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EFFECT OF SNIPER INSECTICIDE (DDVP) ON ANTIOXIDANT BIOMARKERS AND HISTOLOGY OF AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822)

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Abstract

The study evaluated the African catfish's acute toxicity to synthetic Sniper insecticide treatment (DDVP 1000g/l of 2, 3-dichlorovinyl dimethyl phosphate) on Clarias gariepinus with mean weight of 145.6±0.62g and mean length 16.59cm. Exactly 150 juvenile catfish fish were purchased from Rumbun Kifi Fish behind BUK old site, Kano and acclimated in a 1000L tank for 14 days. They were treated with varying sniper concentrations of 0.0, 2.5, 5.0, 7.5 and 10.0mg/L with 3-levels exposure concentrations in a Completely Randomized Design (CRD). Gills tissues were analyzed for antioxidant biomarker activities with the aid of Solarbio Life Science kits. Four days lethal concentration (LC_{50}) value for 96hour was found to be 9.56mg/L. The exposed fish displayed erratic swimming with irregular opercular movement, loss of reflex, mucus secretion and increased air gulping with the increasing concentration of the insecticide relative to the control sample. Oxidative stress biomarker enzymes revealed a corresponding significant increase ($P < 0.05$) in a concentration dependent manner for Catalase (CAT) and Superoxide dismutase (SOD). However, Glutathione S-transferase (GST) significantly decrease ($P < 0.05$) in all the tissues at higher concentrations compared to the control. Histopathological alterations examined in gill tissues are as follows: Hyperplasia, necrosis, primary and secondary lamellae distortion, and epithelial thickening distortion. It can be inferred that changes in the histology and activity of the oxidative stress enzymes in fish tissues after sniper exposure harm the experimental fish. It is therefore advised that the competent authorities create initiatives to reduce the indiscriminate discharge of insecticides in aquatic habitat.

Keywords: *Clarias gariepinus*, Histology, Oxidative stress enzymes, Sniper insecticide, Toxicity

INTRODUCTION

Agriculture is a vital sector in Nigeria, contributing to providing the country's largest food requirements. Pesticides application have been regarded as indispensable sector in harvest losses by reducing pest's damage, thereby maintaining food sustainability and availability throughout the year (Tekwa *et al*., 2010). For instance, Tijjani (2006) reported that without pesticides application in rice and cocoa production, 45% of the entire production could have been lost to pests. This necessitated Nigeria's government advocate for the application of pesticides aiming at promoting agricultural production to meet the country's increasing food demand (Ezik *et al*., 2019). On the course of their application, pesticides enter into aquatic ecosystem via runoff, spray drift and leaching (Kumar, 2016). The release of these agrochemicals into the water bodies could affects non-target organisms such as fish (Sumon *et al*., 2019). Application of systemic insecticides in farming activities could primarily pollute the soil, persist in the environment and convey the residues to the water bodies (Bayo *et al*., 2016). Due to prolong application of these pesticides, their widespread usage, many types of the pesticides have been frequently identified from environmental media, biota, and residential areas leading to habitat destruction, reducing and reproduction impairment (Shefali *et al*., 2020).

Dichlorvos (2, 3 dichlorovinyl dimethyl phosphate), is used as household and agricultural pesticide traded under names sniper and pai-pia (Idi-ogede *et al*., 2016). It is applied to control insects on crops household and stored products, and also to treat external parasitic infections in farmed fish, livestock and domestic animals (Ayoku *et al.*, 2017). It is rapidly absorbed via the gastro intestinal and respiratory tracts and skin, it enters human system mainly by inhalation, dermal and oval routes, and metabolized by the liver and excreted by the kidney (Gautam *et al*., 2014). The mechanism of action of sniper is by blocking of acetylcholine, being an acetylchlorine sterase inhibitor, exposure to high concentration could results to body weakness, headache, blurred vision, salivation, sweating, nausea, vomiting, diarrhea, respiratory failure and abdominal cramps in humans (Veeraiah *et al*., 2015). It is mainly metabolized by esterase to dimethyl phosphate and dichloroacetaldehyde. Dichlorvos has been reported to damage DNA of insects in museum collection (Farah *et al.*, 2011).

Oxidative stress is associated with a high rate of cellular destruction caused by oxygen free radicals and other reactive oxygen species (Ahmad *et al*., 2018). Oxidative stress enzyme activities assessment has been used a biomaker of environmental pollution mainly through free radicals generation and reactive oxygen species (ROS) accumulation (Akinwande *et al*., 2016). Modesto and Martinez (2010) depicted that exposure of fish to pesticides could lead to oxidative damage to biological molecules in the body. Besides, histopathological investigations have long been uesd as a bioindicator of fish toxicity (Rajini *et al*., 2015). Histopathological alterations are usually the incorporation of a variety of interactive physiological processes, which facilitate in recognizing the target organs of toxicity and mechanism of action (Kumar, 2016).

Clarias gariepinus is one of the commonly used fresh water fish species in Africans' aquaculture. It is accepted by many fish farmers in Nigeria due to its fast growth, tolerance to poor water quality high yield, omnivorous feeding habit, adaptability to overcrowding and market value preference (Ayanda *et al*., 2018). For sustainability of fish production, the toxicological assessment for pesticides application, disposal pattern and their residue became imperative. In view of the forgoing this research aimed at assessing the effect of sniper insecticide (DDVP) on oxidative stress biomarker enzyme and histology in African catfish (*C. gariepinus*).

MATERIALS AND METHODS

Source of Experimental Fish and Test Chemical

One hundred and fifty (150) physically healthy juveniles *C. gariepinus* with mean weight of 27.2g and mean length 10.95 -15.5cm were sourced from Rumbun Kifi Fish Farm, behind BUK, Kano State, Nigeria. They were acclimated in a semi-static system prior to the commencement of the experiment for 10 days at 29.7°C in a 1000L dark plastic container filled with borehole water and renewed daily as adopted by Nafiu and Ibrahim (2021). A 2mm pelletized meal (42% crude protein) made by Vital Fish Feed Nigeria Plc was given to them twice at 5% of their body weight. Feeding was stopped 24h prior to the range-finding and toxicity test.

Test chemical Procurement

The stock solution of the DDVP contains 1000g/l of 2,3-dichlorovinyl dimethyl phosphate was procured from registered store at Sabongari market, Kano.

Name	Manufacturer	Manufacturing date	Expiring date	Batch no	NAFDAC No
Sniper 1000EC (DDVP)	Forward (BEIHAI) HEPU Pesticide Co. LTD.	19-04-2021	18-04-2024	FI9003	A5-1267

Water Quality Parameters Measurement

Water quality parameters were measured before and during the acute toxicity test. The water samples were promptly evaluated for the following physicochemical parameters using a multifunction water testing kit (Model no. EZ-9909-SP). Color pH, Total Dissolved Solid (TDS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), and Electrical conductivity were determined.

Range Finding Test and Acute Toxicity Test

Definitive concentration for range-finding test was carried to determine the definitive concentration for testing sniper using the procedure described by OECD (2013) No.236 adopted by Bamidele *et al*. (2018). The concentration of sniper in which 50% of the fishes survived after 96 hours to the lowest concentration that no organism survived were used for the actual acute toxicity test. Forty (40) liters of tap water was poured into each container while 0.0, 0.1, 0.2, 0.3 and 0.4ml of water was removed and replaced with equal volume of sniper from the stock solution into the container to reach 40 liters' mark. Dead fish was removed immediately to avoid possible deterioration of the water quality. Sniper concentrations of 0.0, 0.1, 0.2, 0.4 and 0.4ml were converted to 0.0, 2.50 5.00, 7.50 and 10.0mg/L using the formula described by Ezike *et al*. (2019): $C1VI = C2V2$

Where C_1 = initial concentration of stock solution (g/L)
V_1 = volume of stock used (ml)
C_2 = desired concentration (g/L)
V_2 = required volume of water for dilution (ml)

Experimental Design

The experimental and control juveniles' fishes *C. gariepinus* was subjected to Completely Randomized Design (CRD) with 3-levels of exposure to the varying concentrations of sniper in a 40 x 80 x 40 cm dark plastic tank containing 40L of tap water. set of ten (10) fishes were randomly exposed to sniper concentrations for 96 hours in triplicate Another 10 set of fishes were maintained in water, without test solution which served as control.

Observation of Behavioural Responses

After being exposed for thirty (30) minutes to the fish's behaviours, including irregular swimming, air gulping, loss of reflex and mucous secretion were monitored as adopted by Rakesh and Kumar (2019). When there is no discernible movement or no reaction to light poking, fish are deemed to be dead.

Antioxidant Biomaker Activity Analysis

To lessen blood stains, the liver and gill tissues were removed and rinsed in 5 ml of isolation buffer containing 100 mM Tris-HCl at a pH of 7.20. They were then blended with a mortar and a pestle on ice. According to Mandeep and Rajinder (2017) method, the homogenate was centrifuged at 12000g for 5 minutes to reveal the post mitochondrial contents. According to the manufacturer's instructions, 150µl of each tissue homogenate was added with 1 ml of the extraction reagent and thoroughly mixed on an ice bath. Centrifugation was used to separate the supernatant for 10 minutes at 8000 rpm and 4°C. A glass cuvette was used to collect exactly 90 litres of the mixture, which was then carefully mixed with 240 µL of reagent I, 6 µL of reagent II, and 30 µL of reagent V, and 180 litres of reagent III. After that, 480 L of distilled water was added, and it was let to stand at 4°C for 30 minutes. At the Biochemistry Department of Bayero University, Kano, the mixtures of each tissue sample were tested for the activity of SOD, CAT and GST using microplate ELIZA (Model no: Biobase-EL 10A).

Histopathological Analysis of *C. gariepinus* Tissues

The procedure described by Auwioro (2010) was used to run the fish tissue biopsies at the Histopathology Laboratory at Aminu Kano Teaching Hospital in Kano, Nigeria. The fish were put to death with 40% ethyl alcohol before having their gills were removed for histological analysis. Fish tissues were dehydrated with escalating grades of alcohol, fixed with 10 percent formal saline, cleaned with toluene, and infused with molten paraffin wax. Haematoxylin and Eosin stains were applied to microtome sections (5µm), which were then inspected under a microscope (LEICA DM 750 model) and photographed using an HD camera (LEICA ICE 50 model).

Statistical Analyses

Using SPSS version 20.0, probit analysis was utilized to determine the mortality profile (LC_{50}) of the experimental fish. The Duncan multiple range test with a probability level of 5% was used to compare the means after a one-way analysis of variance (ANOVA) was performed to ascertain the varying sniper concentrations on the mean biochemical variables in the serum of the experimental fish.

Results

Table 1, revealed the range values of the physicochemical parameters of the experimental water used for toxicity study of sniper insecticide. Water temperature, DO, pH, EC, TDS, turbidity ranged between 25.7-31.70°C, 5.2-6.36mg/L, 7.81-8.73, 184-232 µS/cm, 298-332mg/L and 21-29NTU respectively.

Table 1: Physico-chemical Parameters of the Experimental Water Exposed with Sniper (DDVP)

Parameters	Range	Mean (±SD)	Standard limits
Water temperature (°C)	25.7-31.70	27.2±0.03	<40°C*
DO (mg/L)	5.2-6.36	5.5±1.02	5.0-9.0mg/L**
TDS (mg/L)	298-332	311±2.70	500mg/L**
E.C (µS/cm)	184-232	208±5.21	1000µS/cm**
Turbidity (NTU)	21-29	27.7±0.54	25 NTU**
Ph	7.81-8.73	8.32±0.71	6.0-9.0*

***FME (2001), **FAO/WHO (2018)**

Behavioral changes recorded in the experimental fish due to exposure to varying Sniper concentrations as well as in the control treatments are presented in Table 2. Normal swimming pattern was examined in the control treatment throughout the 96hr duration. However, in the highest concentrations of 10.0mg/L, experimental fish were restless and swam erratically. They lose orientation and excessively secret mucus with an increase in opercular movement. With an increase in exposure duration 72-96hr, swimming rate reduce drastically and air gulping decrease in corresponding manner. At highest 96hr period of exposure duration, fish in the highest concentrations air gulping decreased, they became motionless with no sign of opercular movement. They cluster themselves at the bottom of the plastic tank with the operculum wide opened and died.

Table 2: Behavioral Response Observed by *Clarias gariepinus* during 96hour Exposure To Sniper Treatment

Exposure time (hr)	Concentration (mg/L)	Air gulping	Erratic swimming	Loss of reflex	Mucus secretion	Opercular movement
	0.00	-	-	-	-	+++
24	2.50	-	-	-	-	++
	5.00	-	-	-	+	+
	7.50	-	-	-	+	++
	10.0	+	+++	+	++	++
48	0.00	-	-	-	-	-
	2.50	-	+	+	++	+
	5.00	-	-	+	++	+
	7.50	+	+	+	+	+
	10.0	+	+	++	++	-
72	0.00	-	-	-	-	-
	2.50	+	-	+	-	+
	5.00	-	-	++	++	-
	7.50	+	+	++	+	-
	10.0	-	-	+++	-	-
96	0.00	+	-	-	-	-
	2.50	+	-	+	-	-
	5.00	-	+	++	+	-
	7.50	-	+	++	+	-
	10.0	-	+++	+++	++	-

Key: none (-), mild (+), moderate (++), Strong (+++) Adopted from Ani *et al.* (2017)

Mortality profile of Sniper insecticide indicated an increase with increasing concentration and exposure duration and the highest mortality rate was obtained in the highest concentrations. The mortality corresponding to each concentration is presented in Figure 1. The 96hr $LC_{50}$of Sniper determined based on the transformed probit analysis was 9.56mg/L. Linear relationship between the probit mortality and the concentration of the insecticide had a positive correlation of $r^2 = 0.9918$.

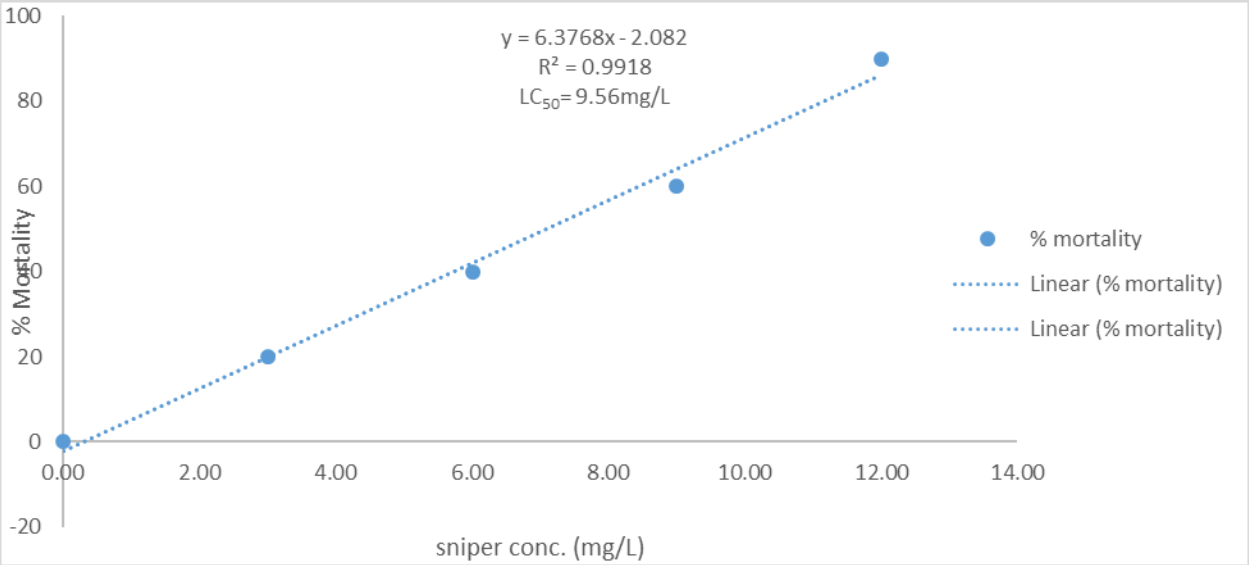


Fig 1: Mortality Profile (%) of *C. gariepinus* Exposed with Different Sniper Concentrations at 96hr Exposure Duration

Table 3, indicates fish survival and percentage mortality at different treatment concentrations in the fish exposed to Sniper. The numbers of survived and dead fish were examined during the exposure duration of 24, 48, 72 and 96hr. highest concentration of the Sniper of 10.0mg/L showed the highest fish mortality of 80% and lowest survival of 40% while no mortality was examined in the control samples throughout the exposure duration.

Table 3: Data on Mortality of Rate and log of Concentration in *C. gariepinus* Exposed to Varying Concentrations of Sniper for 96 hours

Exposed conc.(mg/L)	log conc. (mg/L)	No. of Exposed fish	No. of live fish at varying hours				% Survival	% Mortality	Probit
			24	48	72	96			
0		10	0	0	0	0	100	0	
2.50		10	0	1	1	2	60	40	4.75
5.00		10	0	1	2	3	40	60	5.25
7.50		10	1	1	2	3	30	70	5.52
10.0		10	1	2	3	2	20	80	5.84

Superoxide Dismutase (SOD) Activity

In the gill tissue, the SOD activity shows no significant change ($p > 0.05$) between any of the treatments and the control, but there was a significant difference ($p < 0.05$) between the liver tissues at 80% and the control samples. Fish treated with the highest doses of 80% had significantly reduced mean values of 1.40±.01Unit/mg prot. ($p < 0.05$), but the liver tissue control sample had a higher mean SOD activity of 5.13±0.32Unit/mg prot. The SOD activity

in the gill tissues did, however, significantly decline ($p<0.05$) at the highest wastewater concentrations (80%), from the corresponding control values of 1.53±0.22unit/mg prot (Table 4).

Catalase (CAT) Activity

The liver and gill tissues exposed to the highest concentrations of 80% saw a substantial decrease in CAT activity ($p<0.05$) when compared to the control. The maximum concentrations of the control ranged from 83.78–47.56 and 79.45–37.21 nmol/min/mg prot, in the gills and liver tissues respectively, and showed a significant difference in CAT activity ($p<0.05$) (Table 4).

Glutathione Reductase Activity (GST)

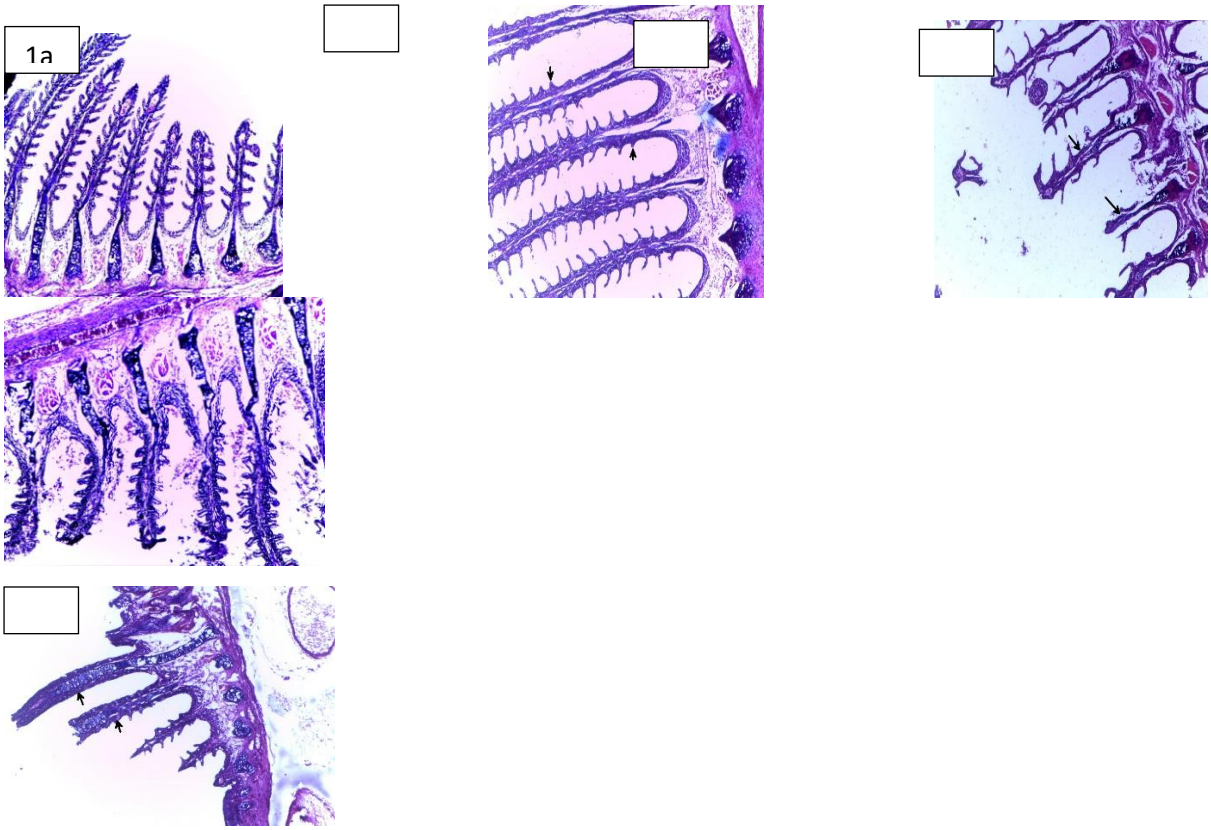
The GST activity was much lower in the liver and gill tissues subjected to the highest concentrations (80% v/v), at 17.50 nmol/min/mg prot and 9.70 nmol/min/mg prot, respectively. The control sample's liver tissue and gills had the highest activity, measuring 34.10 and 46.80 nmol/min/mg prot, respectively.

Table 4: Acute of Oxidative Stress Enzymes Activity in *Clarias gariepinus* Treated with Sniper Insecticide

Organ	Conc mg/L/ Activity	GST (nmol/mg prot)	SOD (unit/ mg prot.)	CAT (Unit/mg prot.)
	0.00	46.80 ±0.01^{b}	1.41 ±0.01^{a}	37.21±2.10^{a,b}
Liver	2.50	32.4 ±1.03^{c}	2.27±0.17^{a}	43.81±1.05^{a,b}
	5.00	28.3 ±0.01^{a}	2.74 ±0.01^{a}	47.54±0.13^{a}
	7.50	21.50 ±0.01^{a}	3.98 ±0.12^{b}	54.90±0.01^{a}
	10.0	17.50 ±1.02^{a}	5.13 ±0.32^{a,b}	79.45±0.71^{a}
	0.00	34.1 ±0.21^{a}	1.53 ±0.22^{b}	47.56±0.01^{a}
Gills	2.50	31.4 ±0.01^{a}	1.65±0.78^{a}	57.32±1.5^{a}
	5.00	28.3 ±0.11^{a,b}	3.80 ±0.07^{a}	61.65±0.54^{a}
	7.50	13.10 ±0.93^{a}	4.71 ±0.91^{a}	76.45±1.03^{a}
	10.0	9.70±0.11^{a,b}	4.85 ±1.00^{a}	83.78±0.66^{a}

Mean values with different superscripts alphabets in a column differed significantly with LSD ($P<0.05$)

Acute toxicity of Sniper indicates treatment-related tissue alterations which intensified with an increase in herbicide concentration. Histological investigation of the gills indicates normal gill architecture of the lamellae in the control group without any discernable pathological change (Plate 1a). After 96hr exposure duration to varying Sniper concentrations, 2.50mg/L revealed lifting of epithelium thickening (Plate 1b). Alterations examined in 5.00mg/L were lifting of the primary lamellae (Plate 1c). Besides, secondary lamellae degeneration and blood congestion were observed at 7.50mg/L (Plate 1d). At 10.0mg/L the gills revealed massive lamellae degeneration, necrosis and distortion of secondary gill lamellae (Plate 1e).



Gills: Photomicrograph of control sample gills tissue of *C. gariepinus* indicating normal gill architecture of the lamellae in the control group without any discernable change (Plate 1a). Lifting of epithelium thickening (Plate 1b). Distortion of the primary lamellae (Plate 1c). Secondary lamellae degeneration and blood congestion (Plate 1d). Massive lamellae degeneration, necrosis and distortion of secondary gill lamellae (Plate 1e). (H&E, mag x 100).

Discussion

Acute toxicity investigation in aquatic environment is commonly applied for toxicity assessment of pollutants on non-target organisms such as fish (Annett *et al*., 2014). Fish's behavioural changes have direct effect on its physiology which is considered as a significant symptom for assessing the pollution status of the environment (Nwani *et al.*, 2013). During the study period, exposure to varying Sniper concentrations made the experimental fish restless, swam erratically, discharges mucus and air gulping increases with an increase in concentration. These alterations could be due to the susceptibility of the exposed fish to the toxic effect of Sniper. Finding from this study demonstrated that Sniper herbicide exerted abnormal swimming pattern and lost reflex in the exposed fish. This in consistent with the findings of Inyang *et al*. (2017), Michael (2018) and Doherty *et al*. (2019) who reported similar behavioural alterations in fish exposed to diazinon and Sniper respectively. Acute toxicity of fish species is have been associated with excessive gulping of air, erratic swimming, restlessness, increased operculum movement, excessive secretion of mucus and body pigmentation, and jerky movement (Inyang *et al*., 2017). Behavioural response have been affected by concentrations of herbicide, species, age, sex and prevailing environmental condition (Karthigayani *et al*., 2014). The air gulping index showed respiratory distress probably due the effect of the toxicants. The decrease in opercular movement and air gulping at the highest concentration is attributed to the response by the fish due to the metabolic changes in fish trying to cope with the toxic effect of the herbicide leading to the decrease in

opercular movement. The air gulping experienced by the experimental fish could be due to the effect of active ingredients in the Sniper to the fish's body physiology as examined by Paulino *et al*. (2012) and Khoshnood *et al*. (2014). Excessive mucus secretion observed in the exposed fish could be due to the herbicides' irritating effect on the fish's body physiology as reported by Doherty *et al*. (2019) and Nafiu and Ibrahim (2021). The decrease in opercular movement could also be associated with the depletion of oxygen in the experimental water making it difficult to cope with the water quality as reported by Micheal (2018). The behavioral changes recorded in the present finding are inconsistent with the finding of Ani *et al*. (2017) on glyphosate.

The LC_{50} values depend on varying chemistries of the compound, age, fish species and the nature of the test solution (Santos and Martinez. 2012). After 96hr of exposure to Sniper, the LC_{50} recorded was 9.56mg/L. The value obtained is higher than 0.553 mg/L reported Doherty *et al*. (2019) when C. *gariepinus* was exposed to sniper but lower than 12.4mg/L and 24.35 mg/L recorded by Khoshnood *et al*. (2014) and Santos and Martinez (2012) in *Caspian kutum* and Neotropical fish on exposer to Sniper. The variation in the LC_{50} might be attributed to the susceptibility to the insecticide, health status, age, size and the fish species. The differences in the LC_{50} could also be attributed to the bioaccumulative capacity, biomagnification and excretion of the chemical in the water (Akinsorotan *et al*., 2019). Khoshnood *et al*. (2014) reported that toxicity of pesticide was both time and concentration dependent, leading to variation in LC50 values.

Oxidative stress experienced by aquatic biota when exposed to pollutants facilitates generation of reactive oxygen species (ROS) and other oxygen free radicals resulting in the alterations of their functions (Hamed, 2015). Antioxidant enzymes such as LPO, SOD, CAT and GST neutralize the toxic effect of ROS in many organisms including fish (El-demerdash *et al*., 2013).

During this study, LPO concentration enhancement in the gills and liver tissues in a concentration dependent manner which could be associated to the failure of antioxidant defense system to cope with an increased reactive oxygen species radicals generation or might be attributed with the metabolism of the sniper causing membrane lipids peroxidation in the tissues examined (Mohamed and Gad, 2008; Ansari and Ansari, 2014). Lipid peroxidation is one of the cellular mechanisms causing degeneration of insecticide-induced oxidative stress in fish tissues (Ansari and Ansari, 2012). The ROS generated during oxidative stress coupled with the presence of unsaturated fatty acids available in cellular membrane facilitate an increased in SOD in the fish tissues. Similar observation was reported by Nwani *et al*. (2013) who depicted that peroxidation of lipids produce free-radicals which disrupt the lipid membrane bilayer structure, leading to cellular damage.

Superoxide dismutase (SOD) is one of the frontline antioxidant enzymes present in a cell protecting organisms against oxidative stress by scavenging superior oxide and hydroxyl radicals against oxygen toxicity (Yang *et al*., 2020). It converts superoxide radicals produced in the peroxisomes and mitochondria to H_2O_2, which finally catalyze to harmless molecules of oxygen and water by another antioxidant enzyme (Catalase) (Ullah *et al*., 2019). During this study, SOD activity revealed an increased in the exposed fish tissues with an increase in the insecticide concentration. An increased in SOD activity revealed a high oxygen radical generation which stimulates the overproduction of SOD activity to an extent exceeding the level of superoxide anions thereby inactivating the radicals (Inyang *et al*., 2018). SOD activity revealed an increased in the exposed fish tissues with an increase in the insecticide concentration. An increase recorded could be due to the high metabolic utilization of the keto acids to gluconeogenesis pathway for glucose synthesis or due to the free amino acids

generation for the synthesis of proteins and ionic regulation (Yildirim and Asma, 2010). An increase might also be attributed to the production of heat shock proteins or destructive free radicals by the insecticide (Ansari and Ansari, 2012).

Catalase an antioxidant enzyme found mainly in the peroxisomes metabolizes hydrogen peroxide into oxygen and water within the cell compartment (Stoyanova *et al*., 2020). An increase in CAT activity in the tissues with an increase in the sniper concentration was recorded. The increase in the activity revealed an inhibition of the enzyme activities by the insecticide' metabolites which is known to disrupting the electron transport between the metabolites and molecular oxygen. This decreases oxygen concentration leading to low ATP production and a decrease in cellular respiration (Wapa *et al.,* 2013).The increase in CAT activity is associated to the high superoxide anion radicals generation explains the accelerated catalase activity (Ezike *et al*., 2019). Yang *et al*. (2020) reported that CAT activity increase in fish tissue due the impairment of the antioxidant defense system which failed to compensate radicals' generation leading to protein oxidation and DNA alteration (Shieh *et al*., 2019). In the present finding, the variation recorded in CAT activity among the tissues might be due to the varying rate for free radicals' generation, body immunity and mobility of radicals within the cellular compartment as reported by (Abdollah *et al*., 2004). Similar observation was reported by Achuba *et al*. (2014) that detoxification system of gill is not as robust as that of liver thus, facilitates its vulnerability towards reactive oxygen species.

GST belongs to phase II biotransformation isoenzyme inhibiting oxidative stress in cells by breaking down superoxide radicals (Mandeep and Rajinder, 2017). An increase in GST activity was examined in gills and liver of the experimental fish compared with the control. The increased in GST activity examined might be due to its effort in catalyzing the metabolites as GST reacts with GSH-group to the metabolites, making them more hydrophilic within the cells (Florescu *et al*., 2020). An increase in GST activity might be due to the immediate effort in responding to the production of superoxide ions by the GST which might suppress protein synthesis. It may perhaps be due to strong cysteine oxidation in resulting in the reduction of the enzyme's activity (Mohamed *et al*., 2019). This agrees with the finding of Mandeep and Rajinder (2017) who reported a decrease in GST activity in liver and gills of *ctenopharyngodon idellus* exposed to chlopyrifos pesticide.

Histopathological alterations among aquatic biota in various tissues/organs are characterized with presence of xenobiotic compounds such as pesticides in the environment (Raibeemol and Chitra, 2016). These chemicals have been reported to cause alteration in gills and liver tissues exposed to Sniper. Epithelial lifting and changes in primary lamellae are known to be nonspecific alterations, leading to disruption of the lamellae examined at 2.50mg/L and 5.00mg/L. Similar observation was reported by Raibeemol and Chitra (2016) and De Moraes *et al*. (2013) who reported severe secondary gill lamellae degeneration in freshwater fish *Pseudetroplus maculatus* exposed to cyhalothrin.

Slight swollen of secondary lamellae and blood congestion observed at 7.50mg/L could be attributed to an increased cellular capillary permeability within the tissues, leading to asphyxiation of the exposed the fish as reported by Faheem and Lone (2017). Asphyxiation due to damage of secondary lamellae has been reported in C. *gariepinus* exposed to Sniper; causing a decline in the gill filaments efficiency and ultimately diffusion of oxygen across the gill lamellae thereby creating a hypoxic compartment within the fish body Michael (2018). At the highest of 10.0mg/L concentrations, massive lamellae degeneration and necrosis were examined in the exposed gills which could be due to thickening of secondary lamella and loss of epithelial cells as observed by Altinok *et al*. (2010). The degree of damage in the present finding were less severe compared to the finding of Michael (2018) who reported thickening

of lamella and sloughing off of the gill filament on exposure to Sniper. Nieves-Puigdoller *et al*. (2007) examined hyperplasia, necrosis in epithelium region and lamellar fusion in Atlantic salmon. Similarly, the gills changes were observed after acute exposure to Sniper in common carp (Velisek *et al*., 2009).

Liver has been regarded as the metabolic organ where detoxification happens (Ullah *et al*., 2019). Liver of the control fish samples had normal hepatocytes architecture whereas in the exposed samples varying degree of damages were identified. At 2.50mg/L, liver tissue displayed lipid granules and necrosis indicating inflammatory response in the liver cells/tissues to the toxicants. The present of lipid granules could be due to the depression in respiratory metabolism by the liver tissues leading utilization of stored intracellular glycogen and the release of hyperglycaemic hormone stimulating the breaking down of more glycogen and glucose in the tissues. Similar observation was reported by Khoshnood *et al*. (2014). On exposure to 5.0mg/L of Sniper, vacuolar degeneration was observed to resulting in stress to fish. Similar observation was reported by Deka and Mahanta (2012) who reported alterations in the liver tissues of *Heteropneustes fossilis* by Malathion pesticides. As the herbicide concentration increases to 7.50mg/L, cellular Infiltration and cytoplasmic vacuolation were examined which might be due to cytoskeletal rearrangement leading to inactivation of liver cells (Nafiu *et al*., 2020). The cytoplasmic vacuolation could be due to the effort by the liver to metabolize Sniper so that it can excrete without causing any significant bioaccumulation in the tissues. This observation is in tandem to finding of Paulino *et al*. (2012). Blahova *et al*. (2014) reported vacuolar degeneration of hepatocyte, dilation of capillaries and hepatic tissue degeneration in common carp as a result of exposure to Sniper. At 10.0mg/L exposure concentration, haemorrhage, necrosis of the hepatocytes and multifocal cellular degeneration were examined in the liver tissues. Multifocal cellular degeneration observed might be due to the energy production stimulation by the liver tissues in combating the stress induced by Sniper concentration as reported by Micheal (2018). Necrosis examined reflects disrupted lipid metabolism an indication of liver dysfunction which corroborates with the finding of Mela *et al*. (2013) who reported necrosis in the liver of neotropical catfish (*Rhamdia quelen*) exposed to Sniper (2-100 μg/L). The haemorrhage recorded could be due to failure of liver to detoxify the Sniper metabolites leading to an increased lesion index within the liver tissue and this may perhaps cause injured liver cells as reported by Micheal (2018).

Conclusion and Recommendations

It was revealed that behavioral, antioxidant enzyme activities and histopathological alterations observed in *C. gariepinus* exposed to Sniper were concentration and time-dependent. Histological alterations are biomarkers of Sniper effect on the exposed fish. Sniper had harmful effect in the experimental fish as it subjected them to varying behavioral and histological distortion. It can be inferred that changes in the histology and activity of the oxidative stress enzymes in fish tissues after exposure to wastewater harm the experimental fish. As a result of their effects on fish and other non-target aquatic biota, it is advised that the competent authorities create initiatives to reduce the indiscriminate discharge of pesticide in aquatic habitat.

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