



Evaluation of Activities of Transferases and Phosphatase in Plasma and Organs of *Clarias gariepinus* Exposed to Fluazifop-p-Butyl

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Abstract

The aim of this study was to evaluate the activities of transferases and phosphatase in plasma, liver and kidney of *Clarias gariepinus*. This study was carried out in the department of fisheries and animal science, Niger Delta University, Wilberforce Island, Bayelsa State, between September, 2014 and April 2015. Adult *Clarias gariepinus* were exposed in four replicates to varying concentrations of fluazifop-p-butyl (ranging from 0.01 -0.03ppm) in a 30 day semi static bioassay. Samples were obtained from the liver, kidney and plasma. A statistically significant increase ($p<0.05$) was recorded in the plasma. Liver alkaline amino transferase (ALT) and Alkaline phosphatase (ALP) unveiled a significance decrease, while liver aspartate amino transferase (AST) showed a clear progressive increase compared to the control. Kidney enzyme values were significant. A progressive decrease in value were recorded (not in a dose dependent pattern). This toxicant could be toxic at high concentration. These parameters could serve as useful biomarkers of sublethal effect of fluazifop-p-butyl in non-target organism in the aquatic environment.

Keywords: Fluazifop-p-butyl, *Clarias gariepinus*, Plasma enzymes, Fish bioassay.

1 Introduction

Pollution via pesticides is one of the major problems in the aquatic environment. Unlike the early 1970's, pesticide production is fast increasing because of its exigency in agriculture and anthropogenic purposes. However, pesticides and related chemicals that destroy the delicate balance between species that characterizes a functioning ecosystem [1]. Pesticides produce many physiological and biochemical changes in aquatic organisms by influencing the activities of several enzymes [1].

These pesticides, even when applied in restricted areas are washed and carried away by rains and flood to large water bodies like ponds and rivers. The offshoot will lead to alteration of physiological properties of water [2]. Pesticides are toxic, not only to fishes but also to other organisms which form food of the fishes [3]. Fishes are very sensitive to a wide variety of toxicants in water, various species of fish show uptake and accumulation of many contaminatants or toxicants such as pesticides [4].

Fluaxifop-p-butyl is a post emergence phenoxy herbicide, it is absorbed rapidly via leaf surfaces and quickly hydrolysis to fluazifop acid. The acid is transported primarily in the phloem and accumulates in the meristems

where it disrupts the synthesis of lipids in susceptible species [5,6]. Fluazifop-p-butyl inhibit acetyl CoA Carboxylase, an enzyme that catalyses an early step in fatty acid synthesis in organisms. This xenobiotics can pass readily into fish tissue, and is highly toxic to fish and other aquatic species including invertebrates [7].

The effect of xenobiotic contamination in an ecosystem can be estimated through analysis of biochemical changes in organism inhabiting that polluted environment [8, 9, 10]. The biochemical response of aquatic organism to pollution is given by changes in several key enzymes, especially those of biotransformation systems. The value of tissue enzyme activities in the diagnosis of the effect of pollutant is an emerging area in aquatic toxicology and remediation programmes [11].

In this study, the native Nigerian fish species *Clarias gariepinus* was chosen as an assay organism due to its hardy nature and being the most cultured fish species in Nigeria [12]. The goal was to access an effect of fluazifop-p-butyl on enzymes in plasma, liver and kidney of *Clarias gariepinus* (a widely cultured fish in Nigeria). This parameter was chosen because of its role in fish metabolism and general physiology.

2 Materials and Methods

2.1 Experimental stock

Fish samples for this study were obtained from biotechnology resource centre, Odi, Bayelsa State, Nigeria. They were transported to the wet laboratory of the

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department of fisheries and animal science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, where the assays were conducted. Thirty (30) healthy adult *Clarias gariepinus* with mean length 16.04 ± 0.23 cm and mean weight 94.04 ± 0.6 g were acclimatized individually in a rectangular aquaria for seven days. Fish were fed once a day at 10.00 – 11.00hrs with 35% crude protein.

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 fluzifop-p-butyl. Four (4) concentrations of the toxicant were prepared from the original (150g/l).

2.3 Test chemical

Sublethal concentrations of fluzifop-p-butyl for the assay (0.01, 0.02 and 0.03ppm) was determined based on the range finding test [13]. These were prepared by transferring 0.02, 0.04, 0.06mls respectively from the original concentration (150g/l) of the toxicant and making it up with borehole water in the test aquaria, 30L of the diluents was used as control.

2.4 General bioassay

Four replications of each treatment level (concentration) and control were set up by introducing fishes individually into each aquarium. The exposed media were renewed every 48hours, the physiochemical characterization of the water used for the bioassay was carried out using standard methods of APHA [14] and the following values were obtained: Temperature $24.05 - 24.20^\circ\text{C}$, pH $6.15 - 6.24$, Conductivity $97.43 - 135.02\mu\text{cm}$, Alkalinity, $11.31 - 17.13\text{mg/l}$, Dissolved Oxygen, $5.21 - 7.13\text{mg/l}$, and Turbidity, $103 - 146\text{NTU}$. The activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) in plasma and organs were assayed using the colorimetric method of Reitman and Frankel [15] while Kind and King [16] method was used for analysis of alkaline phosphatase (ALP).

2.5 Statistical analysis

Data were expressed as mean \pm standard error. The data were subjected to analysis of variance (ANOVA) and Duncan multiple range test (DMRT) were used to test for pair wise significant differences ($p < 0.05$) between treatments.

3 Results

3.1 Plasma enzymes

Table 1 unveiled the result of enzyme parameters of *Clarias gariepinus* exposed to fluzifop-p-butyl. ALT values were statistically significant ($p < 0.05$). A sudden rise in value at 0.01ppm was recorded and subsequent drop in values, albeit higher than the control value. AST values recorded was akin to ALT (fluctuation in values), while ALP values recorded unveiled a significant drop in values and significant rise in values at 0.02 and 0.03ppm (Table 1).

3.2 Liver enzymes

A statistically significant ($p < 0.05$) values were obtained in liver ALT (Table 2). Values decreased as the concentration of the toxicant increases in a dose dependent pattern while AST values increased as the concentration of the toxicant increased except at 0.03ppm (albeit, still higher than the control value). Liver ALP shows a clear elevation of values at 0.01ppm compared to the control and then a sudden drop in values as the concentration of the toxicant increases (Table 2).

3.2 Kidney enzymes

An obvious fluctuation in values were recorded in kidney ALT, albeit experimental values were slightly lower than the control while AST values decrease as the concentration of the toxicant increases in a dose dependent pattern. ALP values were statistically significant at 0.01 and 0.02ppm. The second test concentration (0.02ppm) recorded the highest value (930.00 ± 10.13) compared to the control that had 556.00 ± 8.41 .

4 Discussion

Fish species are sensitive to enzymatic and hormone disruptors. Chronic exposure to low levels of pesticides may have a more significant effect on fish population than acute poisoning [17]. Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behaviour and physiology that impair both survival and reproduction [18]. Biochemical changes induced by pesticidal stress usually lead to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism [19].

4.1 Fish plasma enzymes

Activities of enzymes in fish are essential metabolic processes [20]. We observed an increase in transferases (ALT and AST) in the plasma except AST at 0.03ppm. ALT and AST are found in heart, liver, skeletal muscle, kidney, pancreas, spleen, gill, red cells and brain tissue [21]. When disease or injury affect these tissues and the cells are destroyed, especially liver ALT and AST are released into the blood stream. The amount of ALT or AST is directly related to the number of cells affected by the disease or injury [22]. Increase in activities of these enzymes in plasma may be due to liver damage, which results in the liberation of these intracellular enzymes, hence the elevation of plasma ALT and AST level.

Experiment with common carp, *Cyprinus carpio* exposed to diazinon showed a clear elevation of values in the plasma [22]. Again exposure of Nile tilapia (*Oreochromis niloticus*) to cypermethrin also unveiled a progressive increase in plasma values [23].

A statistically significant ($p < 0.05$) levels was also recorded in ALP in the plasma. This elevation in values is caused by the toxicant, fluzifop-p-butyl. The significant elevation in the activity of ALP in the plasma is an indication of tissue damage to organs that produces the enzyme such as the liver and kidney. According to Nagat

[24], this will eventually lead to leakage of lysosomal enzyme into the cytoplasm and renal necrosis.

Table 1: Activities of ALT, AST and ALP in plasma of *Clarias gariepinus* exposed to fluazifop-p-butyl for 30 days

Conc of Fluazifop (ppm)	ALT (μ /l)	AST (μ /l)	AST (μ /l)
0.00	66.00 \pm 0.12 ^{bc}	267.00 \pm 10.01 ^c	19.50 \pm 1.02 ^c
0.01	102.00 \pm 0.68 ^a	423.00 \pm 10.08 ^a	17.00 \pm 0.09 ^{cd}
0.02	68.50 \pm 1.12 ^{bc}	331.50 \pm 7.11 ^b	20.50 \pm 0.08 ^b
0.03	70.50 \pm 1.08 ^b	240.00 \pm 4.56 ^d	25.30 \pm 0.08 ^a

Means with the same superscript within column are not significantly different ($p > 0.05$)

Table 2: Activities of ALT, AST and ALP in liver of *Clarias gariepinus* exposed to fluazifop-p-butyl for 30 days

Conc of Fluazifop (ppm)	ALT (μ /l)	AST (μ /l)	AST (μ /l)
0.00	137.00 \pm 11.10 ^a	81.10 \pm 0.99 ^c	109.00 \pm 4.00 ^b
0.01	71.00 \pm 3.92 ^b	191.00 \pm 8.43 ^b	211.50 \pm 3.05 ^a
0.02	60.10 \pm 0.07 ^{bc}	376.50 \pm 7.01 ^a	101.00 \pm 0.98 ^c
0.03	55.30 \pm 0.06 ^c	300.00 \pm 10.10 ^a	93.00 \pm 6.41 ^c

Means with the same superscript within column are not significantly different ($p > 0.05$)

Table 3: Activities of ALT, AST and ALP in kidney of *Clarias gariepinus* exposed to fluazifop-p-butyl for 30 days

Conc of Fluazifop (ppm)	ALT (μ /l)	AST (μ /l)	AST (μ /l)
0.00	13.50 \pm 1.23 ^a	248.50 \pm 6.83 ^a	556.00 \pm 8.41 ^b
0.01	11.00 \pm 0.82 ^b	182.50 \pm 3.93 ^b	304.00 \pm 5.12 ^c
0.02	6.50 \pm 0.04 ^{cd}	69.00 \pm 0.05 ^c	930.00 \pm 10.13 ^a
0.03	9.50 \pm 0.03 ^c	68.50 \pm 0.46 ^c	536.00 \pm 3.75 ^b

Means with the same superscript within column are not significantly different ($p > 0.05$)

4.2 Liver and kidney enzymes

The present research revealed a decrease in ALT and ALP values while AST recorded a steady increase except at 0.03ppm (the highest concentration). These enzymes are present in large amount in the liver and kidney of fishes, hence any injury or damage in these organs will eventually lead to a decrease in production. A decrease may also arise a result of leakage of these enzymes into the blood circulation as a result of injury sustained by the probe. Evidently, plasma enzymes in this present study recorded a steady increase, confirming this fact (Table 1). According to Kamen [25], the reduction of these enzymes in the organs may be attributed to interference of the toxicant (fluazifop-p-butyl) with protein metabolism in the hepatic cells or inhibition of the enzymes.

ALP activity is a reflection of changes in endoplasmic reticulum mass. It is also known to occur in the cell membrane and may be involved in metabolite transport [26]. Thus the decrease may denote a decrease in membrane transport of the probe organism.

5 Conclusion

This study confirmed that exposure to pesticide (fluazifop-p-butyl) can result in a significant changes in enzymes of *Clarias gariepinus*. This results indicate that this toxicant could be toxic at high concentration, therefore

further studies are required to evaluate the potential environmental risk of fluazifop-p-butyl, one of the most prevalent herbicide in the Niger Delta, Nigeria.

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