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Assessment of the Toxic of a Cypermethrin, Deltamethrin and Cyhalothrin Insecticide on the Fingerlings of *Clarias gariepinus* (Burchell, 1822)

[1] A. A. Eli, [2] K. E. Lelei, and [2]R. O. Wenibowei,

[1.] Department of Environmental Management and Toxicology, Faculty of Science, Federal University Otuoke, Bayelsa State, Nigeria
Correspondence Email: akayinaboderi@gmail.com; ORCID: 0009-0002-1521-1102
[2.] Department of Biological Sciences, Faculty of Science, Niger Delta University, Amassoma, Bayelsa State, Nigeria
kariyelelei@gmail.com; ORCID: 0009-0002-2321-440X

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Abstract: *Clarias gariepinus fingerlings of 2.620 ± 0.370g and 6.480 ± 0.598cm mean weight and length were exposed to a Cypermethrin, Deltamethrin and Cyhalothrin insecticide at concentrations of 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L in triplicates with a control (0ml/L) for 96hours. To evaluate the effects on the pH, dissolved oxygen-DO, temperature, salinity, total dissolved solids-TDS, and electrical conductivity-EC of the test media; fish mortality (%), the lethal concentration (96hrLC_{50}) using Probit Plot, the risk of the insecticide, and the oxidative stress indices (the antioxidants- Superoxide dismutase- SOD, Catalase activity- CAT, Glutathione-S-transferase- GST and Malondialdehyde- MDA). The mean ± SD of the data obtained were analyzed and tested using One-way ANOVA. Results obtained showed that, the variations (at $p<0.05$) in the values of the parameters analyzed were concentration-dependent. The values for the TDS (92.00±1.0 to 124.50±2.50mg/L), EC (183.50±1.50 to 249.00±5.00µS/cm) and salinity (133.76±3.20 to 159.36±3.20mg/L) were most affected by the exposure concentrations compared to the control; the EC values were higher than the WHO Guidelines. Mortality was 6.67, 10.0, 56.67, 30.0 and 63.33% in the concentrations respectively. The 96hrLC_{50} extrapolated using Probit was 0.00128ml/L, a value that implied high toxicity of the insecticide which was validated by the risk determined to be >1, indicative of high risk. The oxidative stress indicators- SOD, CAT, and GST values determined in the Kidneys and liver of the fish showed reductions with increase in concentration while the MDA had converse values. These values indicated physiological and pathological abnormalities, and oxidative stress in the fish.*

Keywords: assessment, toxic, cypermethrin, deltamethrin, cyhalothrin insecticide, fingerlings clarias gariepinus

INTRODUCTION

Pesticides have been used in the control of pests and have contributed to increase in food production; conversely, their excessive use presents threats for; air, soil and water quality (Zikankuba *et al*., 2019; Barreto *et al*., 2020), non-target organisms (Olaya-Arenas *et al*., 2020) and human health (Lykogianni *et al*., 2021). Pesticides are of concern in environmental matrices due to their toxicity, persistence and mobility (Manjarres-Lopez *et al*., 2021). Centanni *et al*. (2023), opined that, the fates of pesticides are constantly evolving with the diverse water conditions, management strategies and the properties of the pesticides used. Pyrethroid pesticides, esters of chrysanthemic acid (ethyl 2, 2-dimethyl-3-(1-isobutenyl) cyclopropane-1-carboxylate) (Soderlund, 2012) are a class of insecticides that are effective against a wide range of insect pests derived from pyrethrins. Which are insecticidal chemicals present in natural pyrethrum in the flowers of *Chrysanthemum cinerariaefolium* (Evan and Evans, 2009) that are amongst the important classes of pesticides mostly used in the control of domestic insects (Ayaz and Kumar, 2023). Based on the chemical composition, pyrethroid pesticides are grouped into two- Type I Pyrethroid pesticides that lack an alpha-cyano moiety (like Allethrin, Bifenthrin, Bioresmethrin, Resmethrin, Tefluthrin, Tetramethrin, d-phenothrin, Permethrin amongst others) and Type II Pyrethroid pesticides with an alpha-cyano moiety (e.g., Cyfluthrin, Cyhalothrin, Cypermethrin, Deltamethrin, Fenvalerate, Fenpropathrin, Flumethrin, Fluvalinate, and Tralomethrin). Pyrethroids are classified as moderately hazardous (Gajendiran and Abraham, 2018). The type I Pyrethroid pesticides causes Type I Poisoning syndrome- the symptoms include tremors, poor coordination, prostration, seizures and death. The Type II Pyrethroid pesticides cause Type II Choreoathetosis syndrome- symptoms include hyperactivity, hunched back, salivation and tremors, progressing to sinuous writhing movements (Gupta and Crissman 2013).

Pyrethroid pesticides are lipophilic, they enter the body through the skin, digestive system or respiratory system (Hołyńska-Iwan *et al*., 2018) or by ingestion of contaminated materials. Pyrethroid insecticides are neurotoxic, target the voltage-gated sodium channel's receptor site specifically of insects (Valmorbida *et al.,* 2022), causing change in the membrane potential resulting in abnormal state of hyper-excitability in the nerve cells; alterations in insects with sub-lethal incapacitating 'knockdown' effect; killing exposed insects by binding to sodium channels resulting in excitatory paralysis (Davies *et al*., 2007). They are the only type of pesticides that may be applied on insecticide-treated nets (ITNs) and are the cheapest pesticides for controlling malaria vectors (Van den Berg *et al*., 2021), recommended and present in all WHO-prequalified types of ITNs (WHO, 2020; Lissenden *et al*., 2021), used in the production of long-lasting insecticidal nets (LLINs). The wide use of pyrethroids pesticides, especially for insects control threatens the health of the aquatic organisms as they end up in aquatic ecosystems after use, through spray drifts, run-offs and discharges (Bashir *et al*., 2020; Galadima *et al*., 2021; Ahamad and Kumar, 2023). As the usage of pyrethroids have steadily increased because of their high efficacy and low toxicity, they have already been detected in rivers, sediments and wetlands around the world (Zhu *et al*., 2020; Crane, 2021).

Diverse 'branded' and 'unbranded,' liquid and powder insecticide formulations have flooded markets in Yenegoa Municipality in Bayelsa State, Nigeria; there was need to assess the toxic effects of one of such locally produced insecticide (a Cypermethrin, Deltamethrin and Cyhalothrin mixture) using *Clarias gariepinus* fingerlings. Cypermethrin, Deltamethrin and Cyhalothrin are classed as Type II Pyrethroid pesticides which are easily degradable (through photolysis, hydrolysis and oxidation), but combined (as a 'Tri-pyrethroid insecticide' in this case) will have synergistic effects on exposed non-target organisms, this was the thrust of this study.

MATERIALS AND METHOD

Test Organisms

Two hundred and forty fingerlings of *Clarias gariepinus* of mean weight and length, 2.620 ± 0.370g and 6.480 ± 0.598cm respectively were procured from a fish farm in Akenfa, Yenegoa, Bayelsa State, Nigeria. These were kept in holding in plastic tanks of 50L capacity, acclimatized for seven days in the laboratory of the Department of Biological Sciences, Niger Delta University, Bayelsa State using borehole water (Reish and Oshida, 1987). They were fed twice daily with Coppen® feed (0.8-1.2mm) at 5% body weight twice daily during the holding period with change of media to prevent stress and fouling. The fingerlings were monitored for mortality and behavioural changes to allow for stabilization before exposure. There was no mortality during the holding period.

Toxicant

The toxicant used in this study was a commonly sold insecticide, a mixture of three pyrethroids-Deltatmethrin (0.5%), Cypermethrin (0.2%) and Cyhalothrin (0.4%), thus named, a 'Tri-pyrethroid insecticide'. This amongst others is locally produced in the State and was procured from a market in Yenegoa metropolis, Bayelsa State, Nigeria.

Range Finding Test

This was done to determine the threshold concentrations (concentrations at which minimum responses will be elicited from the exposed fish) of the 'Tri-pyrethroid insecticide'. The concentrations of 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L respectively were determined as the acute toxicity exposure concentrations after 0.025, 0.05, 0.10 and 0.20ml/L respectively adapted after Yidi *et al*. (2021) and Mohammad *et al*. (2022) resulted in one hundred percent (100%) mortality within three hours of exposure.

Experimental Design

A static renewal toxicity testing with five exposure concentrations of 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L and a control (0ml/L) made up to 15L with borehole water of acceptable quality in the 50L plastic tanks was carried out for 96hours (during which the fish were not fed to reduce fouling). The experiment consisted of one hundred and sixty (160) fingerlings of *Clarias gariepinus* randomly allocated to the five exposure concentrations in triplicates, and control using Complete Block Design.

Physicochemical Parameters of Test Media (Water)
The dissolved oxygen (DO), temperature, total dissolved solids (TDS), pH, electrical conductivity (EC) and salinity were analyzed to ascertain the suitability of the water for fish survival (Boyd, 2015) before and after exposure. These were measured *in-situ* using Hanna HI 9828 pH/ORP/EC/DO water analyzing device.

Toxicity Testing
This was based on the determination of the mean values of the evaluated physicochemical parameters of the exposure media and the control, toxicological assessment of the insecticide on the fish with respect to concentration and time based on percentage mortalities of the fish in the exposure concentrations and control; determination of the lethal concentration (96hr $LC_{50;}$ the concentration at which 50% of the test population will die), risk of the insecticide, and antioxidants analyses (the levels of the stress indicators- Superoxide dismutase-SOD, Catalase activity- CAT, Glutathione-*S*-transferase- G*S*T, and Malondialdehyde- MDA in the excised Kidneys and liver to ascertain the oxidation stress levels in the exposed fish based on exposure concentration.

Mortalities
During the 96-hr exposure period, observations were made and records taken of the mortalities in the exposure concentrations every 24hrs and the percentage mortalities deduced with respect to time. Probit Plot of mortality was used to determine the median/lethal concentration (96hr LC_{50}).

Determination of Antioxidants
Each of the homogenate was assessed using total protein assay kit (Randox Laboratories Limited, United Kingdom) following the instructions on the assay kit insert.

Superoxide Dismutase (Superoxide Oxido-Reductase)
A modified version was used to determine the activity of SOD. This method involved measuring the ability of SOD to prevent the autoxidation of pyrogallol. Superoxide dismutase (SOD) functions by catalyzing the dismutation of the superoxide radical (O) into oxygen and hydrogen peroxide.

Inhibition of autoxidation by SOD

SOD Activity= Pyrogallol + O $\longleftrightarrow$ $O^{\cdot -}$+ oxidation product

The assay mixture of 1ml that contains the following in a final concentration shown in parenthesis was prepared: 500µl of 0.1M sodium phosphate buffer pH 8.0 (50mM), 33µl of 3.3mM EDTA (0.1mM), 60µl of 8.1mM pyrogallol (0.48mM) and appropriate amount of sample containing 10µg of protein. The change in absorbance at 420nm of the assay mixture was monitored for 2minutes at 25^{o}C against a blank that was made up of all the ingredients except the sample. The activity of SOD was measured in units per milligram protein, where one unit of SOD was the quantity of enzyme required to produce 50% inhibition of pyrogallol autoxidation.

$$\text{Activity of SOD} = \frac{(\Delta A_{Ref} - \Delta A_{Test})\ \text{Total volume}}{\Delta A_{Ref}/2 \times \text{Sample volume}}$$

Where: ΔA= the change in optical density.

Determination of Lipid Peroxidation (for MDA)

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. This method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde: an end product of lipid peroxide during peroxidation. On heating in acidic pH, the product was a pink complex which absorbed maximally at 532nm and which was extractable into organic solvents such as butanol. Malondialdehyde (MDA) is often used to calibrate this test and thus, the results were expressed as the amount of free MDA produced. 30% Trichloroacetic acid- TCA **(**9g of TCA (CCl_3COOH) was dissolved in distilled water and made up to 30ml with same); 0.75% Thiobarbituric acid- TBA **(**was prepared by dissolving 0.23g of TBA in 0.1M HCl and made up to 30ml with same); 0.15M Tris-KCl buffer (pH 7.4) made from 1.12g of KCl and 2.36g of Tris base dissolved separately in distilled water and made up to 100ml with same. The pH was then adjusted to 7.4. An aliquot of 0.4ml of the sample was mixed with 1.6ml of Tris-KCl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45minutes at 80^{o}C. This was then cooled in ice and centrifuged at 3000rpm. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm. The MDA level was calculated. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}Cm^{-1}$.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{E_{532nm} \times \text{volume of sample x mg protein}}$$

Determination of Catalase Activity (CAT)

The assay technique ‘des’ was used to determine catalase activities in this study. It relied on the measurement of the rate of decomposition of H_2O_2 following its reaction with the oxidizing agent potassium permanganate ($KMnO_4$). Catalase catalyzed the quick breakdown of H_2O_2 to water and molecular oxygen. Catalase activity in the sample investigated was directly proportional to the rate of this reaction.

$$2H_2O_2 \text{----------} 2H_2O + O_2$$

Excess $KMnO_4$ reacted with any remaining H_2O_2. The residual permanganate level, measured using spectrophotometry at a wavelength of 480nm indicated the catalase activity. To measure catalase activity, 0.5ml of the sample was mixed with 5ml of 30mM H_2O_2 in an ice-cold test tube, and the reaction was stopped after 3minutes by adding 1ml of 6M H_2SO_4 followed by mixing. The resulting solution was then measured for absorbance at 480nm against distilled water within 30-60 seconds. A blank solution was prepared in the same way as the test, but with 0.5ml of distilled

water instead of the sample. A spectrophotometric standard was also prepared by adding 7ml of 0.01M $KMnO_4$ to a tube containing 5.5ml of 0.05M Phosphate buffer (pH 7.4) and 1ml of 6M H_2SO_4 solution, and its absorbance value was measured at 480nm. The catalase activity was then calculated using the formula:

$$\text{Catalse Activity (Kc)} = \text{Log}[\frac{s0}{s3} \times 2.03]$$

Where; S_0 = Absorbance of standard - Absorbance of blank; S_3 = Absorbance of standard - Absorbance of test.

Determination of Glutathione Peroxidase (for GST)

Glutathione peroxidase catalyzes the following reversible reaction.

$$H_2O_2 + 2GHS \xleftrightarrow{GPx} 2H_2O + GSS$$

The measurement of glutathione peroxidase (GPx) activity was carried out using a coupled enzyme assay system, which was connected to glutathione reductase (GR).

$$H_2O_2 + 2GHS \xleftrightarrow{GPx} 2H_2O + GSSG$$

$$GSSG + 2NADPH \longleftrightarrow 2GSH + 2NADP^+$$

A mixture for the assay was prepared with a final volume of 1ml and the following concentrations: 0.4ml of 250mM potassium phosphate buffer (100mM) with pH 7.0, 0.025ml of 100mM EDTA (25mM), 0.025ml of 80mM sodium azide (2mM), 0.025ml of 20mM reduced glutathione (0.5mM), GR (1.5U), 0.05ml of 2mM NADPH (0.1mM), and the enzyme sample containing approximately 50 µg of protein. The reaction was initiated by adding hydrogen peroxide. The reduction in absorbance was recorded for 5minutes at 25°C, measuring the oxidation of NADPH at 340nm with a spectrophotometer, compared to a blank containing all ingredients except the hydrogen peroxide. Activities of GPx are expressed as unit/gm tissue weight/minute. One unit of enzyme activity is defined as the amount of enzyme required to oxidize one micromole of NADPH per gram tissue wet weight per minute at 25°C. The following formula was used for the calculation of enzyme activities:

$$\text{Activity} = \frac{\Delta A \times \text{Total volume} \times \text{Dilution}}{\text{Volume of sample} \times 6.22 \times \text{Time}}$$

Where; ΔA = the change in absorbance per minute and 6.22 is the extinction coefficient of NADPH at 340nm.

Statistical Analysis

Data obtained were analyzed for mean ± standard deviation. One-way Analysis of Variance (ANOVA) was used to compare the differences in the means at $P < 0.05$ which were tested using Duncan's Multiple Range Test using SPSS® version 2.1.0.

Results

Physicochemical Parameters

The results of the mean values determined for the physicochemical parameters of the test media (Table 1) showed that, there were significant differences ($P<0.05$) in the exposure concentrations; with obvious differences in the values of TDS (which ranged from 92.00±1.0 to 124.50±2.50mg/L in the exposure concentration while the control was 119.30±3.33mg/L), EC (ranged from 183.50±1.50 to 249.00±5.00µS/cm with 253.00±5.23µS/cm in the control) and salinity values (ranged from 133.76±3.20 to 159.36±3.20mg/L while control was 151.50±11.37mg/L).

Table 1: The Mean ± Standard Deviation of the Physicochemical Parameters of the Test Media of the Different Exposure Concentrations of the 'Tri-Pyrethroid Insecticide'

Exposure Concentrations (ml/L)	Temperature (°C)	pH	DO (mg/L)	TDS (mg/L)	EC (µS/cm)	Salinity (mg/L)
0	26.90±0.10^{a}	6.95±0.04^{cd}	4.73±0.09^{ab}	119.30±3.33^{d}	253.00±5.23^{d}	151.50±11.37^{d}
0.0045	27.85±0.05^{b}	6.83±0.10^{ab}	5.37±0.25^{ab}	110.50±8.50^{bc}	221.50±17.50^{bc}	141.76±11.20^{b}
0.0085	27.95±0.05^{bc}	6.59±0.08^{a}	5.31±0.70^{ab}	104.00±3.00^{b}	209.00±5.00^{b}	133.76±3.20^{ab}
0.0110	27.95±0.05^{bc}	6.92±0.03^{c}	4.94±0.04^{a}	92.00±1.00^{a}	183.50±1.50^{a}	117.44±0.96^{a}
0.0125	28.00±0.00^{c}	6.79±0.12^{bc}	5.62±0.30^{b}	124.50±2.50^{d}	249.00±5.00^{c}	159.36±3.20^{cd}
0.0150	28.10±0.00^{d}	7.09±0.01^{d}	5.68±0.17^{b}	115.50±11.50^{cd}	231.00±22.00b^{c}	147.84±14.80^{cd}
WHO (2008)	<40	6.5–8.5	> 4	500	70	<600
USEPA (2011)	-	6.5–8.5	-	500	-	-

Key: Means with the same superscripts down the columns are not statistically different at $P<0.05$; DO = Dissolved Oxygen; pH = Potential Hydrogen; EC = Electrical Conductivity; TDS = Total Dissolved Solids; WHO = World Health Organization; USEPA = United States Environmental Protection Agency

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Mortality

For mortality, there were 32, 9, 6 and 4 dead fish at the 24th, 48th, 72nd, and 96th hour. The exposure concentrations of 0.0045, 0.0085, 0.011, 0.0125 and 0.015ml/L had 6.67, 10.0, 56.67, 30.0 and 63.33% mortality respectively while the control had one (Figure 1). These indicated that, mortality reduced with time (attributed to the sequestration of the insecticide) but increased with increase in concentration, thus, mortality was concentration-dependent.

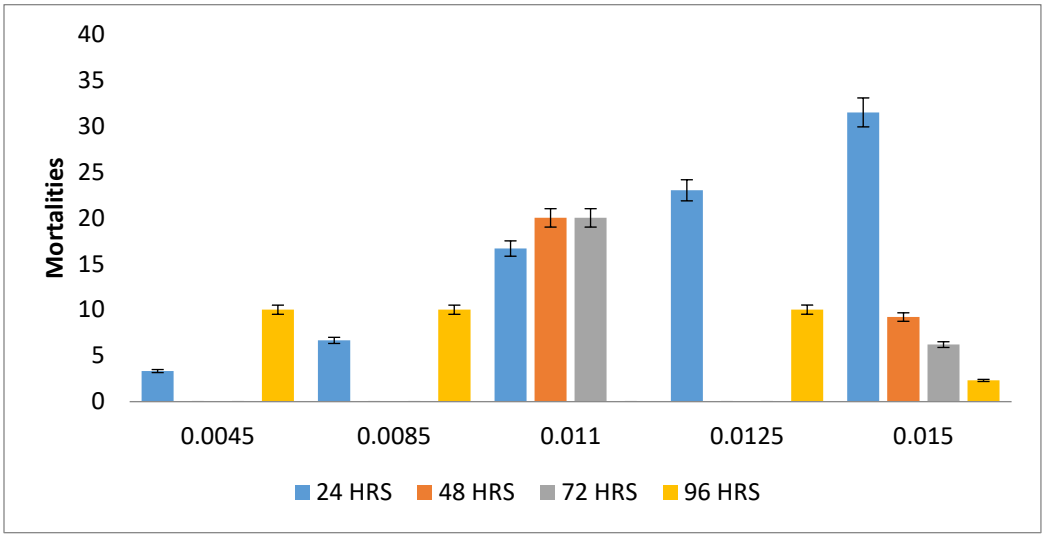

Figure 1: Mortalities of *Clarias gariepinus* fingerlings in the Different Exposure Concentrations of the 'Tri-Pyrethroid Insecticide' with time.

The lethal concentration (96hr LC_{50}) of the insecticide was extrapolated to be 0.0128ml/L which implied that, this 'Tri-pyrethroid insecticide' was highly toxic.

The risk of the insecticide (USEPA, 1998) was extrapolated using 'whole mixture approach', the risk was determined based on:
Risk = Exposure (duration and amount) x Toxicity
And gave a value of >1.

Antioxidants

The mean ± SD values of the antioxidants; Superoxide dismutase (SOD), Catalase activity (CAT), Glutathione-*S*-transferase (G*S*T) and Malondialdehyde (MDA) levels indicated concentration-dependent effects in the kidneys and livers of *C. gariepinus* fingerlings exposed to the different concentrations of the insecticide at 96hr (Figures 2 a-h). There were variations in the mean values which were significant ($P < 0.05$) in the levels of the antioxidants in the kidneys (Figures 2a- d). Generally, in the kidneys, the SOD levels (ranged from 1.850±0.026 to 2.997±0.051U/mg protein), CAT (1.823±0.025 to 2.190±0.036U/mg protein) and G*S*T (2.227±0.025 to 3.373±0.031U/mg protein); their levels reduced with increase in the exposure concentration with the control having

the highest values (4.803±0.045, 3.090±0.026 and 4.240±0.046U/mg protein for SOD, CAT and GST respectively). While the MDA values showed wide fluctuations; the highest exposure concentration of 0.0150ml/L gave a higher value of 4.923±0.045U/mg protein than the lowest exposure concentration (3.673±0.03U/mg protein) while the control had the least value (1.393±0.03U/mg protein).

In the livers of *C. gariepinus* fingerlings from the different concentrations, the values of Superoxide dismutase (SOD), Catalase activity (CAT), Glutathione-*S*-transferase (GST) and Malondialdehyde (MDA) also showed significant variations ($P<0.05$) by the 96th hour (Figures 2e-h). The SOD levels (ranged from 2.57±0.0 to 4.59±0.050U/mg protein), CAT (2.09±0.020 to 3.29±0.030U/mg protein) and GST (2.39±0.02 to 4.10±0.03U/mg protein); these values reduced with increase in the exposure concentration with the control having the highest values (5.39±0.04, 3.71±0.07 and 6.01±0.09 U/mg protein for SOD, CAT and GST respectively). For the MDA (which ranged from 3.41±0.04 to 5.98±0.03U/mg protein), the values generally increased with increase in concentration while the control had the least value (1.70±0.03 U/mg protein).

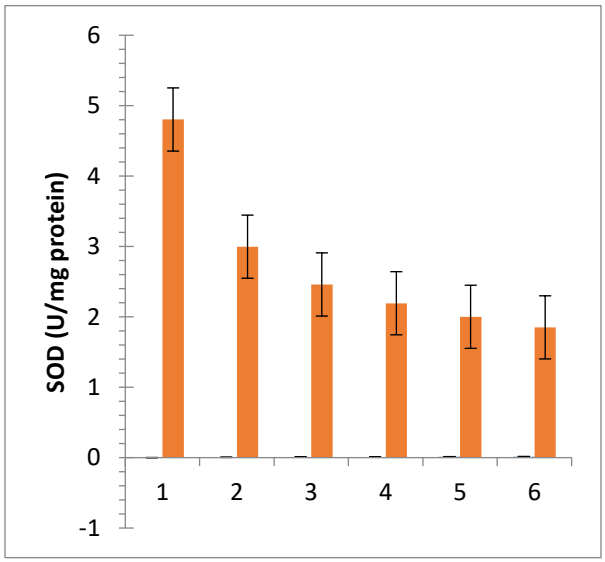

Figure 2a: Showing the mean Superoxide dismutase (SOD) Levels in the Kidneys of *Clarias gariepinus* fingerlings exposed to different concentrations of the 'Tri-Pyrethroid Insecticide' after 96hr

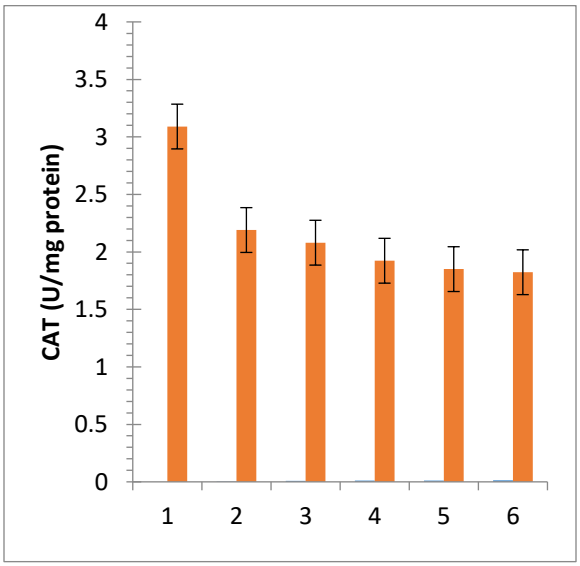

Figure 2b: Showing the mean Catalase activity (CAT) levels in the Kidneys of *Clarias gariepinus* fingerlings exposed to different concentrations of the 'Tri-Pyrethroid Insecticide' after 96hr

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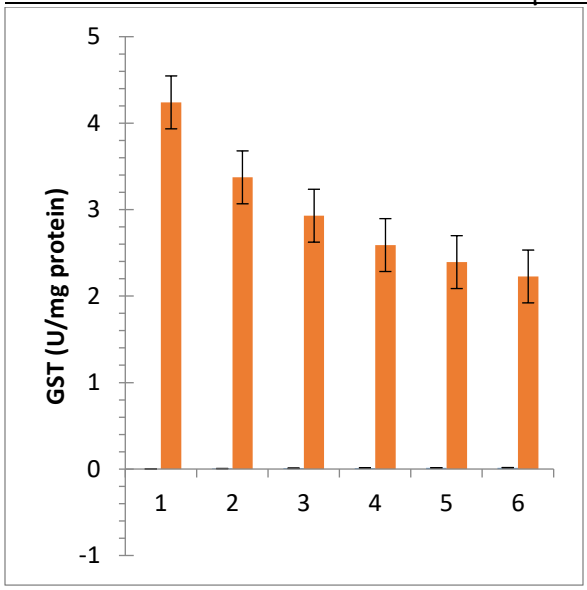

Figure 2c: Showing the mean Glutathione-*S*-transferase (GST) levels in the Kidneys of *Clarias gariepinus* fingerlings exposed to the concentrations of the 'Tri-Pyrethroid insecticide' after 96hr

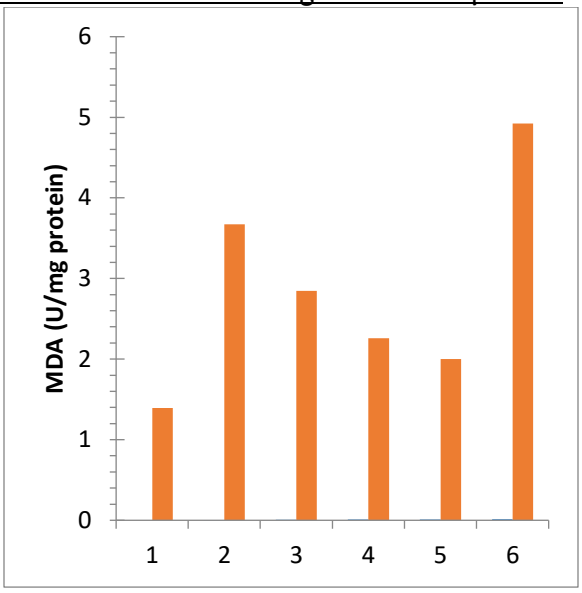

Figure 2d: Showing the Mean Malondialdehyde (MDA) levels in the Kidneys of *Clarias* fingerlings exposed to the concentrations of the 'Tri-Pyrethroid Insecticide' after 96hr

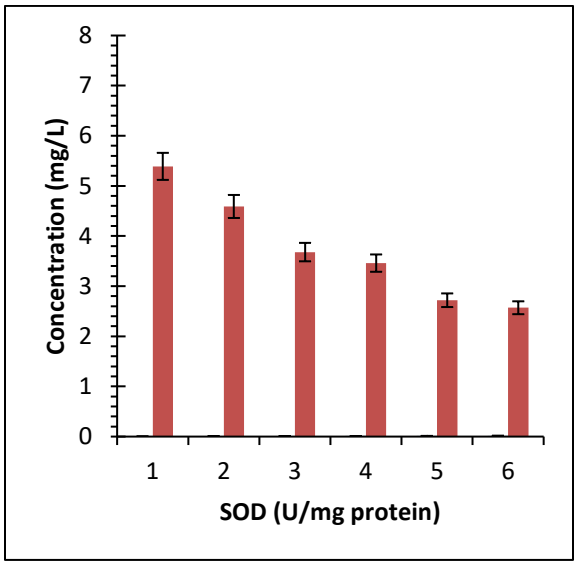

Figure 2e: Showing the mean SOD levels

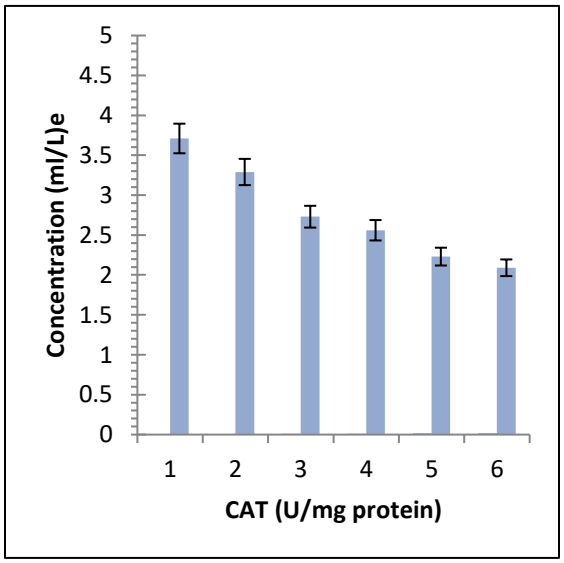

Figure 2f: Showing the mean CAT Levels

in the Liver of *Clarias gariepinus* fingerlings exposed to the concentrations of the 'Tri-Pyrethroid Insecticide' after 96hr

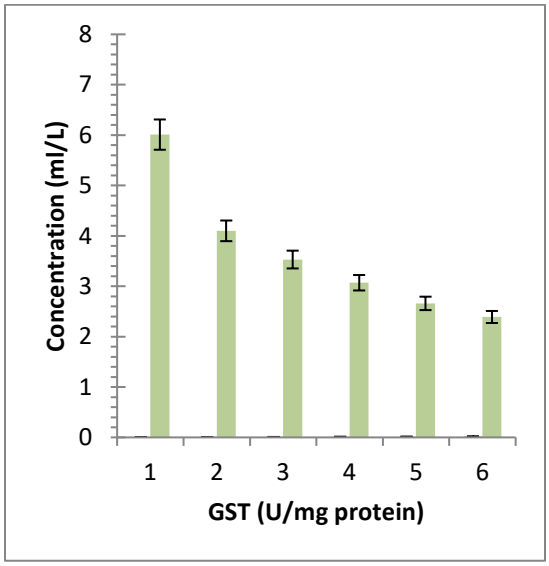

Figure 2g: Showing the mean GST levels in the Liver of *Clarias gariepinus* fingerlings exposed to the concentrations of the 'Tri-Pyrethroid Insecticide' after 96hr

in the Liver of *Clarias gariepinus* fingerlings exposed to the concentrations of the 'Tri-Pyrethroid Insecticide' after 96hr

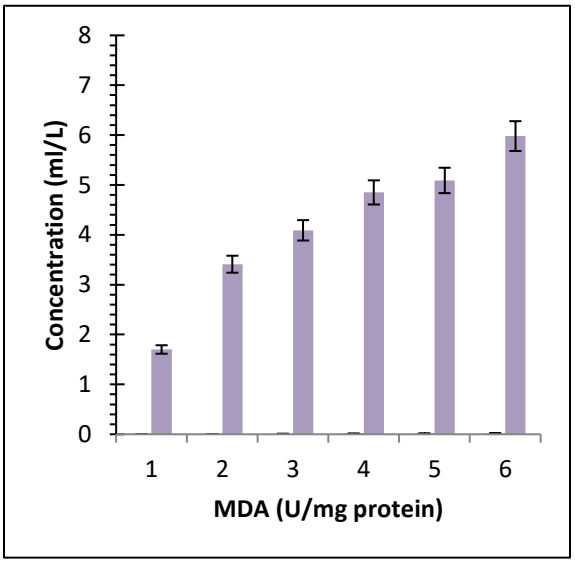

Figure 2h: Showing the mean MDA levels the Liver of *Clarias gariepinus* fingerlings exposed to the concentrations of the Tri-Pyrethroid Insecticide' after 96hr

(N/B: 1, 2, 3, 4, 5 and 6 represent the concentrations, 0.0, 0.0045, 0.0085, 0.0110, 0.0125 and 0.0150ml/L respectively).

DISCUSSION

The variations in the values of the TDS, EC and salinity among the exposure concentrations in the test media (water) were attributable to increased physiological activities in the fish in response to increased stress due to the toxicant (insecticide). The DO and EC levels were observed to be higher than the WHO (2008) and USEPA (2011) guidelines for water thus, fish; implying increased presence of solutes in the test media which indicated increased physiological activities and stress of the fish. The parameters determined were affected by the toxicant and the variations were concentration-dependent with attendant negative implications for fish physiology. These had similar patterns to the findings of Hossain *et al*. (2022), who worked on the effects of Chlorpyrifos on *Oreochromis niloticus.* Salako *et al.* (2020), in their analysis of the physicochemical parameters of the water for *P. reticulata* exposed to varied concentrations of Cypermethrin, Deltamethrin and

Lambda-cyhalothrin pesticides respectively showed that, the parameters were affected in a time-dependent manner which were contrary to the findings (concentration-dependent effects) of this study.

The toxicity of pesticides in aquatic systems is expressed in terms of LC_{50}. The lethal concentration ($96hrLC_{50}$) in this study was extrapolated to be 0.0128ml/L. The lower the LC_{50} value, the higher the toxicity (Shefali *et al*., 2021). The 96hr LC_{50} value from this study was low thus, indicated that the toxicity of the insecticide was high. Using 'whole mixture approach', the risk was extrapolated to be >1 which indicated high risk, validating the toxicity. The magnitude of risk is proportional to both the potency of the chemical and the extent of exposure (USEPA, 1998), this was the case of finding of this study. The Type II Pyrethroid pesticides are classed as moderately hazardous (Gajendiran and Abraham, 2018), as a 'Tri-Pyrethroid (mixture of Cypermethrin, Deltamethrin and Cyhalothrin) insecticide' used in this study, it was determined as highly toxic ($96hrLC_{50}$ value of 0.0128ml/L) and a high risk insecticide.

Prudencio *et al*. (2023), investigated the acute toxicity of the insecticides- Pyrinex Quick 212 EC (Deltamethrin $12gL^{-1}$and Chlorpyrifos $200gL^{-1}$) and Pyro FTE 472 EC (Cypermethrin $72gL^{-1}$and Chlorpyrifos $400gL^{-1}$) on *Clarias gariepinus* juveniles for 96hr at increasing concentrations of each insecticide. The values of the $96hrLC_{50}$ were 0.004 and 0.012 µL L^{-1} for Pyrinex and Pyro respectively, indicating very high toxicity to *C. gariepinus* juveniles. The $96hrLC_{50}$ of Pyro FTE 472 EC (Cypermethrin $72gL^{-1}$and Chlorpyrifos $400gL^{-1}$) was similar to the $96hrLC_{50}$ value of of 0.0128ml/L extrapolated in this study using the 'Tri-pyrethroid insecticide' on *C. gariepinus* fingerlings. Which also reflected in the fish mortalities observed. Salako *et al.* (2020), in their study using the frys of *Poecilia reticulata* (Guppy fish) exposed to acute concentrations of Cypermethrin, Deltamethrin and Lambda-cyhalothrin respectively for 96hrs exhibited varying degrees of toxicity. The exposure concentrations of 60, 20 and 20µg/L resulted in 20%, 15% and 20% mortalities for Lamda-cyhalothrin, Cypermethrin and Deltamethrin respectively. The $96hrLC_{50}$ values were Cypermethrin - 27.07µg/L, Lambda-cyhalothrin - 81.83µg/L and Deltamethrin - 31.51µg/L; with Cypermethrin more toxic to the fish than Deltamethrin and Lambda-cyhalothrin respectively. The fish were observed to have responded in a time-dependent manner with the highest mortality at the 96^{th} hour. These observations were contrary to the findings of this study in which the 'Tri-pyrethroid insecticide' (mixture) was determined to be 'much more' toxic (much lower exposure concentrations and a $96hrLC_{50}$ of 0.0128ml/L) with higher mortalities- a result of the synergistic effect of the mixture (Cypermethrin, Deltamethrin and Cyhalothrin) compared to the individual pesticide as shown in the findings of Salako *et al*. (2020). Though, this study had Cyhalothrin while the former had Lamda-cyhalothrin; and the fish species and stages also differed- the presence of yolk in the fry can confer maternal immunity on the fry thereby, reducing susceptibility to toxicants (Lelei, 2013); which could have accounted for the varied outcomes of both findings. It was evident from the results that, the 'Tri-pyrethroid' insecticide was more toxic than the individual pyrethroids.

Oxidative stress plays a key role in shaping fish's responses to environmental changes and are increasingly being used as biomarkers to indicate, as well as, to understand the mechanisms of various toxicants for ecotoxicological risk assessments. Oxidative stress is a pathological process or causative factor that might have a role in the development of many diseases (Ilona and Högberg, 2011). Oxidative stress due to decrease in antioxidant capacity (Agnieszka *et al*., 2018) as a result of the effects of the insecticide were determined in the excised kidneys and liver of *C. gariepinus* fingerlings, and were concentration-dependent; indicative of toxic effects in the exposed fish. The oxidative stress indices - Superoxide dismutase (SOD), Catalase activity (CAT), Glutathione-*S*-transferase (G*S*T) and Malondialdehyde (MDA) levels, antioxidants that indicate oxidative stress and tissue damage in the kidneys and livers of *C. gariepinus* when compared with control showed that, the levels of SOD, CAT and GST in the kidneys and livers reduced with increase in the exposure concentration. The control had higher values. There were fluctuations in the MDA values in the kidneys, which though increased with increase in concentration, and had the highest level in the highest concentration. The liver showed no fluctuations in the MDA values and increased with increase in concentration. The MDA values were lowest in the control. These findings were similar to those of Parlak (2018); Yidi *et al*. (2021); and Wu *et al*. (2022), on Deltamethrin on Zebrafish, *Channa argus*, and Crucian carp respectively. Yuan *et al.* (2023), in their study using Crucian carp exposed to Deltamethrin (0.61, 1.22, 2.44 and 4.88µg/L) for 24h revealed increased MDA levels but markedly decreased CAT and SOD activities in the exposure groups. Which were also similar to the findings of this study. Paravani *et al.* (2019), reported that, SOD and CAT were activated in Zebrafish after exposure to 0.6µg/L Cypermethrin. Increase in MDA, G*S*T with decrease in SOD and CAT activities were observed in different species of fish exposed to Deltamethrin (Maisnam and Gupta 2014). These affirmed that, the Tri-pyrethroid insecticide induced oxidative stress in the exposed *C. gariepinus* fingerlings. SOD and CAT play important roles in resisting oxidative stress/oxidative defense (Yang *et al*., 2017), G*S*T is involved in toxicant biotransformation. Higher SOD, CAT and G*S*T activities as observed in this study were indicative of oxidative stress in *C. gariepinus* fingerlings. Reactive oxygen species (ROS) are essential for normal cellular functions and play key role in cellular defense (Wu *et al*., 2017), pathological processes and aging. However, oxidative stress/damage to tissues is caused by excessive production of ROS during metabolism (Awoyemi *et al*., 2019). When ROS generation exceeds elimination, it exceeds antioxidant defense and is called 'oxidative stress'; this is associated with the harmful effects of the presence of toxicants (Zhang *et al*., 2020; Zhao *et al*., 2020; Zhao *et al*., 2021) which was the case in this study. The liver is the central metabolic organ, whereas, the kidney is an important site for filtration, re-absorption and detoxification. The plausible reasons for the values of the antioxidant indices being higher in the liver than the kidneys of the test fish in this study.

CONCLUSION

The findings from *Clarias gariepinus* fingerlings exposed to a Cypermethrin, Deltamethrin and Cyhalothrin insecticide for 96hours revealed concentration-dependent effects on the physicochemical parameters of the test media (water); fish mortality (%), lethal concentration

(96hrLC_{50}), risk of the insecticide, and on the oxidative stress indices-SOD, CAT, GST and MDA activities. These findings implied that the 'Tri-pyrethroid insecticide' was highly toxic to the fingerlings of *C. gariepinus* and by extension, other non-target organisms that may be found in environments where such toxicant may be applied. Thus, caution is highly needed in the utilization of insecticides of this sort, especially mixtures.

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