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Changes in Antioxidants Activities in Tilapia Guineensis Exposed to Butachlor under Laboratory Conditions

Chidume, I[1], Ukazu, E. R[2]

[1]*Department of Biotechnology, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria*

[2]*Department of Fish Technology and Product Development, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria*

***Corresponding Author:** **Ukazu, E. R,** Department of Fish Technology and Product Development, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria*

Abstract: *One of the most popular butachlor based herbicides in Nigeria for controlling a variety of weeds in agricultural activities is called Butaforce. Using some of the antioxidants, a biomarker test was performed on Tilapia guineensis plasma to assess the herbicide's oxidative impact. To assess oxidative stress in fish exposed to various butachlor concentrations: 0.00mg/l-control 0.05, 0.10, 0.15, and 0.20, mg/l, some specific antioxidants, such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), lipid peroxidase (LPO), and Glutathione (GSH), were measured in the plasma of T. guineensis exposed to the chemical. Blood samples from juvenile and adult Tilapia guineensis were collected, and Randox test kits were used in the investigation. The antioxidant analysis's findings revealed that, in comparison to the control, CAT and LPO greatly increased in both sizes, but SOD and GSH values dramatically decreased ($P<0.05$). The juvenile fish showed more noticeable changes in comparison to the adult fish, and these changes depended on concentration of the chemical. The outcomes are consistent with using oxidative stress measures in an integrated manner for evaluating the danger of pollution to aquatic ecosystems.*

Keywords: *Oxidative Stress, Aquatic Pollution, Butachlor, Tilapia, Natural resources*.

1. INTRODUCTION

Fish face a variety of pressures as they interact with the aquatic environment. Reactive oxygen species (ROS), which can cause oxidative stress, are regularly produced by the organism in response to environmental stressors [3]. Oxidative stress is defined as a disruption of the prooxidant-antioxidant equilibrium in favor of the former, which may cause damage [1]. The cause is either an increase in ROS, a breakdown in antioxidant defense mechanisms, or an inability to repair oxidative damage [2].

Increased reactive oxygen species (ROS) can interact with biological macromolecules and cause lipid peroxidation, DNA damage, and changes in the activities of antioxidant enzymes like catalase, superoxide dismutase, glutathione reductase, reduced glutathione, and glutathione peroxidase [4]. Somdare et al. [5] state that oxidative stress has been connected to several illnesses, such as cancer, lung problems, and neurological problems.

It is routine practice to undertake toxicity studies and organize and safeguard aquaculture ecosystems using aquatic creatures [6]. Recent years have seen an increase in aquatic contamination as a result of the reckless and widespread use of primary fertilizers like phosphorus and nitrogen. The increased levels of contaminants in the aquatic environment, including pesticides, heavy metals, and industrial chemicals, are the result of several human activities. The increased concentration of organic pollutants in the aquatic ecosystem causes oxygen levels to drop, which raises the mortality rate and makes it more difficult for exposed aquatic creatures to operate normally. When it comes to adverse impacts on aquatic organisms' physicochemical conditions, survival rate, and erratic changes in their health, research and precise data collecting are crucial [7]. Fish, plants, invertebrates, and vertebrates are examples of aquatic animals that are valuable natural resources that reduce the extreme stress caused by human activity [8]. According to research, industrial effluents, residential activities, and agricultural runoff are the main sources of pollution in the aquatic environment [9, 10].

The increasing discharge of household, industrial, and agricultural contaminants into the aquatic environment has caused varied degrees of harm to aquatic organisms [11]. Environmental and health experts are very concerned about the use of pesticides in agriculture because some of these chemicals harm aquatic systems even when sprayed in approved areas because of rain and flooding. Fish are particularly affected [12, 13]. Furthermore, pesticides' high solubility, frequent application, accidental spills, discharge from untreated effluents, and spray drift can all contribute to a substantial accumulation and increased risk of killing aquatic life [14]. The purpose of this investigation was to ascertain the degree of antioxidant enzyme activity in *Tilapia guineensis* plasma subsequent to exposure to varying concentrations of laboratory-produced butachlor.

2. Materials And Methods

- **Experimental Site**

The African Regional Aquaculture Center, located in Buguma, Rivers State, Nigeria, is an outpost of the Nigerian Institute for Oceanography and Marine Research, where the study was carried out. 180 *T. guineensis* fish, 90 of which were juvenile and adult fish taken from ponds at low tide, were acclimated over the course of seven days. Six 50-liter plastic containers with openings were used to carry the fish to the lab.

- **Preparation of Test Solutions and Exposure of Fish**

The herbicide Butachlor, which belongs to the chloroacetanilide class, is well-known for its pre-emergent ability to kill weeds and soil. It is recommended, especially in rice crops, for the pre-emergence control of certain broad-leaved weeds and annual grasses. It is also widely used as a post-emergence herbicide in rice when it is in the form of granules. The herbicide was purchased in Port Harcourt, Nigeria, from a retailer. T. guineensis were exposed to the material in triplicate at doses of 0.05, 0.10, 0.15, and 0.20, mg/L, with 0.00 mg/L serving as the control. Five fish were randomly selected for each test tank. The fifteen-day trial continued. Fresh water was added to the tanks each day. Twice daily, the fish received commercial feed at a rate of 3% of their body weight.

- **Analytical Procedure**

Using a small needle to make a caudal puncture, 2 milliliters of fresh blood were drawn at the conclusion of each experimental session and placed into heparinized sample vials. Samples of blood were immediately centrifuged at 5000 rpm for 15 minutes. Before being analyzed, plasma specimens were pipetted into eppendorf tubes, separated, and kept in a refrigerated at -20°C [15]. A Jenway visible spectrophotometer (Model 6405) with a universal microplate reader was used to read the findings. The approach of Beechey et al. [16] was utilized to assess the antioxidant activity in centrifuged plasma using spectrophotometric analysis. The APHA methods were also used to determine the parameters of water quality [17].

- **Statistical Analysis**

The mean and standard deviation of the mean were used to express all the data. The data analysis was conducted using SPSS Version 22, a statistical software. Two-way ANOVA was used to separate the means, and at 5% ($P<0.05$), the two means were deemed significant.

3. Results

The water quality measures (Table 1) were all within the same range, with the exception of DO, where lower values were recorded with increased herbicide concentrations.

Table 1. *Physico-Chemical Parameters of Water in Experimental Tanks of T.guineensis Exposed To Butachlor*

Concentrations(mg/l)	DO (mg/l)	Temperature (^{o}C)	pH	NH_3 (mg/l)
0.00	6.31±0.55 ^{b}	29.52±2.01 ^{a}	6.64±1.03 ^{a}	0.02±0.01 ^{a}
0.05	5.66±0.88 ^{b}	29.81±7.02 ^{a}	6.68±1.23 ^{a}	0.02±0.01 ^{a}
0.10	5.44±0.33 ^{b}	29.01±1.11 ^{a}	6.61±1.02 ^{a}	0.02±0.01 ^{a}
0.15	5.21±0.11 ^{b}	29.01±3.01 ^{a}	6.69±0.44 ^{a}	0.02±0.01 ^{a}
0.20	4.67±0.33 ^{a}	29.33±5.41 ^{a}	6.54±0.41 ^{a}	0.03±0.01 ^{a}

Means within the same column with different super scripts are significantly different (P<0.05)

The effects of butachlor on the antioxidants in the plasma of juvenile *T. guineensis* are displayed in Table 2. It was demonstrated that the levels of SOD and GSH decreased as the concentration of herbicide rose. Conversely, CAT and LPO increased significantly relative to the control levels. Table 3 displays the antioxidant levels of adult fish exposed to the herbicide. The values of SOD and GSH decreased as the concentration of pesticide rose. Conversely, CAT and LPO increased significantly relative to the control levels.

Table 2. *Changes in Antioxidants Activities in Juvenile Sizes ofT.guineensis Exposed to Butachlor*

Concentration (mg/L)	CAT (mmol/protein)	GSH (mmol/protein)	SOD (mmol/protein)	LPO (mmol/protein)
0.00	60.19±9.01 [a]	7.66±1.12 [c]	12.01±0.51 [b]	7.05±0.55 [a]
0.05	65.22±4.98 [a]	5.02±1.55 [c]	9.12±0.61 [a]	9.03±0.66 [a]
0.10	73.99±3.67 [b]	4.89±1.66 [b]	7.66±0.41 [a]	12.90±3.44 [b]
0.15	78.01±6.98 [b]	3.02±1.12 [b]	6.91±1.44 [a]	14.77±2.92 [b]
0.20	83.77±6.98 [c]	2.99±0.55 [a]	4.77±0.21 [a]	17.77±7.88 [b]

Means within the same column with different super scripts are significantly different (P<0.05)

Key: *CAT- Catalase; SOD- Superoxide dismutase; LPO- Lipid peroxidase; GSH-Glutathione*

Table 3. *Changes in Antioxidants Activities in Adult Sizes of T.guineensis Exposed to Butachlor*

Concentration (mg/l)	CAT (mmol/protein)	GSH (mmol/protein)	SOD (mmol/protein)	LPO (mmol/protein)
0.00	80.55±7.01 [a]	7.31±0.88 [b]	19.98±7.09 [b]	10.01±1.66 [a]
0.05	85.03±9.01 [a]	6.01±1.02 [b]	16.55±2.77 [b]	13.01±0.71 [a]
0.10	90.02±8.01 [a]	5.01±1.44 [a]	14.88±2.77 [b]	15.66±1.99 [a]
0.15	95.02±9.44 [a]	5.21±1.77 [a]	12.70±2.88 [a]	17.77±1.02 [a]
0.20	110.41±9.33 [b]	5.14±0.22 [a]	11.66±2.10 [a]	19.77±0.88 [b]

Means within the same column with different super scripts are significantly different (P<0.05)

Key: *CAT- Catalase; SOD- Superoxide dismutase; LPO- Lipid peroxidase; GSH-Glutathione*

4. DISCUSSION

Reactive oxygen metabolites (ROS), which are created by the toxicity of glyphosate, are what cause oxidative stress. ROS production has the potential to interact with lipid membranes in cells, leading to DNA damage, lipid peroxidation, and physiological process disruption [18]. Butachlor has been linked to cytoplasmic membrane toxicity and oxidative stress, both of which can impair cellular function [19]. An imbalance in the production and removal of ROS leads to the activation of glutathione (GSH), an antioxidant molecule, which balances the concentration of MDA [20]. The fish included in this study exhibited increased GSH activity levels in their plasma. Because they have the ability to catalyze the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen, superoxide dismutases (SODs) are the first line of defense against free radicals. In contrast to the control fish in this experiment, *T. guineensis* was inhibited by butachlor treatment in both sizes. The results of the study show that the plasma of the treated fish had reduced levels of SOD, which would point to a decline in the tissues' resistance to free radicals, which release oxygen. *Oreochromisniloticus* tissues subjected to heavy metal consumption have shown similar results on reduced SOD [21].

The two *T. guineensis* sizes employed in this investigation showed increases in LPO that were time- and dose-dependent. This might be related to butachlor formulations' ability to produce reactive oxygen species (ROS), which might then interact with fish macromolecules to cause cell damage and change antioxidant levels. The results of the present investigation, which indicate that Butachlor increased LPO and caused oxidative stress, are in line with those of Salah et al. [22], who found that LPO increased in grass carp treated with zinc and mercury. In the species *Ranaridibunda,* an increase in LPO that causes oxidative stress has similarly been seen after butachlor therapy [23]. When C. gariepinus was subjected to sub-lethal dosages of deltamethrin, high levels of LPO were observed in its plasma [24]. According to Dar et al. [25], *Carassiuscarassius* treated with endosulfan showed a significant LPO amplification in its blood. One of the targets of ROS produced by lipid peroxidation (LPO) is the lipid membrane. Therefore, LPO estimation has shown to be a helpful method for determining whether pollutants are causing oxidative stress in aquatic species. Fish exposed to

different toxins may exhibit elevated LPO and antioxidant enzyme activity as bioindicators of oxidative stress [26].

The results of this study showed that after 15 days of exposure, the plasma of T. guineensis treated with butachlor exhibited noticeably increased CAT activity. This study's observation of an increase in CAT could represent a physiological response to the drop in ROS generation. When tilapia (*O. niloticus*) are exposed to pesticides in the lab, comparable outcomes have been seen [27]. The reason antioxidant defense enzymes like SOD and CAT are so helpful is that they shield aquatic species from free radicals, which can cause oxidative stress. According to the available data, SOD activity typically dropped as CAT activity rose. Comparing CAT to the other antioxidant enzymes, it was also demonstrated that CAT was the most sensitive antioxidant enzyme. The response to the elevated oxidative stress brought on by chemical exposures may be linked to the rise in CAT activity. Similar increases in CAT activity were observed in other fish species following pesticide exposure [28]. Our earlier work also supported the sensitivity of CAT activity to herbicide exposures [29]. Reduced SOD activity may be a sign of oxidative stress from pesticides harming the antioxidant systems.

5. Conclusion And Recommendations

The current study's findings indicate that glyphosate caused oxidative stress, as shown by increases in CAT and LPO levels and decreases in SOD and GSH levels. When evaluating the risk of pollutants in aquatic ecosystems, the regulatory bodies may find it useful to integrate the use of oxidative stress biomarkers with a fish model. These characteristics can be employed as biomarkers to evaluate the toxicity of pesticides in aquatic environments. To gain a better understanding of the physiological significance of the methyl parathion status in natural populations, more research is required to assess the pesticide's residual effects in various fish body tissues.

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