Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 10, Issue 3, pp: (15-25), Month: May - June 2023, Available at: www.noveltyjournals.com

IMPACT OF LAMDA LAMDA-CYHALOTHRIN ON THE ANTIOXIDANT PARAMETERS OF *Clarias gariepinus*

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DOI: https://doi.org/10.5281/zenodo.7962279

Published Date: 23-May-2023

Abstract: **Widespread applications of pesticides such as Lambda cyhalothrin to boost crops production have frequently led to contamination of the fresh water ecosystem in Nigeria. In this study, *Clarias gariepinus* were exposed to sub-lethal concentrations of Lambda-cyhalothrin pesticide. The 96 h LC_{50} of lambda cyhalothrin to the fish was estimated at 3.98mgl^{-1}. Mortality of 100% and 10% were observed in fish exposed to 12.00 mg l^{-1} and 1.25 mg l^{-1} of Lambda cyhalothrin respectively as compared to no mortality recorded in the control group. Varying degrees of abnormal behaviours like air gulping, hyperactivity, erratic movement, skin discoloration and jerky movements were observed during the 96 h exposure period of the fish to Lambda compared to the control. There was no significant difference ($p>0.05$) between the values of water quality assays in control and treated group. Exposure to sub-lethal concentrations of Lambda cyhalothrin at 0, 0.25, 0.50 and 1.00 mgl^{-1} and for 15 days at 5 days intervals that is 5, 10 and 15days led to changes in the antioxidant physiological functions in the fish in comparison to the control. The result for the oxidative biomarkers showed there was increase in the mean values of Glutathione peroxidase (GPX) and Reduced glutathione (GR) with increase in the concentration of the toxicant when compared with the control. Also, there was significant decrease in the values of the Super oxide dismutase (SOD) and Catalase (CAT) with lipid peroxidase (LPO) showing no significant difference at $P<0.5$ with increase in concentrations of the toxicant when compared to the control. The study reports that lambda could cause oxidative and physiological dysfunction in *Clarias gariepinus*. The biomarkers measured could be useful tools for monitoring effects of other pesticides on aquatic organisms. However, further studies could be done to investigate their mode of action to strike a balance between protection of aquatic biota and discharges of these pesticides and their metabolites to aquatic environments.**

Keywords: **Impact, Antioxidant parameters, Lambda, pesticide.**

1. INTRODUCTION

In order to increase food production due to the increasing human population different types of pesticides are applied to meet up the demand for food production through agricultural practices. Unfortunately, the indiscriminate and uncontrolled applications of these pesticides in agricultural land have resulted to ecotoxicological effects to the aquatic biota when it gets washed into aquatic ecosystem through erosion. Aquatic environment is particularly one vulnerable area as it is the ultimate recipient of pollutants due to basin drainage. The aquatic ecosystems have been known to receive a wide spectrum of pollutants, which may be introduced to them directly or indirectly. The use of pesticides has continued to increase as it is still considered the most effective method to reduce pests and increase crop growth in agriculture. The indiscriminate use

of pesticides has resulted in large scale reduction in aquatic productivity. Pesticides have different diverse impacts on aquatic animals especially fishes which are of economic importance and high value from the point of biological conservation (Mekkawy *et al*., 2013). Environmental pollution by pesticides has become a serious problem in terms of global conservation and animal and human health (Katsumata *et al.*, 2005; Velisek *et al.*, 2010). Lambda-cyhalothrin (LCT) is a synthetic pyrethroid that has immediate and persistent effects activity against a large variety of arthropods, and also harmful both to human and animal health and to vegetal production (WHO, 2005). LCT has been found to accumulate in biological membranes leading to oxidative damage by altering antioxidant systems and increasing lipid peroxidation (LPO) in mammals (Naglaa, 2012).

Fish is highly nutritious, easily digestible and a much sought after food. Nutritional value of fish depends on their biochemical composition, which is affected by water pollution (Prado *et al.*, 2009). The African cat fish, *Clarias gariepinus* is a remarkable and fascinating species, as it is extremely hardy and can withstand adverse environmental conditions and habitat instability. It is hardy and does not easily succumb to disease. It is one of the richest source of animal protein to man. Growth of this fish under natural condition is very fast as they can feed on all types of biowastes. *Claries gariepinus* is useful in biowaste management (Sambhu, 2004). It recycles different types of biowastes such as animal wastes poultry, butcher and fish wastes, and plant protein into fish protein. The fish has predatory, cannibalistic and the voracious feeding habit. It can efficiently assimilate a wide variety of animal and plant proteins and this has made most fish farmers to culture them in natural inland freshwater bodies. Also, the fast growth rate and relatively high market price of this fish has lured many farmers into its culturing. Drained water from fish ponds can be used to irrigate vegetable crops. Fish and aquatic animals are exposed to pesticides in three primary ways. Dermal, direct absorption through the skin by swimming in pesticide-contaminated waters, Inhalation, by direct uptake of pesticides through the gills during respiration, and orally, by drinking pesticides - contaminated water or feeding on pesticide –contaminated prey. There are some secondary causes that cause the exposure of fish and aquatic animals to pesticides and eventually lead to toxicity. Through the consumption of another animal that has been poisoned by a pesticide. The exposure of fish and other aquatic life to pesticides may be a more widespread problem than most people realize. Most pesticide-related fish kills go unreported and, in documented cases, the number of fish killed is often underestimated. Scavengers quickly remove the bodies from the site of murder. Dying and stressed fish may hide in dense cover or leave the area completely.

Bioaccumulation of toxic compounds in fish together with environmental stress can invoke the production of excess ROS commonly known as free radicals- such as superoxide anion (O^{-2}), hydrogen peroxide (H_2O_2), the hydroxyl radical (OH^-) can elicit physiological alterations, oxidative dysfunction such as lipid peroxidation (Islas-Flores *et al.*, 2013; Woo *et al.*, 2006). Different cellular and extracellular components such as the nucleic acids are exposed to high risk of damages by these free radicals causing many degenerative and carcinogenic diseases. Many aquatic organisms show an ability to live in contaminated regions, principally due to their inducible defence mechanisms that allow detoxification, excretion, antioxidant protection, and stress response (Bard, 2000).

Toxicity tests conducted at levels of lambda-cyhalothrin residues measured in water or sediment indicated potential for effects on aquatic organisms including fish and amphipods (Amweg *et al.*, 2005, 2006; Cavas and Ergene-Gozukara 2003; Gu *et al.*, 2007; Heckmann and Friberg 2005; Lawler *et al.*, 2007; Maund *et al.*, 1998; Van Wijngaarden *et al*., 2005; Wang *et al.*, 2007; Weston *et al.*, 2004). Concerns have therefore been raised about the widespread use of lambda-cyhalothrin and its potential impact on aquatic ecosystems. The aim of this study was to determine the impact of Lambda of African catfish, *Clarias gariepinus.*

2. MATERIALS AND METHODS

2.1. Experimental Site

The experiment was conducted at Heldin's Fisheries Unit, Old Airport Road Thinkers Corner, Emene, Enugu State. Experiments were conducted to determine the impact of lambda cyhalothrin on African catfish *C. gariepinus* respectively. The agro pesticide Lambda cyhalothrin was used in the experiment. A total of one hundred and forty juveniles of *C. gariepinus* (mean weight 27 ± 0.07 g; mean length 13.48 ±1.01cm) were used for the experiment. The fishes were exposed to both acute toxicity test for 96 h and sub-lethal concentration of the pesticide for 15 days at 5days interval. .

2.2. Collection and Acclimation of the Experimental Fish

All the fishes were obtained from Rojenny Tourist Game Village Idemmiri Local Government Area of Anambra State through the help of local fish farmers. They were transported to the Laboratory using 50litre gallon. The fishes were transferred into different fiber reinforced plastic (FRP) tanks, containing 15l of de-chlorinated tap water. Aeration was provided to all the containers round the clock with the help of aerator in order to maintain dissolved oxygen contents. This was made possible by providing the tanks with air stones and regulator valves to control the air pressure uniformly to all the tanks. The fishes were acclimated for two weeks before the commencement of the experiment. During the acclimation and throughout the exposure period, the fish were fed at 2% body weight with commercial fish diet (Coppens Fish feed for aquaculture by .5700 Am Helmond, Holland) with active ingredients of crude fibre 42%, crude fat 13%, crude fiber 2.8%, crude ash 6.6%, phosphorus 0.85%, sodium 0.2% and calcium 1.2%

2.3 Experimental Design

Completely randomized design (CRD) was used for the experiment. One hundred and eighty fishes were distributed into eighteen plastic aquaria. Each treatment was replicated six times with 10 fish per container. Fishes were exposed to different sub lethal concentrations of the pesticides as treatments. The experiment was replicated in triplicates with the exception of the control. The different concentrations were measured and introduced into experimental containers containing 10litres of tap water. The mixture was allowed to stand for 30 minutes before introducing the fish to be tested.

2.4 Experimental set-up for Acute Toxicity Test (Range finding test)

The acute toxicity test of the pesticide to *C. gariepinus* was carried out according to Environmental Protection Agency (EPA) (2002) and United Nation Environmental programme (UNEP) (1989) in a static renewal system by using 15L capacity plastic tanks. Five experimental concentrations of each of the pesticides were prepared for each of the experiments. The five concentrations of the pesticide were prepared from the original solution using the formula described by Solbe (1995). The concentrations of the trial test were prepared by pipetting different volumes of the original concentration of the pesticide into 10 L of water in five static tanks at a time to make five different solutions. In the experiment, the concentrations of Lambda cyhalothrin used were 1.25, 1.50, 3.00, 6.00, and 12.00 mg L^{-1}. Each concentration was prepared in replicate and used for stocking of ten fish. One group was exposed to only de-chlorinated tap water which served as control. Feed was not offered to the fish 48 h before and during 96 h of test period. The physico-chemical parameters of the test water were analyzed using standard methods APHA (2005). The research was conducted in an indoor experimental outfit. The ethical guidelines for the Animal of Ministry of Agriculture, Enugu state were strictly adhered to. In order to avoid fouling the experimental media, the containers were checked daily, while the dead fishes were recorded and removed using scoop net. The water quality and appropriate concentrations of the pesticide were maintained by renewing the test water and the toxicant daily. The behavioral changes in the exposed fish and control were also observed. Finney's probit analysis method (Finney, 1971) was followed to determine the 96 hLC_{50} of the pesticides on the exposed fish

2.5 Long-term Exposure to Sub-lethal Concentrations of the Agro Pesticides

The study was conducted in a static renewal system after the acute toxicity test. Each of the experiment was conducted by exposing the fish to different LC_{50} of the various pesticides at 96h. The concentrations of these pesticides were prepared in arithmetic series and were not sufficient to cause immediate mortality of the experimental fish. The concentrations of Lambda cyhalothrin used were 0.25, 0.50 and 1.00 mg L^{-1}. A total of one hundred and forty fishes from the acclimatized group were distributed randomly to the container, 10 fish per container. The fishes were fed twice daily at 2% total body weight at 9.00 and 16.00h with commercial fish diet, having 30% crude protein. Fifty per cent of the exposed solution was renewed every other day to maintain the water quality of the test media and normal concentration of the pesticides. Aeration was provided to all the containers round the clock with the help of aerator in order to maintain dissolved oxygen contents. This was made possible by providing air stones and regulator valves to control the air pressure uniformly to all the containers. The experimental containers were cleaned daily. The test fish along with the controls were sampled on days 5, 10 and 15 to determine the toxic effects of the pesticides on the exposed fish. On each sampling day, two fish from each concentration were used for the analysis of the antioxidant parameters.

2.6 Physico-chemical Parameters

Water quality parameters such as temperature, pH, and dissolved oxygen were recorded during the experimental period.

Novelty Journals

ISSN 2394-966X

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2.6.1 Temperature, pH and CO_2

These parameters were measured with a hand-held Hanna Combo instrument (HI 98129). The pH and temperature were measured by setting the pH mode on the instrument. The probe was submerged in the water for about one minute and the reading taken when the stability symbol on the top left corner of the LCD disappeared. The pH value was displayed on the primary LCD of the instrument while the secondary LCD displayed the temperature. The probe was submerged in water and the readings were taken as in the case of pH above. The primary LCD showed readings for both pH and CO_2 while the secondary LCD displayed the temperature of the sample.

2.6.2 Dissolved Oxygen (DO)

Dissolved oxygen of the diluting water was measured according to the Winkler's method described by APHA (2005). This is described in the steps below:

- Water sample was poured into a 300ml biological oxygen demand (BOD) bottle. 2ml $MnSO_4$ solution was added followed by the addition of 2ml alkali-iodide azide reagent and the bottle stoppered with care to exclude air bubbles
- The sample was then mixed gently by inverting the bottle a number of times until a clear supernatant was obtained
- The sample was then allowed to settle for two minutes followed by the addition of 2ml H_2SO_4, allowing the acid to run down the neck of the bottle
- The bottle was stoppered again and gently inverted until dissolution was complete
- 100ml of the prepared solution was poured into a conical flask and titrated with 0.0125N $Na_2S_2O_3.5H_2O$ solution to a pale straw/yellow colour
- 3ml of freshly prepared starch solution was added, making the solution become blue in colour

Titration was continued by adding the thiosulphate drop-wise until the blue colour disappeared. The amount of thiosulphate used in the titration represented the amount of dissolved oxygen in the sample.

2.6.3 Alkalinity

Total alkalinity was determined titrimetrically as described by APHA (2012) using the following reagents: 0.1 N tetraoxosulphate (IV) acid (H_2SO_4) and methyl orange indicator.

Procedure: One hundred millilitres (100 ml) of water was put in a conical flask and 2-1 drops of methyl orange indicator were added. Then it was titrated with 0.1 NH_2SO_4 until the yellow colour changed to orange indicating the end point. The total alkalinity (mg/l) was calculated from the following formula:

$$\text{Alkalinity (mgl/l)} = \frac{\text{ml x N x 50,000}}{V}$$

N = Normality of titrant

V = Volume of water sample (100 ml)

ml= Volume of titrant used.

2.7 Oxidative Stress Enzymes Activity

2.7.1 Superoxide Dismutase

Superoxide dismutase (SOD) was determined following methods adopted by Mishra and Fridovich (1972) with some modifications. Aliquot of 30 µl enzyme from the tissue homogenate of muscle was pipetted into a cuvette, containing 1.5 ml, 0.05 M carbonate-bicarbonate buffer (pH 10.2), with 10^{-4} M EDTA. After running a blank with distilled water instead of sample, the cuvette with the buffer and sample mixture was set in its position and 0.5 ml, 0.01 M epinephrine (molecular weight, 183.2 g) was pipetted into the mixture. Immediately, change in absorbance at 480 nm for 3 min at 30 sec interval was recorded, using Libra – UV spectrophotometer. Each sample was carried out in triplicate. SOD was expressed in units of activity, where one unit of activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

2.7.2 Catalase

Catalase (CAT) activity was assayed following methods described by Takahara *et. al.*(1960). A mixture of 2.5ml, 50mM phosphate buffer (pH 7) and 30 µl enzyme sample (liver) was prepared in a cuvette and was set in Libra UV-spectrophotometer at 240nm. Freshly prepared 1 ml, 3% H_2O_2 was pipetted into the mixture and immediately, change in absorbance for 3 min at 15 sec interval was recorded for each sample in triplicates. A blank before the sample was run with buffer only to zero the reading. Catalase enzyme specific activity was expressed as units of CAT, where one unit is explained as the millimole of H_2O_2 decomposed per minute per milligram protein. Molar extinction of H_2O_2, $\varepsilon_{240} = 43.6M^{-1} C^{-1}$, was used to calculate the enzyme specific activity.

2.7.3 Total Reduced Glutathione

The tissue total reduced glutathione (GSH) concentration was measured using the method described by Ellman (1959). Samples were treated with trichloroacetic acid (TCA, 5% w/v) and decanted (4000 rpm for 10 min). Fifty microliter of clear supernatant was mixed with Tris–HCl buffer (230 µl, 0.8 M Tris/HCl, 0.02 M EDTA, pH 8.9) and 20 µl of 0.01 M DTNB (2, 2'-dinitro-5, 5'-dithiobenzoic acid, Ellman's reagent). Reaction mixture was incubated for 5 min at room temperature. The absorbance of GSH–DNTB conjugate was determined at 412 nm, and the concentration (nM GSH/mg protein) was calculated using standard calibration.

2.7.4 Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was measured using the method described by Paglia and Valentine (1967). One milliliter of reaction mixture containing 0.2 ml of phosphate buffer (0.4 M, pH 7.0), 0.2 ml of EDTA, 0.1 ml of azide (10 mM), 0.1 ml of H_2O_2 (1 mM) and 0.2 ml of tissue supernatant and 0.1 GSH (61.4 mg GSH in 100 ml distilled water; make 1.0 ml of this solution to 10 ml with distilled water). The sample was incubated at 37°C for 15 minutes. At the end of incubation period, the reaction was terminated by adding 0.5 ml of 10% TCA. Tubes were centrifuged at 10,000 rpm for 5 min and the supernatant was collected in separate tubes and 0.2 ml of Tris buffer (0.1 M, pH 7.4) and 50 µl of DTNB (0.4 mg/ml) were added to 1.0 ml of reaction supernatant. The absorbance was immediately read at 420 nm. The GPx activity was expressed as nM GSH oxidized min^{-1} mg^{-1} protein.

2.7.5 Lipid Peroxidation

Lipid peroxidation (LPO) in the liver was determined according to Sharma and Krishna-Murti (1968) by estimation of thiobarbituric acid reactive substances (TBARS). One ml of the tissue homogenate was incubated at 37°C for 30 minutes. Proteins were precipitated by adding 1 mL of 10% trichloroacetic acid (TCA) and then centrifuged at 2000 x g for 15 minutes. One ml of the supernatant was taken as an aliquot in a separate tube to which 1 ml of thiobarbituric acid reacting substances (TBA) solution was added. The tubes were kept in boiling water bath for 10 minutes. The tubes were allowed to cool. After cooling, the optical density was read at 535 nm. The specific activity is expressed in nanomoles of TBARS mg^{-1} protein.

2.7.6 Statistical Analysis

Data obtained were analysed, using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago, Illinois, USA). Differences in test concentrations and control were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests to determine level of significance at 5% probability level. Results were expressed as mean ± standard deviation

3. RESULTS

3.1 Effects of Lambda cyhalothrin on Behavioural Response and Mortality of *Clarias gariepinus*

The fishes exposed to an acute concentration of Lambda cyhalothrin for 96 h, showed to some varying degrees of behavioural disorder before death. The behavioural irregularities displayed by the exposed fish increased with increasing concentrations of the Lambda cyhalothrin thus, exhibiting a positive correlation with the concentration.

At the onset of the experiment (12-24 hours post exposure), behavioural changes in fishes were rather rapid (Table 1). There was an immediate burst of activity (hyperactivity) in all the toxicant-exposed groups. This was characterized by abnormal or agitated/erratic swimming, colliding and hitting of tails against wall of the aquarium, sudden or quick movements as well

as general restlessness. With progression of exposure time (48-72 hours post exposure), activity of fish in the exposed groups decreased (hyperactivity). There was air gulping (rapid opercular movement), loss of balance or equilibrium characterized by fish swimming backwards or in circles, free fall, as well as vertical positioning. Fish exhibited startle or panic responses to stimulus with sudden darts of energy and holding out their pelvic and pectoral fins. At the time of quiescence, fish exhibited highly reduced activity during which they remained vertically still with much reduced faint and irregular opercular beats. This period was followed by death. However, no abnormal changes were observed in the control experiment throughout the exposure duration.

Table 1: Behavioural response of *Clarias gariepinus* juvenile exposed to acute concentrations of Lambda cyhalothrin

		EXPOSURE DURATION			
Behavioural changes	Concentration (mgL-1)	24HRS	48HRS	72HRS	96HRS
Air Gulping	Control	-	-	-	-
	0.25	+	+	+	
	0.50	+	++	+	++
	1.00	++	+	+	+++
Hyperactivity	Control	-	-	-	-
	0.25	-	++	+	+
	0.50	-	+++	++	+++
	1.00	+++	+++	+	+++
Erratic movement	Control	-	-	-	-
	0.25	+	+	+	+
	0.50	++	++	+	++
	1.00	++	++	+	+++
Skin Discoloration	Control	-	-	-	-
	0.25	++	+	++	+
	0.50	++	+	+	++
	1.00	++	++	+	+
Jerky movements	Control	-	-	-	-
	0.25	+	+	+	+
	0.50	+	++	+	+
	1.00	++	+	-	+++
Equilibrium Status	Control	-	-	-	-
	0.25	+	+	++	+
	0.50	+	++	+	+
	1.00	++	++	+	+++

Keys:

None - Moderate ++ Strong +++

Mild +

3.2 Physicochemical Parameters after Exposure to Lambda cyhalothrin.

Mean values of the water quality assay of both acute and long-term recorded during the exposure of *C. gariepinus* to Lambda cyhalothrin are presented in Table 2. The result showed that dissolved oxygen ranged from5.5 to 7.50 mgL^{-1}, temperature 27.50-28.00°C, pH 7.7-8.9 alkalinity 15.00-18.20 mg L^{-1}, while free carbon dioxide ranged from 4.2 -4.32 mg L^{-1} for acute test. In long term sub-lethal concentration test, dissolved oxygen ranged from 6.8 to 8.50 mg L^{-1}, temperature 27.50-28.00 °C, pH 7.8-9.1 alkalinity 18.00-22.20 mg L^{-1}, while free carbon dioxide ranged from 4.68-4.98 mgL^{-1}. Statistical analysis indicated that there was no significant difference ($P > 0.05$) in water quality parameters between the exposed tanks and the control.

Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 10, Issue 3, pp: (15-25), Month: May - June 2023, Available at: www.noveltyjournals.com

Table 2: Mean values of physico-chemical parameter recorded during the exposure period

Parameter	Control		Acute test		Long-term exposure	
	Mean	Range	Mean	Range	Mean	Range
DO (mg L^{-1})	7.00	6.1-7.9	6.5	5.5-7.50	6.85	6.0-7.9
Temp (°C)	27	27.0	26.25	27.5-28	28.25	27.5-29
pH	8.50	7.8-9.2	8.30	7.7-8.90	8.45	7.8-9.1
Alkalinity.(mgL^{-1})	17.85	16.2-19.5	17.1	16-18.20	17.75	16.0-19.5
CO_2 (mg L^{-1})	4.20	4.1-4.30	4.29	4.25-4.32	4.23	4.20-4.25

TABLE 3: Mean mortality and Probit values of *Clarias gariepinus* exposed to various concentrations of Lambda cyhalothrin.

Lambda Cyhalothrin (mgL^{-1})	Log Concentration	Mean Mortality (%)	Probit values
0.00	0.00	0	0
1.25	0.09	0	0
1.50	0.18	10	3.72
3.00	0.48	25	4.33
6.00	0.78	90	6.28
12.00	1.08	100	8.72

At 12.00 and 1.25 mgL^{-1} of Lambda cyhalothrin, 100 and 10% mortalities respectively, were observed in exposed fish while no mortality was recorded in the control group (Table 4).The 96 h LC_{50} of Lambda cyhalothrin to the exposed fish was found to be 3.98 mg L^{-1}.

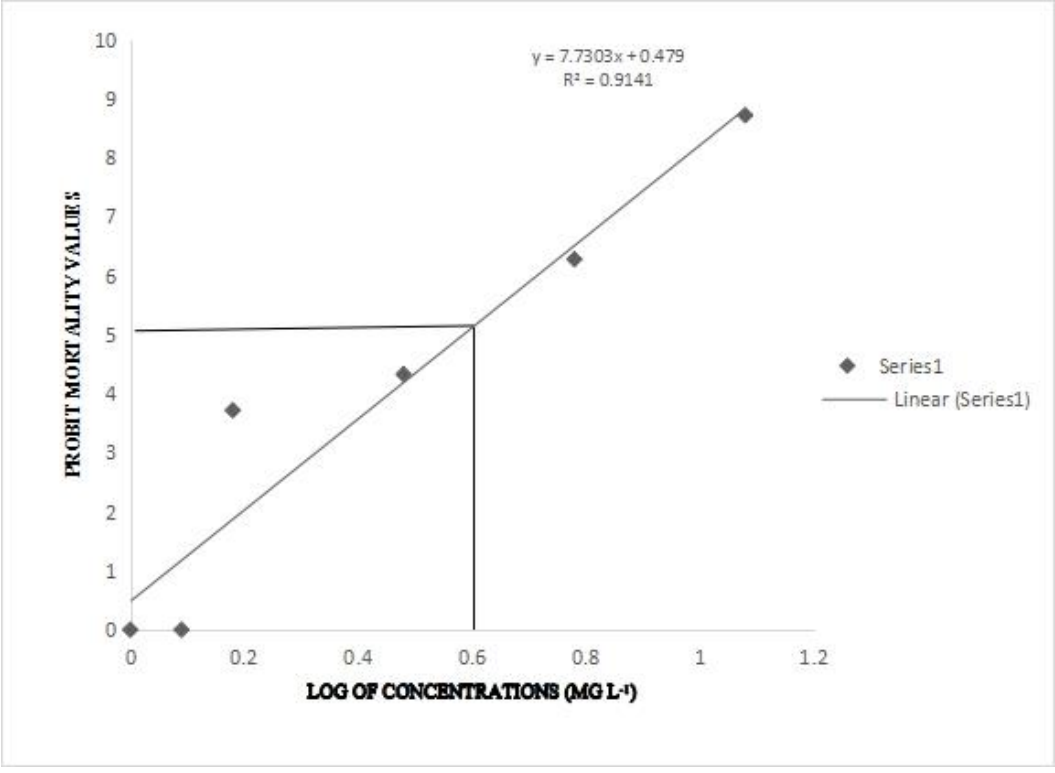

Fig. 2: Logarithmic concentration–probit line for determination of 96 hrs LC_{50} of Lambda cyhalothrin to C. gariepinus.

Novelty Journals

ISSN 2394-966X

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3.3 Effect of Lambda cyhalothrin on the Oxidative Stress Parameters of *Clarias gariepinus*

From the result of the oxidative indices (Table 4), it was observed that there was significant difference (P<0.5) in the oxidative parameters analysed. There was increase in the mean values of GPx and GR with increase in the concentration of the toxicant than in the control. The highest value recorded for GPx was 10.42±0.00 in the concentrations of 1.25 mgL^{-1} on the 5th day and 1.75 mgL^{-1} on the 15th day while the lowest value recorded was 8.54±0.04. There was also significant increase in GR compared to the control with the highest mean value (18.35±0.00) recorded on the 10th Day at 1.50 mgL^{-1} concentration with the lowest values recorded as 8.54±0.04. Unlike the values recorded for GPx and GR, there was significant decrease in the values of the SOD and Catalase while LPO showed no significant difference at P<0.5 with increase in concentrations of the toxicant compared to the control. The SOD was recorded to have a significant increase (12.11±0.04) on Day 15 at 1.75 mgL^{-1} and the lowest value record was 3.98±0.44. There was a decrease in value of SOD as concentrations increased. There was a drastic increase in MDA (8.42±0.00) on the 10th day at 1.75 mgL^{-1} concentration.

Table 4: Oxidative parameters indicator of *Clarias garipienus* to Sub-lethal Concentrations of Lambda cyhalothrin

Parameter	Concentrations (mg^{-1})	Exposure Duration (Days)		
		Day 5	Day 10	Day15
GPx (nmol min^{-1}mg $protein^{-1}$)	Control	9.9 ± 0.46^{2b}	7.11 ± 4.01^{1b}	10.36 ± 0.00^{3b}
	1.25	10.42 ± 0.00^{3d}	8.54 ± 0.04^{1c}	9.24 ± 0.00^{2b}
	1.50	9.28 ± 0.00^{2d}	8.99 ± 0.00^{1d}	10.26 ± 0.00^{3b}
	1.75	10.12 ± 0.00^{2c}	6.32 ± 0.00^{1a}	10.420 ± 0.00^{3c}
GR	Control	13.98 ± 0.54^{3c}	11.36 ± 4.37^{1a}	13.39 ± 0.00^{3c}
	1.25	14.26 ± 0.00^{1a}	16.34 ± 4.04^{3b}	14.26 ± 0.00^{2b}
	1.50	13.19 ± 0.00^{1}a	18.35 ± 0.00^{3c}	15.64 ± 0.00^{2c}
	1.75	15.76 ± 0.482^{2c}	20.48 ± 5.82^{3d}	14.36 ± 0.00^{2c}
MDA	Control	3.59 ± 0.00^{2d}	10.40 ± 5.80^{3d}	1.36 ± 0.00^{1a}
	1.25	2.41 ± 0.00^{2b}	4.59 ± 0.00^{3a}	1.38 ± 0.00^{1b}
	1.50	2.32 ± 0.00^{2a}	5.29 ± 0.00^{3b}	1.37 ± 0.00^{1a}
	1.75	0.37 ± 0.05^{1a}	8.42 ± 0.00^{3c}	2.19 ± 0.00^{1c}
CAT	Control	0.42 ± 0.00^{1b}	0.41 ± 0.24^{1b}	0.72 ± 0.03^{3b}
(mmol min^{-1}mg $protein^{-1}$)	1.25	0.35 ± 0.00^{2a}	0.39 ± 0.00^{1b}	0.88 ± 0.00^{3c}
	1.50	0.42 ± 0.00^{2b}	0.16 ± 0.00^{1a}	0.69 ± 0.00^{3a}
	1.75	0.18 ± 0.62^{1d}	0.13 ± 0.00^{1a}	0.67 ± 0.00^{3a}
SOD	Control	9.58 ± 0.29^{2c}	13.05 ± 4.03^{3d}	11.14 ± 0.51^{2b}
(µmg $protein^{-1}$)	1.25	8.46 ± 0.00^{2a}	8.32 ± 0.00^{1c}	11.24 ± 0.00^{3b}
	1.50	9.12 ± 0.00^{2b}	7.34 ± 0.00^{1b}	10.24 ± 0.00^{2a}
	1.75	3.98 ± 0.44^{2b}	6.26 ± 0.00^{1a}	12.11 ± 0.04^{3d}
LPO	Control	6.24 ± 0.29^{3c}	5.41 ± 1.06^{1b}	5.39 ± 0.00^{1b}
(nmol TBARS mg $protein^{-1}$)	1.25 mgL^{-1}	6.34 ± 0.00^{3c}	4.25 ± 0.00^{1a}	5.66 ± 0.00^{2b}
	1.50 mgL^{-1}	5.42 ± 0.00^{2a}	6.22 ± 0.00^{2c}	5.33 ± 0.00^{1b}
	1.75 mgL^{-1}	9.9 ± 0.46^{2b}	4.28 ± 0.00^{1a}	5.22 ± 0.00^{2a}

Mean with different alphabetic letters show significance difference (p<0.05) among Lambda cyhalothrin concentrations within the rows while different numeric superscripts indicate significant difference among durations of exposure within the horizontal as determined by Duncan's multiple Range.

Bioaccumulation of toxic compounds in fish together with environmental stress can invoke the production of excess ROS commonly known as free radicals- such as superoxide anion (O^{-2}), hydrogen peroxide (H_2O_2), the hydroxyl radical (OH^-) and can cause physiological alterations, oxidative dysfunction such as lipid peroxidation (Islas-Flores *et al.*, 2013; Woo *et al.*, 2006). Fish antioxidant responses are very sensitive to environmental contamination and frequently used in aquatic environmental health monitoring (Sturve *et al.*, 2008).

4. DISCUSSION

The physico-chemical parameters of the test water measured during both acute and sub-lethal toxicity bioassay were within suitable ranges for the survival and normal growth of *C. gariepinus*. Hence changes in fish behaviour and subsequently death could not have arisen from poor water quality of the test water. On the optimum pH scale for fish growth developed by Badiru (2005), the range of pH for this study (7.8-9.26) corresponds to the desirable range (6.5-9) for fish production. However, dissolved oxygen range for this study (6.1-9.5 mg/L) spans the range for slow growth following long term exposure (15mg/L) of the dissolved oxygen scale for warm water fishes by Badiru (2005). Similarly, the temperature range for this study (16.4-20°C) is within the normal range of temperature in the tropics to which fish are adapted (22-35°C) as reported by Howerton (2001).

Changes in behaviour observed in this study are similar to those reported by several authors. Hyperactivity of fish in exposed groups during 12-24 hours could be attributed to an attempt to escape the toxic environment. Hyperactivity of fish on introduction to an unfavourable environment has been suggested as the primary and principal sign of nervous system failure due to pesticide poisoning which affects physiological and biochemical activities. Ramesh *et al.*, (2009) reported similar behavioural responses of common carp to atrazine exposure which include increased opercula movement, mucous secretion, jerky movement, floating on the sides and hypersensitivity showing violent erratic and fast swimming, and opined that the abnormal behaviour of the fish indicates the toxic effect of Lambda cyhalothrin on the central nerves system (CNS) and cardiovascular system. Mekkawy *et al.*, (2013) also reported hyperactivity in *C. gariepinus* exposed to atrazine which was characterized by rapid and erratic swimming or darting, partial loss of equilibrium, rapid pectoral fins and opercula movements, reduction in the feeding activity, fins haemorrhage and loss of some skin parts. In the course of metribuzin poisoning in rainbow trout, Velisek *et al*., (2008) reported similar clinical symptoms such as accelerated respiration, loss of movement coordination, fish lying on their flanks and moving in this position.

Swollen abdomen and discolouration of the skin were also observed in fish exposed to the toxicant. This is attributable to necrotic damage to the gut of fish and suggests that toxicity of both pesticides is not restricted only to the outside. Annune *et al*. (1994) and Olusegun (2001) reported similar findings. Nwani *et al.* (2013) also reported skin discolouration in *Tilapia zilli* exposed to the chloroacetanilide herbicide butachlor. Ikele *et al.* (2011) similarly observed that the *C. gariepinus* normal darkly pigmentation in the dorsal and lateral parts was changed to very light pigmentation when exposed to diethyl phthalate. Generally, it is argued that behavioural studies gives a direct picture of response of the fish to pesticides and related chemicals and the behavioural activity as well as morphological responses of organisms represents the final integrated result of a diversified biochemical and physiological processes

The present study demonstrated that C. gariepinus exposed to lambda-cyhalothrin showed concentration and duration dependent on significant increases ($P< 0.05$) in radical activities. Oxidative stress is mechanism for toxicity leading to cell death and disturbance of the physiological processes in fish (Banaee, 2013). It is related to reactive oxygen species production and can occur when the antioxidant and detoxifying systems are deficient not able to neutralize the active intermediates that are produced by xenobiotics and their metabolites. Changes in the levels of antioxidant enzyme activities could be used as biomarkers in different aquatic organisms (Orbea, Ortiz-Zarragoitia, Sole´, Porte, and Cajaravile, 2002). It was observed that the oxidative stress parameter results for Lambda cyhalothrin showed some fluctuations though they both exhibited similar trends. This fluctuation corroborates with the findings on increase in antioxidant activities in several fishes exposed to herbicides (Elias *et al.*, 2002; Faronbi, *et al.*, 2008; Kadry, *et al.*, 2012).

Oxidative stress in aquatic organism is induced by many chemical pollutants which may stimulate the production of reactive oxygen species and oxygen free radicals that can lead to alteration in antioxidant systems (Kadry, *et al*., 2012). In this experiment, the exposure of fish to Lambda cyhalothrin showed that there were significant ($p<0.05$) elevation in GPx and GR activity throughout the duration of the experiment when compared to control. This is in line with the reviewed reports on the effect of pesticides on oxidative activities of *Clarias gariepinus* (Elias *et al.*, 2002; Faronbi, *et al.*, 2008). GPx enzyme plays important role in protection of animals against oxidative dysfunction by reducing lipid hydro peroxides to alcohols (Velma and Tchounwou, 2010).

Decreased hepatic reduced glutathione (GSH) production results to oxidative stress and hepatocellular necrosis (Messiha and Abo-Youssef, 2015). Unlike GPx and GR, there was no evident change in MDA for the fish exposed to Lambda cyhalothrin. This contradicts the reports of Kadry, *et al.* (2012) who reported an increase in all oxidative indices of *Clarias*

gariepinus exposed to sub-lethal doses of pesticide. In vertebrates, superoxide dismutase is one of the most important antioxidant enzymes that detoxify superoxide anion radical (O_2^-) while catalase (CAT) reduces hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2). Thus CAT and SOD provide the first line of defense against stress. Both enzymes (CAT and SOD) are inducible and may have been produced in response to the toxicity of Lambda cyhalothrin. Decrease in catalase activity was observed in this study in the Lambda cyhalothrin. Activities of anti-oxidant enzymes of *C. gariepinus* indicated a significant dose-dependent increase in catalase as well as superoxide dismutase activities and the highest activity was recorded in the fish exposed to the highest concentration of the toxicant. Antioxidant enzyme activities have been used as an early warning sign of environmental pollution (Rosety *et al.*, 2005). In vertebrates, superoxide dismutase is one of the most important antioxidant enzymes that detoxify superoxide anion radical (O_2^-) while catalase (CAT) reduces hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2). Thus CAT and SOD provide the first line of defence against stress. Both enzymes (CAT and SOD) are inducible and may have been produced in response to the toxicity of Lambda cyhalothrin. Increase in catalase activity observed in this study could be attributed to the production of hydrogen peroxide (H_2O_2). As catalase is responsible for its detoxification to water and oxygen, more activity of catalase is observed. Superoxide dismutase catalyses the dismutation of the superoxide anion radical to water and hydrogen peroxide, which is detoxified by catalase. The increase in superoxide dismutase activity after Lambda cyhalothrin administration appears to be an adaptive response to increased generation of reactive oxygen species. Elevated SOD activity indicates increased production of superoxide anion and the enzyme responsible for its metabolism (Zheng *et al.*, 2009; Chanu *et al.*, 2013).

Ullah *et al.* (2014) had reported elevated levels of catalase activity in muscles and brain of *Tor putitora* exposed to cypermethrin. Nwani *et al.* (2014) also reported elevated levels of catalase activity in primextra exposed *C. gariepinus*, with 4.80%–23.76% and 6.20%–37.81% increase in the muscle and liver tissues respectively at different exposure levels. Mirvaghefi *et al.* (2015) also observed that catalase activity was significantly higher in diazinon-exposed groups compared to the control group of Rainbow trout. Topal *et al.* (2015) reported a reduction in the activity of CAT in rainbow trout on exposure to nickel chloride.

Manjunatha *et al.* (2015) reported increased levels of catalase and superoxide dismutase in *Labeo rohita* during atrazine exposure. Kadry *et al.* (2012) similarly reported that atrazine exposure also led to a significant increase in the activities of catalase (CAT) and superoxide dismutase (SOD) in *C. gariepinus*. Reduction in the activity of CAT may be a result of influx of superoxide radicals which resulted in elevation of H_2O_2 production in the cell that consequently decreased CAT activity in the tissues (Rhee *et al.*, 2010).

Nwani *et al.*, (2010) also reported a concentration-dependent increase in the antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) thus suggesting the use of these antioxidants as potential biomarkers of toxicity associated with contamination in freshwater fishes.

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