pesticide increased.

Table 2: Behavioral Responses Examined by *Clarias gariepinus* during 96h Exposure to Lambda-cyhalothrin (Karate)

Exposure time (hr)	Concentration (mg/L)	Air gulping	Erratic swimming	Lost of reflex	Mucus secretion	Opercular movement
24	0.00	-	-	-	-	-
	0.63	-	-	-	-	-
	1.25	-	-	-	+	-
	1.87	-	-	-	+	++
	2.50	+	+	+	+	++
	3.12	+	+++	+	++	+++
48	0.00	-	-	-	-	-
	0.63	-	+	+	+	+
	1.25	-	-	-	+	+
	1.87	-	+	+	+	+
	2.50	-	+	-	-	-
	3.12	++	+++	+	+++	-
72	0.00	-	-	-	-	-
	0.63	+	-	+	-	+
	1.25	-	-	+	+	+
	1.87	+	+	+	+	+
	2.50	++	-	++	-	-
	3.12	+++	-	++	-	+
96	0.00	+	-	-	-	-
	0.63	+	-	+	-	+
	1.25	++	+	+	+	-
	1.87	++	+	+	+	-
	2.50	++	+++	++	+++	-
	3.12	+++	+++	+++	+++	-

Key: =none -, mild +, moderate ++, Strong +++ adopted from Ani *et al.* (2017)

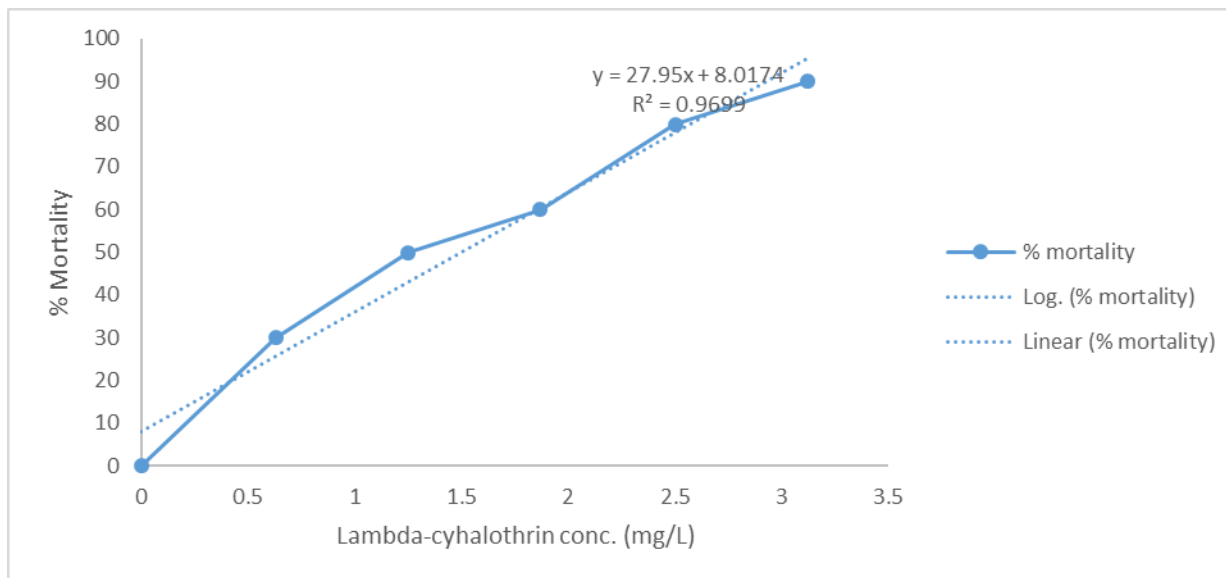


Fig 1: Mean mortality (%) of *C. gariepinus* Challenged with Varying Lambda-cyhalothrin Concentrations at 96 hours

Fish survival and cumulative mortality profile at varying test concentrations in the experimental fish exposed to lambda-cyhalothrin are presented in Table 3. The number of survived and dead fish were examined depending on the exposure duration (24, 48, 72 and 96 h) in *Clarias gariepinus*. The Lambda-cyhalothrin concentration of 3.12mg/L showed the highest fish mortality of 100% and lowest survival of 0% while no mortality was recorded in the control throughout the experiment (Table 3). This shows that increase in mortality rate results to decrease in survival rate of the experimental fish.

Table 4, revealed the acute effects of lambda-cyhalothrin on haematological parameters in *C. gariepinus*. PCV, haemoglobin (Hb), RBCs, lymphocytes and monocytes revealed no significant difference ($p > 0.05$) between the all treatments and control, with exception of the highest concentration of 3.12 mg/L in

which significant difference ($p < 0.05$) was recorded in RBCs and lymphocytes count. Control sample had significantly higher mean PCV value of 34.69% while the lowest mean value of 17.82% was recorded from treatment exposed with 3.12 mg/L. The haemoglobin (Hb) concentrations did not differ significantly ($p > 0.05$) between the control and treated fishes at all concentrations with the exception of 2.50mg/L treatment which differed significantly ($p < 0.05$) with the control. The haemoglobin concentration in the control sample had the highest mean value of 15.40g/dl while at the highest concentration (3.12mg/L) the concentration declined to 9.87g/dl (Table 4). The RBCs count of the experimental fish reduced significantly ($P < 0.05$) from 6.01×10^{12} cells/L in control samples compared to the highest concentration of 3.12 mg/L treatment in which 2.98×10^{12} cells/L was recorded. Lymphocytes and monocytes revealed no significant difference ($P > 0.05$) between

the all the treated and control fish. Their values decreased with an increase in the pesticide concentrations from 43.21 to 29.91% and 17.81% to 9.98% respectively (Table 4).

Results of the effect of lambda-cyhalothrin on WBCs count revealed differed significantly ($P < 0.05$) among all the exposed groups and control with exception of the highest concentration

(3.12mg/L) which did not differed significantly ($P > 0.05$). WBC increased with an increasing concentration of the test chemical. WBC count in the lowest (2.5mg/L) to the highest concentrations (3.12mg/L) ranged from 7.90 to 12.70×10^9 cells/L, eosinophils of 4.66 to 6.84%, neutrophils 31.05 to 36.89%, MCV 28.79 to 34.82fl, MCH 23.71 to 31.76pg and MCHC 36.70 to 41.46 g/dl.

Table 3: Data on Cumulative Mortality Profile of *C. gariepinus* Exposed to Varying Concentrations of Lambda –cyhalothrin (Karate) for 96 hours

Exposed conc.(mg/L)	log conc. (mg/L)	No. of exposed fish	No. of live fish at different duration (hours)				% survival	% mortality	Probit
			24	48	72	96			
0		10	0	0	0	0	100	0	
0.63	-0.200	10	0	1	1	1	70	30	4.48
1.25	0.096	10	0	2	1	2	50	50	5.00
1.87	0.271	10	1	1	2	2	40	60	5.25
2.50	0.397	10	1	1	3	3	20	80	5.84
3.12	0.494	10	1	2	3	3	10	90	6.28

Table 4: Effect of Lambda-cyhalothrin on Haematological Indices in *C. gariepinus* After Acute Exposure

Concentrations(mg /ml)/Parameters	0.00(control)	0.63	1.25	1.87	2.50	3.12
PCV (%)	34.69±0.10 ^{a,b}	33.8±0.56 ^a	32.9±1.01 ^a	31.83±0.17 ^a	25.73±0.91 ^a	17.82±0.18 ^{a,b}
RBC (× 10 ¹² cells/L)	6.01 ±0.56 ^a	5.80±1.41 ^a	5.64±0.06 ^a	3.67±0.33 ^a	3.53±0.74 ^a	2.98±0.85 ^{a, b}
WBC (×10 ⁹ cells/L)	7.67±1.01 ^c	7.90 ±0.01 ^a	10.43±0.56 ^a	11.63 ±1.01 ^a	12.41±0.30 ^a	12.70±0.52 ^b
Hb (g/dl)	13.10±0.01 ^{a, b}	10.74±0.15 ^a	10.81±0.82 ^a	10.10±0.93 ^a	9.86±0.64 ^a	9.70±0.13 ^a
Lymphocytes (%)	43.21 ±1.19 ^b	41.85±0.31 ^a	38.75±0.14 ^a	36.09±0.80 ^a	32.57±0.16 ^a	29.91±0.81 ^b
Neutrophil (%)	31.02±0.14 ^a	31.05±0.72 ^a	32.80±0.07 ^a	34.76±0.45 ^a	35.82±0.16 ^a	36.89±0.10 ^{a, b}
Monocytes (%)	17.81±1.49 ^a	16.74±2.01 ^a	13.52±1.01 ^a	12.64±0.13 ^a	10.02±0.10 ^a	9.98±1.05 ^{a, b}
Eosinophil (%)	4.31± 0.47 ^a	4.66±1.01 ^a	4.68±0.01 ^a	4.78±0.02 ^a	4.93±1.96 ^a	6.84±0.01 ^{a, b}
MCV (fl)	24.41±0.15 ^a	28.79 ±1.63 ^a	31.78±0.81 ^a	31.89±1.23 ^a	32.70±0.12 ^a	34.82±0.12 ^b
MCH (pg)	21.86±1.08 ^{a,b}	23.71 ±0.10 ^a	26.96±0.01 ^a	29.98±0.52 ^a	31.41±0.93 ^a	31.76±0.66 ^a
MCHC (g/dl)	33.01±0.31 ^{a,b}	36.70 ±0.10 ^a	36.81±0.01 ^a	37.72±0.08 ^a	38.61±0.63 ^a	41.46 ±0.66 ^{a, b}

Mean values with different superscript alphabet in a row differed significantly (P<0.05)

DISCUSSION

Behavioural changes have direct impact on the physiological condition of an organisms, which serves as a vital symptom for assessing the pollution status in vertebrates including fish [13]. During the 96hr exposure duration to varying lambda-cyhalothrin concentrations, the experimental fish displayed various stressful pattern such as air gulping, mucus secretion, inconsistent jumping, loss of reflex and erratic swimming which were noticeable particularly among fish exposed to the highest concentrations. Air gulping, mucus secretion and inconsistent jumping observed indicates respiratory impairment due to the alterations in habitat suitability of the exposed fish or due to mobility of the surfactant in the insecticide to the fish's body system as reported by Sánchez-Bayo *et al.* [32] and Ogamba *et al.* [33]. Excessive mucus secretion in the exposed fish could be regarded as a non-specific response against toxicants with sole aim of minimizing direct toxicant contact [7]. It may also serve as a barrier between the fish' body and the toxic environment, thereby reducing the insecticides' irritating effect or by absorbing via epidermal mucus layer as reported by Ahmad *et al.* [34].

In polluted aquatic environment, aquatic biota including fish display irregular, erratic swimming pattern and vertical surfacing [35]. Pyrethroid insecticides (lambda-cyhalothrin) are axonic poisons in the nervous system of the fish affecting sodium channels leading to the delay in the inactivation of the Na⁺ channels, thereby causing neuronal excitability [8]. Disturbance to these channels during exposure to toxicant causes different neurobehavioral changes, affecting

energy metabolism, homeostasis and neuromuscular functions [8]. The above features observed could be responsible for erratic swimming and loss of reflex identified in experimental fish exposed to the varying concentrations of lambda - cyhalothrin as reported by Tu [36] and Ahmad *et al.* [34]. Similar observation was also reported by Omoniyi *et al.* [37] who examined lateral and upward movement, respiratory distress and spontaneous air gulping in *Clarias gariepinus* (juveniles) exposed to dichlorvos. The behavioral changes observed might also be due to metabolic alterations in *C. gariepinus* trying to withstand with the toxic effect of lambda-cyhalothrin. The present finding corroborates with that of Ilavazhahan *et al.* [38] who reported increased mucus secretion, rapid jerk movement and increased movements of opercula *Catla catla* on acute exposure to Methyl parathion. The general body weakness, loss of reflex and eventual mortality occurred at 96hr exposure period among the highest concentrations of the test insecticide. These changes might be attributed to the depletion of oxygen in the experimental water which might cause disruption of carbohydrate metabolism and the exposed fish which could not cope with the water condition, became unconscious and eventually die as observed by Ogundiran *et al.* [39] and Mishra [40]. Alterations in the normal behavioral responses in fish could also be considered as an index of the stress experienced by the exposed fish as obtained by Ezenwosu *et al.* [24]. Similarly, discolouration, surfacing activity and intense hyperactivity were also recorded in *Clarias batrachus* exposed to lambda cyhalothrin [41]. The observed behavioral changes recorded in the present finding is in tandem with

previous observation by Rani and Kumaraguru [42] on lambda-cyhalothrin and with other pesticides such as Devi and Mishra [43] on chloropyrifos, Ani *et al.* [44] on glyphosate and Parvaiz *et al.* [45] on Cypermethrin.

The present finding revealed that the 96 hr LC₅₀ value of lambda -cyhalothrin (karate) was 1.169mg/L. The LC₅₀ values has been reported to depend on organism (fish), pesticides bioaccumulation factor, varying chemistries of the compound forming the pesticide, the test conditions and the reactions of the organisms receiving the toxicant [46,8]. The low 96 hr LC₅₀ value recorded could be attributed to its high bioconcentration factor (BCF) of 2,240 as approved by PAN [47] in fish species. It could also be due to the high mean water-tissue organic carbon partition coefficient (KOC), indicating affinity to organic matter and high tendency to adsorb on suspended particulate materials in the water column and tissues [47]. The LC₅₀ observed in the present study is higher than 5.00µg/L and 0.0008163µg/L recorded by Kumar *et al.* [48] and Ezenwosu *et al.* [24] in *C. batrachus* and *C. gariepinus* exposed to lambda-cyhalothrin respectively. Similar trend of LC₅₀ was reported in pyrethroid insecticide of the same family by Ayoola and Ajani [49] of 63mg/L and Veni and Veeraiah [49] of 5.13 µg/L in *Clarias gariepinus* and *Cirrhinus mrigala* respectively. Ahmad *et al.* [34] also recorded 96 hr LC₅₀ value of 4.57µg/L in *Cirrhinus mrigala* (Indian major carp) exposed to cypermethrin (35%EC). The variation in the LC₅₀ values examined in the present study could be attributed to insecticides sensitivity, exposure duration and concentrations and varying surfactants in the test chemicals. The LC₅₀ value obtained in the present finding

could also be due to the certain level of resistance displayed by the experimental fish to the insecticide. Similar observation was reported by Kumar *et al.* [48, 51] in acute toxicity of cypermethrin and λcyhalothrin and cypermethrin respectively. The LC₅₀ values variations in many fish species have been attributed to the respective capability of the fish to cope and metabolize the lambda-cyhalothrin compounds [24]. Variation in the LC₅₀ might be due to pesticide formulation, water quality status, and physiological condition of the fish, size, age and sex of test organisms [52, 53]. This imply that, many LC₅₀ values can be obtained from similar pesticide/fish species [8]. The low 96hr LC₅₀ led to the mortality rate acceleration and decline in the survival rate as the exposure time increases at varying concentrations. This could be attributed to the harmful effect of the pesticides on the fish survival as reported by Olele and Zelibe [54].

Haematological alteration in fish due to pesticides exposure among other pollutants have been used as a health condition bioindicator of the fish (Ezike *et al.* [7]). After 96hr exposure to varying lambda-cyhalothrin concentration, there was a decrease in RBCs, Hb concentrations and PCV value in all the treated groups compared to control. The decrease in RBCs count could be attributed to the effects of the varying pyrethroid insecticide (lambda - cyhalothrin) concentrations on the fish's haematopoietic system by blocking the erythropoiesis via transferrin disruption as reported by Ullah *et al.* [22] in *Tor putitora* exposed to Cypermethrin (pyrethroid). The decreased in RBCs could also be due to the alteration in iron synthesis in the blood or due to the inhibitory effect of the surfactants in the

lambda -cyhalothrin on the enzymatic processes meant for haemoglobin synthesis [23]. The declined in the RBC count on the course of exposure to the varying insecticides treatment could be due to the hypoxia generation which might stimulate RBC disruption as results of insufficient of Hb content in the cellular medium as reported by Ullah *et al.* [22]. The decreased in PCV and Hb with an increase in the concentration of the test chemical compared with control sample revealed haemolysis of the RBC count; as a result of distorted osmoregulation causing decrease in dissolve oxygen in the experimental water. Similar observation was reported by of Akinrotimi and Gabriel [55]. The decrease in PCV value examined after exposure duration (96hr) to lambda-cyhalothrin could be attributed to the low oxygen in the blood circulation of *C. gariepinus* as reported by Ezike *et al.* [58] in *C. gariepinus* exposed to Dichlorovos. Decreased in haemoglobin concentration in exposed fish relative to the control sample implied the fish's capability to maintain sufficient oxygen to the cells and tissues is inhibited, causing a reduced physical activity as reported by Akintade *et al.* [56]. A decrease in Hb and PCV has been examined in previous findings by David *et al.* [57] on deltamethrin of carp (*Cirrhinus mrigala*) and [23] on mixture of atrazine and metolachlor of *C. gariepinus* respectively. George *et al.* [23] depicted that decrease in PCV is attributed to alterations in osmoregulation leading to anaemia and haemodilution. Reduction of RBCs count in fish exposed to pyrethroid insecticides has been observed to alter liver functions and heam synthesis [8]. The examined reduction in RBC count, haemoglobin and PCV during the 96hr exposure period is in tandem with what was reported in other

aquatic biota challenged with the pesticides [16, 7].

Lymphocytes and monocytes count decreased with an increase in the insecticide concentration relative to the control. The reduction in lymphocytes and monocytes could be due to the interactive effect of lambda-cyhalothrin being an axonic poisons that affect the nerve fiber of an organism through binding to a protein that regulates the voltage-gated sodium channel as reported by Ezenwosu *et al.* [24]. The reduction in lymphocytes could be due to their effect on α -cyano group in the lambda-cyhalothrin chemical structure on the metabolic activities in the fish lymph tissues. This agrees with the observation of Akinrotimi *et al.* [16]. The decrease in lymphocytes and monocytes could also be due to the effect of active ingredient in the pesticide which stimulate cellular oxidative damage and ultimately the decrease in lymphocytes and monocytes [8].

White blood cells count defense body against infectious agent caused by toxicants [68]. In the present study, WBCs count increased with increased in treatment concentrations compared with control samples. The elevated WBC count is attributed to the stimulation of immune system due to cellular/tissue dysfunction or due to the activation of the experimental fish's defense mechanism as reported by George *et al.* [23]. WBC count is responsible mainly in the control of immunological function in an organisms; therefore, increased in the WBC in all the treated fishes could facilitate a decline in non-specific immunity of the experimental fish or protective response in the fish under pesticide exposure stress. Similar observation was reported

by Akinrotimi *et al.* [16] in *C. gariepinus* exposed to cypermethrin. Elavation in WBC count examined in the present study compared with control could also be attributed to the stimulation of the defense mechanism by the treated fish to cope with the impact of the insecticide. This is in tandem with the present finding of Ezike *et al.* [7]. Increased WBC count in experimental fish exposed with acute concentrations indicates leukocytosis related with leucocytic response in the fish under environmental stress as reported by Akinrotimi *et al.* [16]. High lymphocytes count under toxicant stress could led to an increased in WBC count as observed by Akinrotimi *et al.* [16] on exposure of *Clarias gariepinus* to Cypermethrin. Ullah *et al.* [22] reported an increase in white blood cells (WBCs) count and a decline in RBCs count on exposure of *Tor putitora* to cypermethrin. Changes in haematological indices due to pesticides exposure have been attributed to the hematopoietic system's failure and stimulated defense mechanism which alters WBCs count [59]

Erythrocyte indices such as MCV, MCH and MCHC has been reported to alter the homeostatic processes of fish physiology due to exposure to toxicant [16]. In the present finding, the MCV, MCH, and MCHC values increased in all treated fishes compared with the control. Increased in MCV, MCH and MCHC observed in the exposed fish indicates that the treated fish developed macrocytic hyperchromic anemic status induced by the pesticide due to impairment for utilization of vitamin B₁₂ or liver dysfunction as reported by [22]. Macrocytic hyperchromic anemia is attributed to disruption in DNA synthesis during erythropoiesis [60]. The increase in MCV, MCH and MCHC is in agreement with the

work of Akinrotimi and Amachree [61] following acute exposure of *Tilapia guineensis* to detergent. The red blood cells of the exposed fish in the present study were normocytic as their MCV values did not differ significantly from the control. The MCV, MCH and MCHC increased indicates normocytic hypochromic anaemia during the 96hr exposure duration. This could be attributed to the increased destruction of the red blood cells by the pesticide higher than the production ability of the bone marrow as reported by Ali and Kumar [62].

There was an elavation in the neutrophils and eosinophils count compared with the control. An increase in the neutrophils and eosinophils count reported could be due to the detoxifying effort against the pesticides-induced toxicity on the WBCs indices as reported by Ullah *et al.* [22] and George *et al.* [23]. An increase in neutrophils and eosinophils might be due to the lipophilic nature of pyrethroids insecticide on biological membrane tissues where they are readily absorb leading to induced oxidative stress in tissues and ultimately facilitate the increase in the neutrophils and eosinophils count as reported by Kingsley *et al.* [63] in wistar rats exposed to Dichlorvos. The significant alterations observed in the haematological indices of the exposed fish under the applied experimental condition is in tandem with the conclusion reached by Gholami-SeyesKolaei *et al.* [64], Ezike *et al.* [58] and Bamidele *et al.* [65]. Alterations in haematological variables of many fish species were reported by Saedi *et al.* [66] who studied the effect of diazinon on haematological parameters of fry rainbow trout (*Oncorhynchus mykiss*), [30] on cypermethrin to haematological

alterations in *Tor putitora* and [67] on *Cyprinus carpio* exposed to pyrethroid (Permethrin).

Conclusion and Recommendations

From the present findings it was revealed that behavioral changes and haematological alterations recorded were mainly at higher concentrations and time-dependent. Behavioral characteristics are obviously sensitive indicators of toxicant's effect on the experimental fish. It was indicated that lambda cyhalothrin exert toxic effect on the exposed *Clarias gariepinus* as it predisposes them to behavioral and haematological alterations. Thus, the continuous application of synthetic lambda-cyhalothrin for agricultural purposes could adversely affect aquatic biota. It is therefore recommended that appropriate authority should minimize the indiscriminate use of synthetic insecticides due to their impact on aquatic biota such as fish in order to reduce its potential risk to other non-target organisms.

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