

**NSAP****47th Annual
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**SECURING ANIMAL
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GLOBAL CHALLENGES****LIVER BIOCHEMICAL AND ANTIOXIDANT ENZYMES RESPONSE OF *Clarias gariepinus* (BURCHELL, 1822) JUVENILES EXPOSED TO BUTACHLOR (HERBICIDE)***Salim, A. M., Hadiza, Y. B. and Yusuf, M. A.**Department of Fisheries and Aquaculture**Federal University Dutsin-Ma, Katsina State.*Corresponding author: sahmad@fudutsinma.edu.ng; +2347039528683; +2348064663682**Abstract**

The present study was conducted to investigate the biochemical and antioxidant response of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of butachlor. The experiment consisted of three treatments and a control (0.040, 0.052 and 0.078mg/L), the study lasted for a period of eight weeks. At the end of the experimental period, three fish from each treatment were dissected and the liver was removed and ground using laboratory mortar and pestle. Super oxide dismutase (SOD), glutathione (GHS), catalase activity (CAT) and lipid peroxidation were determined. The result showed a significant ($p > 0.05$) dose dependent increase in CAT, SOD and GHS whereas lipid peroxidation values revealed a dose dependent decrease. Butachlor was found to induce oxidative stress in the exposed groups as revealed by the dose dependent increase of the antioxidant enzymes.

Keywords: Liver, Biochemical, Antioxidant, *C. gariepinus*, Butachlor

Introduction

In recent years, freshwater ecosystem has experienced serious threats from human activities such as industrialization, agricultural activities, urban waste management issues, and increase in urbanization (Meijide *et al.*, 2018; Zhu *et al.*, 2018).

Numerous studies have quantified contaminants in fish organs to evaluate environmental quality, seeking causal relationships with fish health, and, based on these, the liver is likely to be the best choice, followed by the kidney and gills. The liver is reported to be the primary organ for bioaccumulation and thus, has been extensively studied in regards to the toxic effects of xenobiotics. The liver is also a target organ due to its large blood supply which causes noticeable toxicant exposure. In addition, liver is a detoxification organ and it is essential for both, the metabolism and the excretion of toxic substances in the body (Monteiro *et al.*, 2013).

Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. The biochemical processes represent the most sensitive and relatively early events of pollutant damage (Begum, 2004). Severe or prolonged biochemical and physiological alterations will lead to structural alterations, which will become more pronounced over time and the severity will increase (Van Dyk *et al.*, 2009). Antioxidant enzymes in the fish liver are also considered to be important parameters for testing water quality and negative effects of pesticides, as well as for detecting potential adverse effects and relatively early events of pollutant damage (Koenig *et al.*, 2012). Livingstone (2001) has stated that the oxidative stress caused by pesticides in aquatic organisms may lead to ROS production and alterations in antioxidants enzymes. The production of ROS may attack nearby molecules resulting in damage of the molecular structure and function or dysfunction of many organs and systems. The excessive production of ROS production and their damaging effects can be minimized by the cellular antioxidant systems (Dorts *et al.*, 2012). Generally, organisms under stress conditions use the antioxidant enzymes to adapt to environmental stress and altered activities depend on the dose, species

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and route of exposure (Gravato *et al.*, 2006). The present study is aimed at investigating the biochemical response of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of butachlor.

Materials and method

Experimental Design

Exposure of fish to the herbicide was carried out using glass aquaria of 30 x 30 x 45cm dimension. A completely randomized design was used, consisting a control and three treatments in duplicate (4x2). Ten fishes were randomly selected and stocked in each aquarium. A static-renewal system was used throughout the experiment OECD, (1992). The study lasted for eight weeks.

At the end of the study, three fish from each treatment were sacrificed for the collection of the organ (Liver). The liver was collected by dissecting the fish samples with the aid of a dissecting kit. It was then weighed in grams and digested.

Determination of lipid peroxidation

Lipid peroxidation was measured by the modified method of Niehaus and Samuelson (1968) as described by Akanji *et al.* (2009).

Determination of Antioxidant Enzymes parameters

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) was determined by the method described by Fridovich (1989).

Principle: The ability of superoxide dismutase (SOD) to inhibit auto oxidation of adrenaline at pH 10.2 forms the basis of this assay.

Procedure:

Tissue homogenate of 0.1ml was diluted in 0.9ml of distilled water to make 1:10 dilution of micro some. An aliquant mixture of 0.2ml of the diluted micro some was added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of d distilled water. The Absorbance was measured over 30 seconds up to 150 seconds at 480nm.

Catalase (CAT) activity

Catalase (CAT) activity was measured using Abei's method (1974). 10uL of tissue homogenate was added to test tube containing 2.8mL of 50mM potassium phosphate (buffer pH 7.0).The reaction was initiated by adding 0.1mL of freshly prepared 30mM H₂O₂ and the decomposition rate of H₂O₂ was measured at 240nm for 5minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041mM-1cm-1 was used to calculate the Catalase Activity.

Catalase conc. = Absorbance of sample/molar extinction coefficient (E)

Catalase Activity= Absorbance of sample/molar extinction coefficient (E) x Protein Conc. (mg/ml)

Unit: One unit is the amount of Catalase that decompose 1μmol of H₂O₂ per minute at pH 7.0

Glutathione (GHS) concentration

Reduced glutathione (GSH) concentration measurement was done according to Ellman (1959) as describe by Rajagopalan *et al.* (2004).

Procedure: 150μl of tissue homogenate (in phosphate - saline buffer pH 7.4), 1.5ml of 10% TCA was added and centrifuge at 1500g for 5 minutes. 1 ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.

Data analysis

The data obtained was subjected to one way analysis of variance (ANOVA) at p<0.05 alpha level using SAS version 9.0. Where significant differences are observed, Duncan's Multiple Range Test (DMRT) was employed to separate the means.

**Results**

Table 1 shows the sub lethal effects of butachlor on some liver biochemical parameters of *C. gariepinus* juveniles. The results shows that all the biochemical parameters checked (Catalase activity CAT, Superoxide dismutase SOD, Gluthathione GHS, and Lipid peroxidation MDA) varies significantly ($P < 0.05$) between the control group and the exposed groups with mean values of CAT ranging from 23.15 ± 0.6077 in the control and 35.55 ± 0.6077 in the highest concentration of the exposed group, SOD mean value of 31.35 ± 1.4878 in control group and 45.05 ± 1.4878 in the highest concentration, GHS mean values of 37.35 ± 1.9650 in the control and 57.35 ± 1.9650 in the highest concentration and MDA mean values of 19.45 ± 0.4683 in the control and 15.70 ± 0.4683 in the highest concentration of the exposed group.

The values of CAT, SOD AND GHS increases with an increase in concentration of the toxicant, furthermore the values of the aforementioned parameters in the exposed groups were found to be higher than the control. However, between 0.040 and 0.052mg/L the values of MDA tends to decrease with an increase in concentration.

Table 1: Sub Lethal effects of butachlor on some liver biochemical parameters of *C. gariepinus* juveniles

Conc. (mg/l)	CAT (μ /mg protein)	SOD ((μ g/ml)	GHS (ug/mg protein)	MDA (umols/mg protein)
Control	23.15 ^c	31.35 ^b	37.35 ^b	19.45 ^a
0.040	27.50 ^b	34.40 ^b	42.00 ^b	16.85 ^b
0.052	28.25 ^b	34.70 ^b	44.90 ^b	14.55 ^{bc}
0.078	35.55 ^a	45.05 ^a	57.35 ^a	15.70 ^c
SE	0.61	1.45	1.01	0.47

Means with the same superscript along the columns were not significantly different ($P > 0.05$)

Key:

CAT = Catalase activity, SOD= Superoxide dismutase, GHS= Gluthathione and MDA= Malondialdehyde

Discussion

The concentration dependent increase of SOD, CAT and GHS activities observed in the liver of the exposed groups in the present study might be due to the production of superoxide anions, as SOD converts the superoxide radical to H_2O_2 . The increase of CAT activity in the same organ may be due to the SOD stimulated production H_2O_2 since CAT is responsible for the detoxification of hydrogen peroxide to water. The increase might also be attributed to the fact that in cases of increased levels of reactive oxygen species and oxidative damage, cells will usually increase the accumulation of a number of enzymatic antioxidants which SOD, CAT and GHS are all included (Downs *et al.* 2002). The higher values of the antioxidants enzymes observed in the present study further confirms that the fish in the exposed groups are exhibiting oxidative stress (Fatima *et al.*, 2006). Previous reports have associated SOD and CAT as working together to dismutize oxygen radicals at physiological conditions (Achuba and Osakwe, 2003). Therefore, the observed dose dependent increase in CAT and SOD activities in the liver might be due the aforementioned reason. The increase may also be due to the fact that SOD-CAT system represents the first line of defense against oxidative stress. However, the levels of MDA which is the measure of lipid peroxidation decreases with an increase in concentration, this decrease is probably

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because liver is more resistant to oxidative stress due to the large spectrum of antioxidant enzymes. The concentration dependent decrease might also be attributed to the attempt by the defense system of the exposed fish to remove the already generated ROSs (Bayero, 2017). In contrary an increased level of MDA as the concentration of the toxicant increases were reported (Farombi *et al.*, 2008; Nwani *et al.*, 2010; Hameed, (2016). In general, the elevation of antioxidant activities in the exposed groups indicates adaptive responses of the organism to counteract the oxidative effect of generated reactive oxygen species (Hegazi *et al.*, 2010).

Conclusion: Butachlor induces a dose-dependent increase in antioxidant defense enzymes (SOD, CAT and GHS) (23.15-35.55, 31.35-45.05 and 37.35-57.35) respectively in the liver of the exposed groups. This shows that there is increased production in reactive oxygen species (ROS) and therefore there is oxidative stress.

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