OXIDATIVE STRESS IN BLACK JAW TILAPIA (*Sarotherodon melanotheron*) EXPOSED TO DIMETHOATE IN THE LABORATORY

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Abstract

One of the most often utilized organophosphate insecticides in Nigeria for a variety of pest control applications in agricultural activities is dimethoate. Some of the antioxidants were used as a biomarker test to assess the oxidative effect of the herbicide in Sarotherodon melanotheron plasma. The plasma of S.melanotheron exposed to dimethoate was tested for specific antioxidants, such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), lipid peroxidase (LPO), and Glutathione (GSH), in order to assess oxidative stress in fish exposed to varying concentrations of the chemical: 0.05, 0.10, 0.15, 0.20, and 0.25 mg/l. Blood samples were taken from S. melanotheron juveniles and adults, and Randox test kits were used for analysis. Antioxidant study results revealed that, in comparison to the control, CAT and LPO were significantly ($P<0.05$) enhanced in both sizes, but SOD and GSH values decreased significantly ($P<0.05$). The juvenile fish showed more noticeable changes than the adult fish, and these changes were concentration-dependent. The obtained findings are consistent with the integrated application of oxidative stress metrics in aquatic ecosystem pollution risk assessment evaluation.

Keywords: *Dimethoate, Antioxidants, Contaminants, Toxicology, Aquatic Environment.*

INTRODUCTION

Organizing and safeguarding aquaculture environments, as well as conducting toxicity assessments, are common uses for aquatic organisms [1]. Because main nutrients like phosphorus and nitrogen are used so extensively and indiscriminately, there has been an increase in aquatic pollution recently. The elevated levels of pollutants in the aquatic environment, including industrial chemicals, pesticides, and heavy metals, are caused by a variety of human activities. The high concentration of organic pollutants in the aquatic environment causes a reduction in oxygen levels, which raises the death rate and impairs the ability of exposed aquatic creatures to operate normally. It is crucial to conduct research and gather precise data regarding the harmful impacts on aquatic organism health, survival rate, and random fluctuations in an organism's physicochemical conditions [2]. Fish, plants, invertebrates, and vertebrates are examples of aquatic species that are valuable natural resources that help to mitigate the extreme stress caused by human activity [3]. According to studies, home activities, agricultural runoffs, and industrial effluents are the main sources of pollution in the aquatic environment [4–5], which has significant negative consequences on environmental health.

The increased release of household, industrial, and agricultural contaminants into the aquatic environment has caused varied degrees of harm to aquatic organisms [6]. Health and environmental experts are gravely concerned about the use of pesticides in agriculture as some of these chemicals are eroded by rains and floods to nearby aquatic systems even when sprayed in restricted areas. Fish are particularly affected [7, 8]. Furthermore, the high solubility of pesticides, their frequent application, accidental spills, discharge from untreated effluents, and spray drift can result in a considerable accumulation and enhance their potential to poison aquatic life [9]. The molecules of these contaminants in water can cling to suspended particles, accumulate in sediment, or be ingested by aquatic organisms. These compounds affect the physiology and survival of aquatic creatures, but they can also interact with their genetic makeup to induce mutations and/or cancer [10].

It is known that eating contaminated agricultural products, sediments, zooplankton, phytoplankton, aquatic weeds, and fish can expose people to toxic substances such as drug residues, heavy metals, insecticides, fungicides, herbicides, and various other industrial wastes [11]. Fish and other aquatic organisms are among the most delicate kinds of invertebrates. They show nearly all physical and biochemical changes brought on by exposure to hazardous substances, and they are commonly utilized as warning systems to keep an eye on the aquatic ecosystem's normal state. Fish are the most significant and well-known species among the several organisms in the aquatic food chain [12] because of their capacity to absorb, metabolize, and concentrate toxic substances found in the water. Because aquatic organisms can develop highly reactive oxygen species through oxidative stress, they are particularly vulnerable to increased metal exposure [13]. The mechanism of metal toxicity is represented by the generation of oxygen reactive radicals, which interact with proteins, lipids, and nuclei to produce genetic, cellular, and metabolic disorders that ultimately result in the death of the living being[14]. Oxidative stressors lead to metabolic, cellular, and genetic responses.

A disturbance of the prooxidant-antioxidant equilibrium in favor of the former, which may result in damage, is referred to as oxidative stress [15]. Reactive oxygen species (ROS) are on the rise, antioxidant defense systems are compromised, or oxidative damage cannot be repaired, which is the cause [16]. Fish experience a variety of stresses as they interact with the aquatic environment. The body regularly produces reactive oxygen species (ROS) in reaction to environmental stress, which can lead to oxidative stress [17]. High levels of ROS may interact with biological macromolecules to cause lipid peroxidation, DNA damage, and modifications in the activities of multiple antioxidant enzymes, such as glutathione reductase, catalase, superoxide dismutase, reduced glutathione, and glutathione peroxidase [18], according to Talas et al. Oxidative stress has been connected to several illnesses, such as cancer, respiratory problems, and neurological problems, according to Somdare *et al*. [19].

The oxidative stress brought on by the hazardous compounds can be stopped by antioxidants [20]. Fishes' antioxidant defense mechanisms rely on antioxidants in their enzyme systems and low molecular weight proteins. Because fish produce a lot of reactive oxygen species, have a lot of peroxidases, and have low glutathione levels, exposure to heavy metals can elicit dose- and time-dependent oxidative stressors in fish [21]. Previous research has shown that certain pesticides and toxicants can have a variety of detrimental consequences, including decreased growth, immunological response changes, oxidative stress induction, genotoxic effects, and metabolic changes [22, 23,24]. Thus, the purpose of this work was to ascertain the antioxidant enzyme activity in the plasma of *S.melanotheron* that had been exposed to various lab-based dimethoate concentrations.

Materials And Methods

Experimental Location and Fish

The study was carried out in African Regional Aquaculture Center, an outstation of Nigerian Institute for Oceanography and Marine Research, Buguma, Rivers State, Nigeria. A total of 180 *S.melanotheron* comprised of 90 each of juvenile and adult sizes were sourced from ponds during the low tide. The fishes were transported in six open 50l open plastic containers to the laboratory and acclimated for a period of seven days.

Preparation of Test Solutions and Exposure of Fish

In the present study, commercial formulation of dimethoate is a broad spectrum organophosphate insecticide having systemic, contact and stomach mode of action. Dimethoate is a acetylcholinesterase inhibitor affecting the central and peripheral nervous system producing depression. The pesticide was purchased from a commercial outlet in Port Harcourt, Nigeria. *S.melanotheron* were exposed to the chemical at the concentrations of 0.00 control, 0.05, 0.10, 0.15, 0.20 and 0.25 mg/L in triplicates. Five fish were randomly distributed into each test tank. The experiment lasted

for a period of 15 days. The water in the tanks was renewed daily. The fish were fed twice daily at 3% body weight with a commercial feed.

Analytical procedure

At the end of each experimental period, 2ml of fresh blood sample was collected by making a caudal puncture with the help of fine needle and poured in heparinized sample bottles. Blood samples were centrifuged immediately for 15 minutes at 5000 rpm. Plasma specimens were separated, pipetted into eppendorf tubes and stored in a refrigerator at -20°C until assayed [25]. The results were read using a universal microplate reader on a Jenwayvisible spectrophotometer (Model 6405). The activity of antioxidants in centrifuged plasma was determined spectrophotometrically using the method of Beechey *et al.* [26]. Water quality parameters were also determined using the methods APHA [27].

Statistical Analysis

All the data were expressed as mean and standard deviation of mean. The statistical package, SPSS Version 22 was used for the data analysis. The means were separated using two ways ANOVA and the two means were considered significant at 5 %($P<0.05$).

Results

The water quality parameters (Table 1) were within the same range except in DO, where a lesser values were obtained at higher concentration of the pesticide. The effects of Dimethoate on the antioxidants in the plasma of *S.melanotheron* juveniles are presented in Table 2. It was observed that the values of SOD and GSH decreased with increasing concentrations of the pesticide. While CAT and LPO increased significantly when compared to the control values. The same trend was observed in the antioxidants of adult fish exposed to the pesticide (Table 3), where the values of SOD and GSH decreased with increasing concentrations of the pesticide. While CAT and LPO increased significantly when compared to the control values

Table1: Physico-Chemical Parameters of Water in Experimental Tanks of *S.melanotheron* Exposed To Dimethoate Formulations

Concentrations (mg/l)	DO (mg/l)	Temperature (°C)	pH	NH_3 (mg/l)
0.00	6.02±0.22 [b]	29.91±2.11 [a]	6.67±1.66 [a]	0.02±0.01 [a]
0.05	5.77±0.02 [b]	29.81±3.02 [a]	6.64±1.02 [a]	0.02±0.01 [a]
0.10	5.51±0.71 [b]	29.57±1.77 [a]	6.66±1.69 [a]	0.02±0.01 [a]
0.15	5.28±0.88 [b]	29.91±3.09 [a]	6.64±0.61 [a]	0.02±0.01 [a]
0.20	4.88±0.67 [a]	29.81±5.77 [a]	6.66±0.33 [a]	0.03±0.01 [a]
0.25	4.11±0.99 [a]	29.71±4.02 [a]	6.63±0.77 [a]	0.03±0.01 [a]

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

Table 2: Antioxidants Levels in Juvenile Sizes of *S.melanotheron* Exposed to Dimethoate Formulations

Concentration (mg/L)	CAT (mmol/protein)	GSH (mmol/protein)	SOD (mmol/protein)	LPO (mmol/protein)
0.00	62.22±9.77 [a]	8.00±1.05 [c]	12.01±0.77 [b]	6.89±0.44[a]
0.05	65.88±9.02 [a]	5.02±1.04 [c]	9.33±0.08 [a]	8.03±0.45 [a]
0.10	70.07±7.02 [b]	4.01±1.55 [b]	7.02±0.55 [a]	11.77±3.03[b]
0.15	75.03±5.11 [b]	3.27±1.11 [b]	6.55±1.81 [a]	15.03±2.33 [b]
0.20	80.77±4.01 [c]	2.01±0.65 [a]	4.32±0.69 [a]	18.89±2.33 [b]
0.25	85.68±5.02 [c]	1.01±0.77[a]	3.77±0.99 [a]	19.02±2.71 [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: Antioxidants Levels in Adult Sizes of *S.melanotheron* Exposed to Dimethoate Formulations.

Concentration (mg/l)	CAT (mmol/protein)	GSH (mmol/protein)	SOD (mmol/protein)	LPO (mmol/protein)
0.00	80.01±4.44 [a]	7.05±0.55 [b]	18.11±2.66 [b]	10.99±1.09 [a]
0.05	83.66±9.58 [a]	6.02±1.01 [b]	15.07±2.55 [b]	13.07±0.81 [a]
0.10	90.02±7.04 [a]	5.68±1.07 [a]	13.63±2.99 [b]	15.66±1.55 [a]
0.15	95.03±6.02 [a]	5.47±1.77 [a]	12.04±2.77 [a]	17.88±1.33 [a]
0.20	111.99±6.03 [b]	5.22±0.66 [a]	11.19±2.48 [a]	19.03±0.54 [b]
0.25	137.03±9.66 [b]	5.01±0.11 [a]	10.33±1.81 [a]	22.99±1.69[c]

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

Discussion

Reactive oxygen species (ROS) are produced by mild oxidative stress as a compensatory response under stressful physiological conditions, and their elimination can shield organisms from oxidative damage [Dar et al., 28]. Under chemical stress, antioxidant activity can either be increased or decreased depending on the type, duration, and susceptibility of the exposed species. Given that fish plasma serves a variety of purposes related to the metabolism of toxicants, the drop in GSH values seen in this study could be the result of either enhanced peroxidase activity or direct scavenging of radicals [29, 30]. The current investigation's two sizes of *S.melanotheron* showed dose- and time-dependent increases in LPO. This could be attributed to the ability of dimethoate formulations to produce reactive oxygen species (ROS), which could interact with the fish's macromolecules and cause cell damage and modifications to antioxidants. The results of the present investigation regarding the rise in LPO caused by dimethoate and the oxidative stress that followed are in line with those of Salah *et al*. [31], who noted an increase in LPO in grass carp exposed to zinc and mercury. An increase in LPO that results in oxidative stress has also been seen in the species *Rana ridibunda* after treatment with fenthion [32]. Plasma from *C. gariepinus* subjected to sub-lethal doses of deltamethrin exhibited high levels of LPO [33]. According to Dar et al. [34], there was a significant LPO amplification in the blood of *Carassius carassius* exposed to endosulfan. One of the targets of ROS that proceeds via lipid peroxidation (LPO) is the lipid membrane [35]. Therefore, LPO estimation has also been effectively used to indicate oxidative stress caused by pollutants in aquatic animals [36]. Fish exposed to different toxins may exhibit antioxidant enzyme activation and an increase in LPO, which can be considered as bioindicators of oxidative stress [37].

Due to their 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 [38]. When compared to the control fish in the current investigation, it was inhibited by dimethoate exposure in both sizes of *S.melanotheron*. Reduced SOD levels in the treated fish's plasma in this study suggest that the tissues' capacity to withstand oxygen-producing free radicals has diminished. In the tissues of *Oreochromis niloticus* subjected to heavy metal consumption, similar results on reduced SOD have been found [39]. The results of this investigation showed that after 15 days of exposure, there was a significant ($P<0.05$) increase in CAT activity in the plasma of *T.guineensis* treated to dimethoate. The current study's rise of CAT could represent a physiological response to the reduction of ROS formation. Tilapia (*O. niloticus*) subjected to pesticides in the lab have shown comparable outcomes [40]. Aquatic creatures greatly benefit from antioxidant defense enzymes like CAT and SOD because these enzymes shield them from free radicals that can cause oxidative stress. The current findings demonstrated that when CAT activity rose, SOD activity generally decreased. Comparing CAT to the other antioxidant enzymes, it was also discovered that CAT was the most sensitive. While the decrease in CAT activity may be attributed to the potential direct binding of metal ions to the -SH groups on the enzyme molecule, the rise in CAT activity may be connected to coping with the increased oxidative stress generated by chemical exposures. Following pesticide exposure, higher CAT activity was also seen in a variety of fish species [41]. Our earlier findings also supported the sensitivity of SOD and CAT activity to metal exposures [42]. Reduced SOD activity may be a sign of pesticide-induced harm to the antioxidant systems.

In this investigation, the values of GSH decreased noticeably as the pesticide concentration rose. The fish's cells may be reducing their GSH levels as a defense against oxidative stress caused by dimethoate. Fish exposed to other toxicants have also been observed to have similar decreases in GSH levels [43]. The drop in GSH in the exposed fish, regardless of size, might be a sign of the introduction of superoxide radicals and the antioxidant's restricted ability to counteract oxidative stress. According to one theory, fish tissues exposed to pesticides had lower GSH levels because of their organ-specific reactions [44]. Fish's decreased GSH level is most likely the result of their defensive mechanism shielding them from oxidative stress. Pesticide-induced decreases in GSH levels may be due to GSH's ability to bind to toxins and neutralize their harmful effects. Initially, GSH provided quick defense against oxidative stress by purifying the ROS produced by oxidative stress directly or through the GSH redox cycle [45].

Conclusion and Recommendations

The current study's findings showed that dimethoate generated oxidative stress, as seen by drops in SOD and GSH levels and increases in CAT and LPO levels. When determining the risk of pollutants in aquatic ecosystems, the regulatory authorities may find it useful to incorporate the use of oxidative stress biomarkers with a fish model. More studies on the toxicokinetics and dynamics of dimethoate are needed to have a better understanding of the mechanisms of action that result in the development of oxidative stress. These characteristics can be employed as biomarkers to evaluate the toxicity of pesticides in aquatic environments. To further grasp the physiological importance of the methyl parathion status in natural populations, more research has to be done to assess the pesticide's residual effects in various fish body tissues.

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