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The Effect of *Lambda cyhalothrin* on Transferases, Urea and Creatinine in Organs of *Parohiocephalus obscurus,* a Common Niger Delta Wetland Fish

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Abstract

The aim of this study was to unveil the effects of lambda cyhalothrin on transferase, urea and creatinine in *Parophiocephalus obscurus*. Thirty adult fish (mean length, 15.50 ±0.2cm) were acclimatized to laboratory condition for 10 days and then exposed to varying sublethal concentration of the toxicant (0.012, 0.024, 0.036mg/l) in semi static bioassay for 14 days. Tranferases (aspartate amino tansferase (AST), alanine amino transferase (ALT) were determined in the liver and muscle while creatinine and urea were determined in the muscle and kidney. Transferase values were significant ($p<0.05$) in the organs tested. A dose dependent decrease was recorded in the liver AST and muscle ALT while a dose dependent increase was recorded in the liver ALT and muscle AST. Muscle and kidney urea were not significant unlike creatinine in the muscle and kidney. The enzymes tested could be more useful biomarkers of sublethal effect of lambda cyhalotrin on *Parophiocephalus obscurus*. The results clearly unveiled the potential effect of lambda cyhalothrin

Keywords: Aspartate amino transferase, Alanine amino transferase, Lambda cyhalothrin, Urea, creatinine

1 Introduction

Effect of pesticide on aquatic environment has gained much attention all round the world in the last few decades as they are posing a serious threat to non targeted organisms [1, 2]. Pesticides enter into the aquatic ecosystem through various routes affecting adversely to the aquatic biota [3-5. Pesticides and related chemicals destroy the delicate balance between species that characterized a functioning ecosystem [6]. Pesticides produce many physiological and biochemical changes in fresh water organisms by influencing the activities of several enzymes [6].

Lambda cyhalothrin is a pyrethroid insecticide [1, 7]. Pyrethroids are synthetic chemical analogues of pyrethins which are naturally occurring insecticidal compounds produced in the flowers of *Cyrysanthemum cinerariaefolum.* The three major group of pyrethroid insecticides are permethrin, cypermethrin, and bifenthrin [8]. Insecticidal products containing pyrethroids have been widely used to control insect pest in agriculture, public health, homes and gardens [9]. In agriculture, target crops include cotton, cereals, ornamentals potatoes, and vegetables [9]. Various researchers have reported the effect of cyhalothrin on aquatic organisms [10, 11]. Residues of lambda cyhalothrin have been detected in irrigation and storm water run-off resulting from agricultural, public health and residential applications [10]. Lambda cyhalothrin is toxic to aquatic organisms including fish and amphipods [1, 7, 8].

Fishes are very sensitive to a wide variety of toxicants in water, various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides [12]. Due to accumulation of pesticides in tissue, many physiological and biochemical changes occurs in fish and other aquatic organisms as a result it influence on enzymes and metabolites [13 – 15].

This present research is targeted at identifying the effect of cyhalothrin on some metabolites and enzymes of *Parophiocephalus obscurus*, a common Niger Delta wetland fish.

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2 Materials and Methods

2.1 Experimental stock

Thirty adult *Parophiocephalus obscurus*, mean weight 42.20±0.15gSD and mean length 15.50 ±0.02cmSD were obtained from a private farm at Tombia town, Yenagoa, Bayelsa State. They were transported in 20 liter trough to

the wet laboratory (Ecotoxicology unit) of the Department of Biological Sciences, Niger Delta University, where the assays were conducted from October to December, 2015. Fishes were acclimatized individually in a rectangular aquaria for 14 days during which they were fed once a day 9.00 – 11.00hr) with 35% crude protein at 1% biomass.

2.2 Experimental design

Completely randomized design (CRD) was used for the experiment. There were four treatment levels with three replicates. A range finding test (trial test) was carried out using the toxicant lambda cyhalothrin. Four concentrations of the toxicant were prepared from the original solution (25g/l). The test solution was renewed daily and it lasted for 14 days.

2.3 General Bioassay technique

Sublethal concentrations of lambda cyhalothrin for the assay (0.012, 0.024 and 0.036 mg/l) were determined based on the range finding test [14]. These were prepared by transferring 0.01, 0.02 and 0.03mls of the original concentration of the toxicant and making it up 30L with borehole water in the test aquaria. 30L of the diluent (water) was used as control. Fishes were introduced individually into each aquarium. The exposure period lasted for 14 days during which the media were renewed daily. The physico-chemical characterization of the water used for fish bioassay was carried out using standard methods of APHA [16] and the following values were obtained: temperature 26^{o}C, pH 6.16 – 6.36, dissolve oxygen 5.40-7.30mg/l, alkalinity 15.30-16.39mg/l, conductivity 98.51 – 138.08µs/cm, turbidity 0.42 – 0.53NTU.

2.4 Biochemical and Metabolites assay technique

After the 14 days exposure period, fishes were sacrificed and dissected for the collection of liver, muscle and kidney. A quantity (0.5g) of each of the organs was macerated with pestle and mortar and 5mls of perchloric acid was added for stabilization (metabolite samples). While samples for enzymes analysis, 5mls of physiological saline was added. After addition of these diluents, the samples were centrifuged at the rate of 3,000rpm for 15 minutes. The supernatant were then removed and stored in plain bottles at -20^{o}C for analysis [17]. The activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were assayed using the Colorimetric method of Reitman and Frankel [18] while the activities of metabolites (urea and creatinine) were determined via Colorimetric methods of Weatherburn [19] and Sehismeister *et al.* [20] respectively.

2.5 Data analysis

The data analysis was subjected to analysis of variance (ANOVA). Where difference exist, Ducan multiple range test (DMRT) was used to test for pair wise significant differences (P<0.05) between treatments.

3 Results and Discussion

3.1 Enzymes

The liver AST values were significant (p<0.05). A dose dependent decrease in values were recorded (Table 1) as the concentration of the toxicant increases, while the liver ALT values recorded a sudden increase at 0.012mg/l and 0.024mg/l and a drop in values at 0.036mg/l (the highest concentration). Muscle AST values increased as the concentration of lambda cyhalothrin increases (not a dose dependent pattern) while a dose dependent decrease characterizes the levels of muscle ALT values (Table 1)

3.2 Metabolites

Muscle urea values were not significant (p<0.05). A slight increase in values were recorded as the concentration of the toxicant increases while muscle creatinine values were significant (not in a dose dependent pattern). The highest value was recorded at 0.02mg/l (363.00±2.10µmol/l). Kidney) Kidney urea values not significant, albeit a slight decrease in values were observed while creatinine values decreases compared to the control (Table 2). The first concentration (0.012mg/l) had the highest value (4.00±0.00mg/dm) while the control recorded 32.00±0.0mg/dm.

3.3 Muscle Urea and Creatinine

Metabolic parameters such as creatinine and urea can be used as a biomarker for assaying the physiological function of muscle and kidney [14]. Muscles are responsible for the generation of mobility as well as providing shape and structural support in fishes; hence any distortion in the metabolic composition of the muscle leading to a reduction in energy generation due to protein reduction may be detrimental to the exposed fishes. The values of urea (Table 2) increased as the concentration of cyhalothrin increases except at 0.012mg/l (the least concentration). The elevation in values may possibly indicate that the toxicant is lethal to the organs responsible for the regulation of urea levels in the probe organism. Again, the increased in values in the muscle compared to the control may possibly indicate high level of energy required by the muscles of the fish to maintain their physiological functions due to introduction of the xenobiotic (cyhalothrin) into the system. The high amount of waste generated (urea) is produced in response to high metabolic process taking place in the muscle cell due to the toxicant effect [21].

Creatinine is a nitrogenous waste product formed from the metabolism of creatine in the skeletal muscle. It diffuses freely throughout the body fluid (water). It is filtered from the extracellular fluid by the kidney and excreted in the urine [22]. Creatinine and adenosine triphosphate (ATP) are absolutely involved in contractile process in skeletal muscle mediated by the enzyme creatine kinase, the low values obtained in the muscle is a clear evidence that creatinine metabolism was high in the skeletal muscle as a result of the effect of cyhalothrin on the muscle fibres [23].

Table 1: AST and ALT in the liver and muscle of *Parophiocephalus obscurus* exposed to lambda cyhalothrin for 14 days.

Con. of lambda cyhalothrin (mg/l)	Liver		Muscle	
	AST (µ/l)	ALT(µ/l)	AST (µ/l)	ALT(µ/l)
0.000	339.50±9.01[a]	44.00±0.32[b]	171.20±7.30[c]	51.00±0.30[a]
0.012	266.10±10.0[b]	63.23±1.10[a]	204.50±6.10[b]	32.21±0.63[b]
0.024	254.25±7.31[b]	57.11±0.30[a]	314.12±4.93[a]	27.19±0.06[b]
0.036	180.00±3.80[c]	25.00±0.03[c]	289.00±3.60[ab]	27.00±0.06[b]

Means within with different superscript are significantly different ($p<0.05$)

Table 2: Urea and creatinine in the muscle and kidney of *Parophiocephalus obscurus* exposed to lambda cyhalothrin for 14 days

Con. of lambda cyhalothrin (mg/l)	Muscle		Kidney	
	Urea (µmol/l)	Creatinine (mg/dm)	Urea (µmol/l)	Creatinine (mg/dm)
0.000	1.10±0.00[a]	163.00±0.09[c]	0.50±0.00[a]	32.00±0.02[a]
0.012	0.80±0.01[a]	363.00±2.10[a]	0.15±0.00[a]	4.00±0.00[d]
0.024	1.25±0.00[a]	204.50±4.03[b]	0.2±0.01[a]	10.50±0.01[c]
0.036	1.80±0.02[a]	224.00±6.11[b]	0.20±0.01[a]	22.00±0.02[b]

Means within column with different superscript are significantly different ($p<0.05$)

3.4 Kidney Urea and Creatinine

A clear decrease in concentration of urea and creatinine were recorded compared to control. The presence of increasing concentration of urea and creatinine as reasoned by Calbreath [24] suggested the inability of the kidney to excrete these products, which further indicated decrease in glomerula filtration rate. A decreased concentrations of these metabolites suggested that the kidney may not be affected by the toxicant. Similar result was also obtained by Inyang (2008) when he exposed *Clarias gariepinus* to diazinon (an organophosphate insecticide).

3.5 Transferases (AST and ALT)

AST and ALT test are part of standard laboratory tests to detect health abnormalities in organisms [25, 26]. Analysis of these enzymes can provide important information about the internal environment of organisms [27]. Alterations in transferases in fish have been reported Inyang *et al.*[15], Samanta *et al.* [28], Inyang [29]). Elevated levels of AST in the muscle indicate the enhanced transformation of amino acids in the muscle, which may provide keto acids to serve as precursor in the synthesis of essential organic elements [30]. Enhanced AST in fish was also reported by Inyang *et al.* [15] when they exposed *Clarias lazera* to deltamethrin.

Decreased values of AST in liver may be as a result of inhibition nature of the toxicant in the enzyme. According to Karmen *et al.* [31], the reduction in activities of transaminases in the liver may be attributed to interference of the pesticide (cyhalothrin) with protein metabolism in the hepatic cells or inhibition of the enzymes. The authors added that the decrease in values may be an offshoot of leakage of the enzymes from the liver into the serum.

Alanine amino transferase (ALT) plays a vital role in the synthesis and deamination of amino acids during stress imposed conditions for meeting the high energy demand of organisms. Liver ALT increased at 0.012 and 0.024mg/l then dropped at 0.036mg/l (the highest concentration). Increased activity of ALT in the liver in the present study indicated tissue damage which may be due to disturbance in normal physiological and biochemical processes such as kreb's cycle, TCA cycle and subsequent leakage of this enzymes from the liver cytosol via membrane into the blood stream [28]. The present result was in agreement with the results of Jee *et al.* [32] when they exposed Koren rock fish, *sebastes schlegeli* to cypermethrin. Muscle ALT decreases down the experimental group as the concentration of the toxicant (cyhalothrin) increased. The reduction in the activities in the muscle is an indication of stress imposed by the toxicant and inhibition.

4 Conclusion

Exposure of fish for a long time will surely pose a problem to their health; hence humans are at risk if these intoxicated fish find their way into the food chain. Several reports have implicated pesticides as toxicants capable of altering metabolic and biochemical indices in fishes. This present research unveiled the effects of lambda cyhalothrin on *Parophiocephalus obscurus* (a common Niger Delta wet land fish). These parameters could serve as a biomarker for evaluation of sublethal effect of this toxicant on the organism. Again further research should be carried out to determine the safe dose for all stages of the probe organism (fingerlings, post fingerlings, juveniles and adult).

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