RESPONSE OF METABOLITES IN BRAIN TISSUES OF CLARIAS GARIEPINUS EXPOSED TO SUBLETHAL CONCENTRATIONS OF THE ORGANOPHOSPHATE INSECTICIDE RAIDER® (CHLORPYRIPHOS 20%EC)

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Abstract

The effect of the insecticide perfect killer on the metabolites in the brain of *Clarias gaiepinus* was investigated. The study was done to simulate the possible impact of the insecticide on fish metabolites in the brain due to consequences of flooding and erosion. The fish were exposed to different concentrations of the toxicant (0.25ml, 0.5ml and 1.0ml) in 30litres of water in plastic basins for a week. The control tank (0ml) had no toxicant. Fish were sacrificed and brain tissue samples collected for analysis of creatinine, total protein and total glycerides. Data were analysed for means and standard deviations. Analysis of variance (ANOVA) was employed at the 95% confidence level to determine the variability and similarities in the measured variables. Turkey HSD post Hoc test was thereafter used to separate means. SPSS 20.0 statistics tool kit was used to aid the analysis. The result of the Study reveal an increase in creatinine from control (0ml) to 0.25ml inclusion of the toxicant from a mean of 26.0±11.35 to 67.33±32.88 before reducing as the toxicant increased to 1.0ml. There was a significant difference ($P<0.05$) between 0.25ml and 1.0ml toxicant inclusions. There was also an increase in total protein and total glyceride from control (0ml) to 0.25ml respectively. There was no significant difference ($P>0.05$) between control and all inclusion levels of toxicant in total protein. The study shows that the insecticide perfect killer® hinders proper metabolism of *Clarias gariepinus.* Therefore care should be taken in its application, as the contamination of surface waters may affect a wide range of fishes causing damage to vital organs and even death. The prospects of biomagnification and bioaccumulation is also worrisome.

Key words: *Metabolites, creatinine, total protein, total glycerides sublethal, Clarias gariepinus, Chlorpyriphos*

1.0 INTRODUCTION

The use of pesticides, insecticides and other agrochemicals to fight pest and weeds in eliciting global attention. Proponents and opponents are divided as to the validity of its usage. While it is impossible and economically naïve to conduct commercial or large-scale agriculture without the usage of these chemicals, it is not also environmentally sustainable to do so. Oftentimes, these chemicals exceed their intended usage or purposes and affect non-target

organisms including humans. Insecticides for instance used to control soil insects and for preserving woods used for roofing from insect attack may find its way into surface and portable water systems through sewage run-off after precipitation. This is the nexus of all arguments against the continued use of these chemicals.

In low lying cities and potential flood plains like Yenagoa, this problem may be amplified as most of its land surfaces are completely inundated by water in the peak of the rainy season. Non target organisms like fish may be seriously impacted resulting in deformities, incapacitation and even death. Worse still, chemical infested fish may be consumed by humans resulting in bioaccumulation and biomagnification of these harmful chemicals. There is a global consensus therefore to monitor the effect of those chemicals on fish and other aquatic organisms.

As *Clarias gariepinus* are important fish stocks with high commercial acceptability and high consumption there is a need to simulate the effect of these chemicals on its body metabolism. This will provide us with vital information as to the safety of these chemicals and a reason to protect these fish stocks.

2.0 MATERIALS AND METHODS

2.1 Test Organism: Fish (Procurement and Transport)

Sixty (60) juveniles of *clarias gariepinus* were procured from Amassoma, market. They were transported in plastic baskets containing borehole water to the Biology laboratory of the Niger Delta University, Amassoma, Bayelsa State.

2.2 Toxicant (Chlorpyriphos 20% EC)

The organophosphorus insecticide with trade name Perfect Killerr® containing Chlorpyriphos 20% EC as active ingredient was purchased from Ekeki market within Yenagoa metropolis of Bayelsa State. The insecticide is a selectively active pesticide that is used to control termites and other soil insects in the field and crop plantation as well as in industry, furniture and buildings.

2.3 Acclimatization

In the laboratory, the fish were acclimatized in separate containers using borehole water. During the acclimatization process the fish were fed twice daily with 4mm pelletized compounded feed to satiation. The fish were acclimatized for 4 days before the commencement of the experiment.

2.4 Range finder test

Some of the fish samples were exposed in three different plastic containers to three different concentrations of the toxicant (1ml, 1.5ml and 2.0ml) in 30 litres of tap water for 96 hours in order to determine the sublethal concentrations for the definitive test. It was observed that all the fish died in the cotainers with toxicant concentrations of 1.5ml and 2.0ml respectively. Therefore the concentration of 1ml that did not produce any mortality was taken as the highest concentration in the sublethal definitive test.

2.5 Definitive test (Experimental Test)

Test concentration of 1ml, 0.5 and 0.25ml of the toxicant Perfect Killer was added to 30 litres of water in basins containing the experimental fish. Proper mixing was ensured by using a stick to stir the water. The fish were fed daily for one(1) week during this period in a static daily removal system. The fish were exposed to toxicant concentrations in replicate containers.

2.6 sample collection

In the laboratory the fish were dissected for the collection of the brain. The brain was grounded by pounding it with mortar and pestle and perchloride acid was added for stabilization. The samples were put in test tubes and centrifuged at the rate of 300 revolutions per minute for 15 minutes. The supernatant (floatable) were removed and stored in plain EDTA bottles for analysis.

2.7 Laboratory analysis

2.7.1 Total creatinine(TC)

Total creatinine was determined using the Jaffes method. In an alkaline medium, creatinine reacts with picric acid to produce a pink color, which is directly proportional to the concentration of the creatinine in the sample.

2.7.2 Total protein

Colometric determination of total protein was done based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue color complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the Protein concentration.

2.7.3 Total glyceride

Total glyceride was obtained using linearity process. The reagent is linear up to 1000mg/dl. If the concentration is greater than linearity (1000mg/dl), sample was diluted with normal saline and the assay repeated. The result was multiplied with dilution factor, mix and incubate for 5 minutes at 37°c. Measurement was done for the changes in absorbance for standard and sample against the reagent.

3.0 RESULT AND DISCUSSION

3.1 Result

The result of the study is displayed in Tables 1 and 2 and Figures 1 to 4.

Table 1 show the metabolite values of *C gariepinus* exposed to 0.25ml and 0.50ml and 1.0ml inclusion of the insecticide Chlorpyrifos The Table show that the creatinine value increased from control (0ml) to 0.25ml inclusion of the toxicant before reducing as the toxicant increased to 1.0ml. There was a significant difference ($P<0.05$) between 0.25ml inclusion and 1.0ml inclusion of toxicant, but there was no significant difference ($P>0.05$) between control and all other levels of inclusion. Also, Total protein values increased from control to 0.25ml inclusion of the toxicant before reducing below control values. There was no significant difference ($P>0.05$) between control and all inclusion levels of Chlorpyriphos. Total glyceride also rose sharply from control to 0.25ml inclusion of the toxicant before dropping sharply at inclusions of 0.5ml and 1,0ml respectively. There is no significant difference ($P>0.05$) between control and 0.50ml and 1.0ml inclusions but significant difference ($P<0.05$) between 0.25ml inclusion and control with other levels of inclusion of the toxicant'

Table 1: Mean response of metabolites with different concentrations of insecticide

S/N	CHLORPYRIFOS (ml)	CREATININE (NMOL/L)	TOTAL PROTEIN (g/L)	TOTAL GLYCERIDE (mmol/L)
1	0	$26^{ab} \pm 11.35$	$3.3^{a} \pm 1.68$	$0.28^{a} \pm 0.20$
2	0.20	$67.33^{a} \pm 32.88$	$4.33^{a} \pm 3.09$	$1.473^{b} \pm 0.71$
3	0.50	$44.33^{ab} \pm 4.04$	$1.833^{a} \pm 0.70$	$0.1467^{a} \pm 0.020$
4	1.0	$19^{b} \pm 10.81$	$2.4667^{a} \pm 0.90$	$0.333^{a} \pm 0.221$

Mean±Standard Deviation. Means with the same letter superscript along the same column are not significantly different (P=0.05).

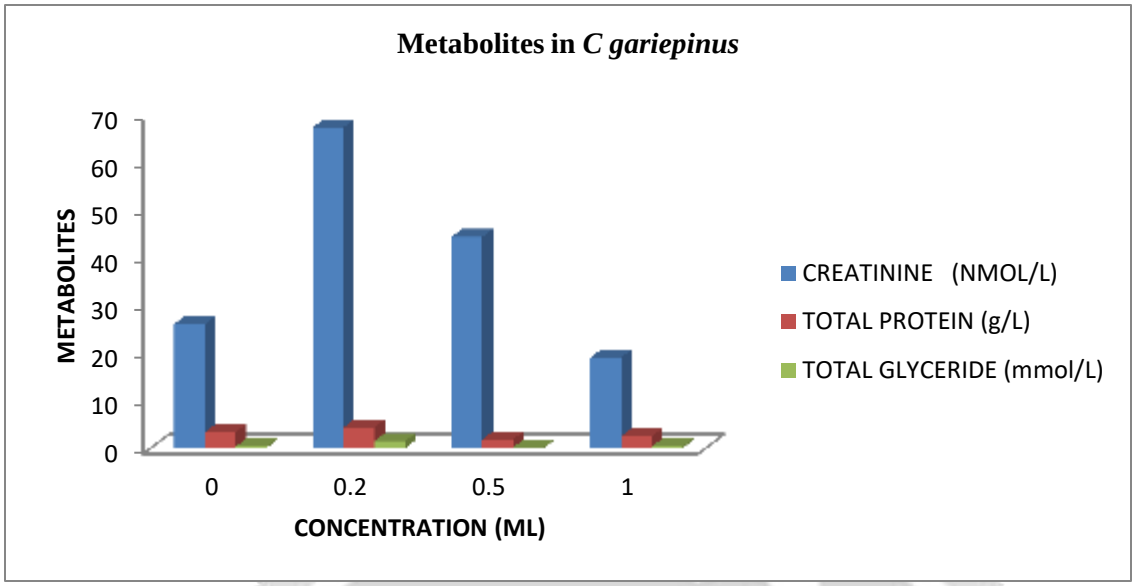

Figure 1: Mean metabolites of *C gariepinus* with different concentrations of insecticide

Figure 2 show a rise in creatinine levels from control to 0.25ml inclusion before falling drastically below control at 1.0ml inclusion of toxicant.

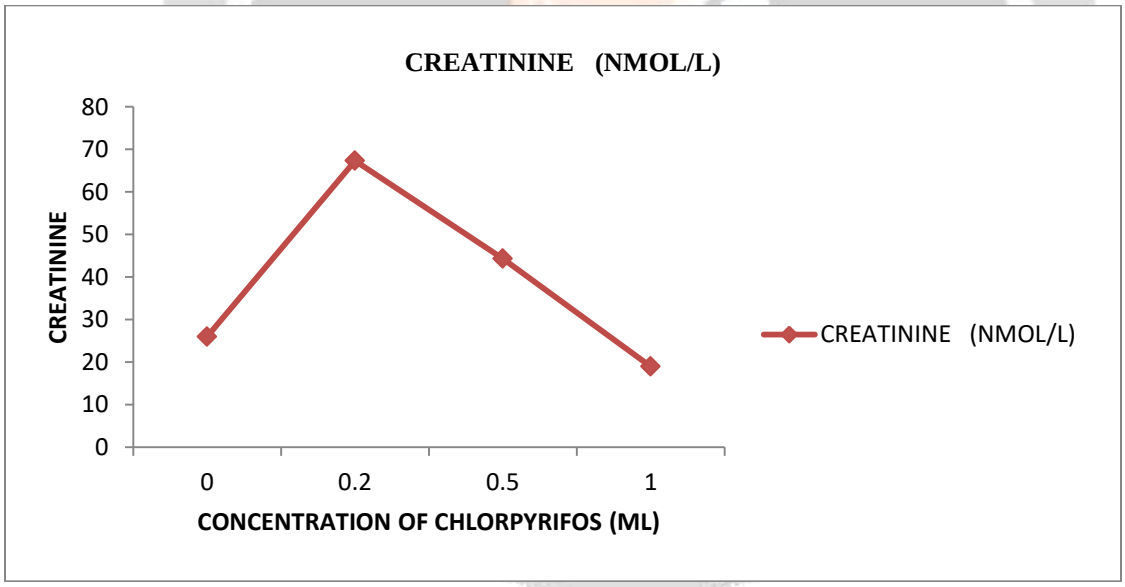

Figure 2: Creatinine of *C gariepinus* with different concentrations of insecticide

Figure 3 below show a gentle rise in Total protein level from control to 0.25ml inclusion level before falling below control level at the 1.0ml inclusion concentration of chlorpyrifos.

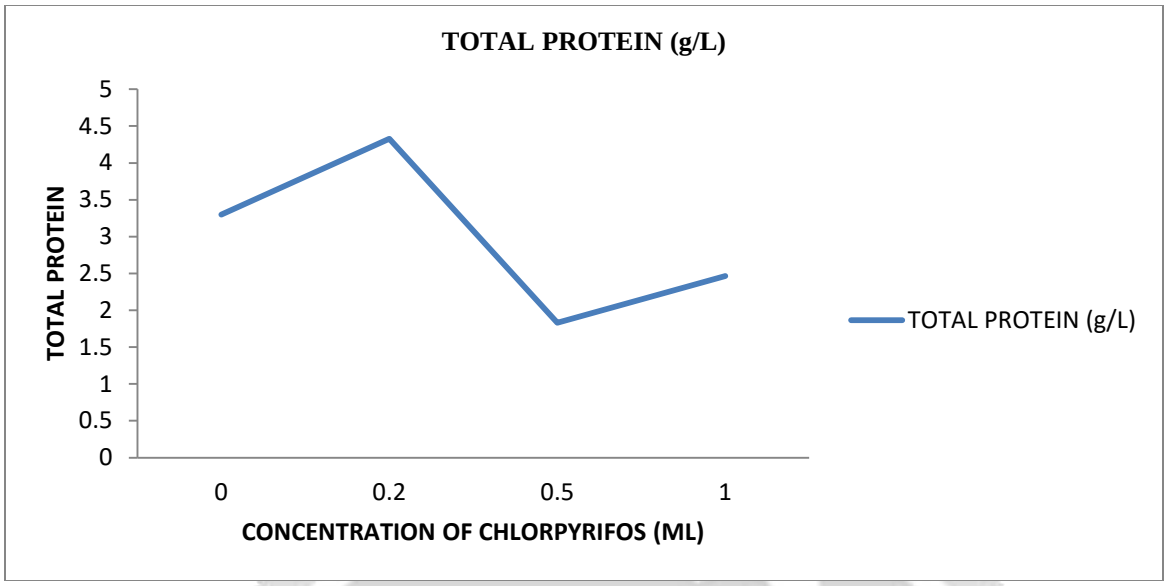

Figure 3: Total protein of *C gariepinus* with different concentrations of insecticide

Figure 4 below show a rapid rise in Total glyceride level from control to 0.25ml inclusion level before falling below control level at the 1.0ml inclusion concentration of chlorpyrifos.

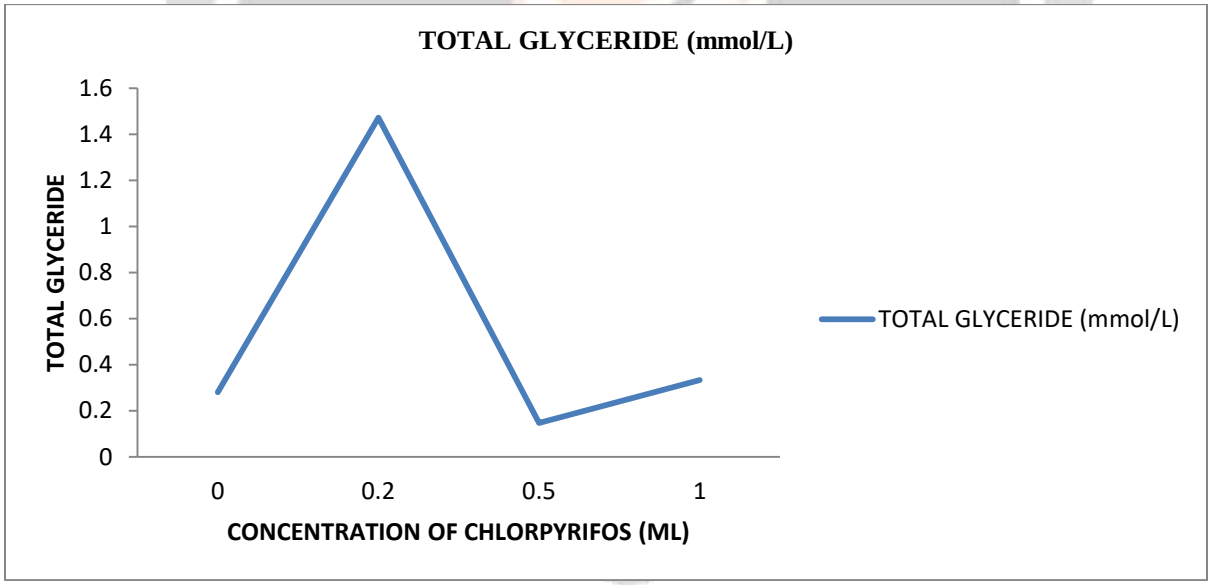

Figure 4: Total Glyceride of *C gariepinus* with different concentrations of insecticide

Table 2 below shows the correlation of the different variables. Creatinine is significantly correlated with Total glyceride (0.770).

Table 2: Pearsons Correlation of Variables of metabolites

S/N	Variables	Chlorpyrifos	Creatinine	Total Protein	Total Glyceride
1	Chlorpyrifos	1	-0.205	-0.314	-0.211

		-	0.522	0.320	0.511
		12	12	12	12
2	Creatinine	-0.205	1	0.412	0.770**
		0.522	-	0.183	0.003
		12	12	12	12
3	Total Protein	-0.314	0.412	1	0.560
		0.320	0.183	-	0.058
		12	12	12	12
4	Total Glyceride	-0.211	0.770**	0.560	1
		0.511	0.003	0.058	-
		12	12	12	12

****Correlation is significant at 0.01 level (2-tailed)**

3.2 Discussion

In this study the alterations and fluctuations of metabolites is connected to the water chemistry used in the experiment after the introduction of the toxicant. The study recorded an increase in creatinine from control(0ml) to 0.25ml inclusion of the toxicant, rising from a mean of 26.00(nmol/l) to 67.33(nmol/l) before reducing to 19.00(nmol/l) in the 1.0ml inclusion of the toxicant. Also, total protein value had an increase from control to 0.25ml inclusion of the toxicant, rising from a mean 3.3(g/l) to 4.33(g/l) before declining below the control. The total glyceride also had a rapid spike sharply from control to 0.25ml inclusion of the toxicant at a mean of 0.28(nmol/l) to 1.47(nmol/l) before dropping sharply at inclusion of 0.50ml and 1.0ml with means of 0.14(nmol/l) and 0.33(nmol/l) respectively.

The finding of the subsequent increase in creatinine, total protein and total glyceride in this study is not in conformity with the results of some previous scholars, whose work did not observe a subsequent increase in creatinine, total protein and total glyceride when exposed to different concentration of toxicant. In a study of the pesticide Diazinon on fish, result showed a value in plasma that were significantly higher in the control fish than all the treatment concentrations of Diazinon (Inyang et al, 2010). Similar observations have been made for DDT and malathion in *Sarotherodon melanotheron* (Ramalingam and Ramalingam, 1982), diquat in carp (Magdy et al, 1993) and cypermethrin in the Koren Rockfish, *Sebastes Schlegeli* (Jee et al, 2005).

A decrease in the concentration of the metabolites (Urea and creatinine) suggest that the kidney may not have been affected by the toxicant. Additionally a decrease in values of these metabolites may suggest that the kidney is under stress to remove these metabiotic waste due to toxicity of diazinon on the probe organism tissues.

In another study, Edori et al, (2013) observed that the total protein levels in the viscera decreased in content when compared to the control value. The average value of total protein in the tissue of *Tympanotonus fuscatus* depreciated in value in all the test solutions when compared to the control.

The increase in creatinine, total protein and total glyceride before declining in this study maybe as a result of variation in the various metabolites due to changes in the rate of synthesis and metabolism resulting from the toxicant. Sudden stress can appear to cause depression by increasing levels of protein in the brain that decreases the availability of an important chemical that regulates our mood. Under normal conditions, large molecular proteins are not found in urine for instance however during stressful conditions large molecular proteins may intrude into the filtration process.

On the other hand, the sudden decrease from 0.25ml of all the metabolites respectively is difficult to explain. However, the most important factors decreasing fish growth consist of disorder in feeding behaviors, decrease in feeding rate, dysfunction in metabolism process and waste of energy to overcome the stress caused by insecticide exposure (Tripathi et al, 2003). For example, disorder in the metabolism of carbohydrates, proteins and lipids in various tissues, particularly liver of fish exposed to insecticides, may reduce their growth rates. Begum (2004) found out that protein and carbohydrate metabolism in the liver and muscle tissue is disrupted on the exposure to a carbofuran insecticide. Decreased total protein levels may be due to starvation, malnutrition and chronic liver diseases (Martin et al, 2010).

In summary, the observation in this study show and eratic pattern in the metabolites of *Clarias gariepinus* exposed to the insecticide. It can be concluded that the insecticide has a marked negative effect on the metabolites of *Clarias gariepinus.*

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