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Haematological Responses and Growth Performance of *Clarias gariepinus* (Burchell, 1822) Fingerlings exposed to a Cypermethrin, Deltamethrin and Cyhalothrin Insecticide

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Abstract: *The fingerlings of Clarias gariepinus of 2.620 ± 0.370g and 6.480 ± 0.598cm weight and length were exposed to a Cypermethrin, Deltamethrin and Cyhalothrin insecticide at 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L in triplicates with a control (0ml/L) for 96hours. Some haematological parameters and blood performance (BP) were assessed to determine the 'health status' of the fish. The growth performance of the survivor fish from the exposure concentrations two weeks after exposure were also evaluated based on some growth parameters- mean weight gain (WG), specific growth rate (SGR%), relative growth rate (RGR%), and condition factor (K). The means ± SD of data obtained were determined and analyzed using One-way ANOVA. Results showed that the variations (at $p<0.05$) in the values of the parameters analyzed were concentration-dependent. The red blood cells, haemocrit, haemoglobin, mean corposular haemoglobin counts reduced with increase in concentration while the white blood cells, lymphocytes, eosinophils counts, and mean corposular haemoglobin concentration reduced with increased concentration, the basophils and monocytes counts were also affected. The BP values reflected the negatively impacted physiological condition and 'health status' of the fish. The growth performance premised on the WG (1.20±2.580 - 6.02±4.820g), SGR (11.06 - 22.68%), and RGR (54.9 - 217.46%) gave values that were comparatively better with increased concentration, than the control (2.29±2.772g, 14.96% and 109.56% respectively) which implied good recovery of the survivor fish. The K value, indication of the state of well-being of the fish had values from 0.69 - 1.60 from the concentrations while the control was 0.50, indicative of better well-being in the survivors with the removal of the toxicant.*

Keywords: Pyrethroid insecticide, *Clarias gariepinus*, blood parameters.

INTRODUCTION

Pyrethroid pesticides commonly utilized around the world since the 1980s, are a type of synthetic organic insecticides used due to their high efficiency and low toxicity when compared to the organophosphate and carbamate groups of pesticides (Yoo *et al*., 2016). Pyrethroid pesticides, esters of chrysanthemic acid (ethyl 2,2-dimethyl-3-(1-isobutenyl) cyclopropane-1-carboxylate) (Soderlund, 2012) are effective against a wide range of insect pests. Derived from pyrethrins, these insecticidal chemicals present in natural pyrethrum in the flowers of *Chrysanthemum cinerariaefolium* (Evan and Evans, 2009), are amongst the important classes of pesticides mostly used in the control of pest populations and domestic insects (Ayaz and Kumar, 2023). There are over thirty pyrethroids; based on chemical composition, pyrethroid pesticides are grouped into two- Type I Pyrethroid pesticides which lacks an alpha-cyano moiety (Allethrin, Bifenthrin, Bioresmethrin, Resmethrin, Tefluthrin, Tetramethrin, d-phenothrin, and Permethrin) and Type II Pyrethroid pesticides with an alpha-cyano moiety (Cyfluthrin, Cyhalothrin, Cypermethrin, Deltamethrin, Fenvalerate, Fenpropathrin, Flumethrin, Fluvalinate, and Tralomethrin) are classified as moderately hazardous (Gajendiran and Abraham, 2018). The Type I Pyrethroid pesticides causes Type I poisoning syndrome, or "T syndrome," the symptoms include tremors, poor coordination, prostration, seizures and death. The Type II Pyrethroid pesticides cause Type II Choreoathetosis syndrome, or "CS syndrome," with symptoms like hyperactivity, hunched back, salivation, tremors, progressing to sinuous writhing movements (Gupta and Crissman 2013). Pyrethroid insecticides have several other sites of action- affect the sodium channels in the nerve membranes which is responsible for causing the repetitive neuronal discharge; some of them cause the inhibition of Ca^{2+}, Mg^{2+}, -ATPase which results in the interference with calcium removal from nerve endings, causing the release of neurotransmitters in the postsynaptic gap (Shefali *et al*., 2021). 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 mosquitoes (Van den Berg *et al*., 2021), recommended and present in all WHO-prequalified types of insecticide-treated nets (WHO, 2020; Lissenden *et al*., 2021), used in the production of long-lasting insecticidal nets (LLINs). There are over thirty pyrethroids widely used in insecticide formulations; even combined to reduce insect resistance and improve the effectiveness of formulations. The wide use of pyrethroids pesticides threatens the health of the aquatic organisms as they end up in aquatic ecosystems 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), especially in non-target organisms like fish.

There has been influx of varied 'labeled and unlabeled', 'powder and liquid' insecticide formulations of recent into markets in Yenegoa, Bayelsa State, Nigeria, there was need to assess the toxic effects of a locally produced insecticide (a Cypermethrin, Deltamethrin and Cyhalothrin mixture) using *Clarias gariepinus* fingerlings. Easily degradable (through photolysis, hydrolysis and oxidation), Cypermethrin ($C_{22}H_{19}Cl_2NO_3$), Deltamethrin ($C_{22}H_{19}Br_2NO_3$) and Cyhalothrin ($C_{23}H_{19}C1F_3NO_3$) are Type II Pyrethroid pesticides but when combined as 'a Tri-pyrethroid insecticide' will have synergistic effects on exposed non-target organisms, this was the focus 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 acclimatized for seven days, kept in holding in plastic tanks of 50L capacity 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, Cypermethrin and Cyhalothrin, thus named, a 'Tri-pyrethroid insecticide'. This 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

Toxicological assessments of fish to the insecticide with respect to concentration and time were carried out based on haemolotogical analyses, blood performance (BP), and growth performance of the survivor fish two weeks post-exposure. For which the mean lengths (cm) and weights (g) of the survivor fish from the exposure concentration after two weeks were used to determine the growth performance based on; the mean weight gain (WG), Specific growth rate (SGR%) which is the measure of the daily growth rate, and relative growth rate (RGR%) which is the increase in growth of the fish during the post-exposure phase. The condition factor (Fulton's condition factor-K) which is the well-being of the fish (Ricker, 1975) from the different exposure concentrations during the growth period were also determined against the control.

Haematological Analyses

Blood was obtained from the test fish (*Clarias gariepinus*) from the different exposure concentrations (0.0045, 0.0085, 0.011, 0.0125 and 0.015ml/L) and control (0.0ml/L) after 96hr of exposure by cardiac puncture using heparinized needles (1.0 mm). This was transferred into EDTA (Ethylenediamine tetraacetic acid) bottles for haematological analyses. The haematological parameters analyzed were: Haematocrit (Ht), Haemoglobin (Hb), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Mean Corposular Haematological Count-MCHC: Eosinophils, Basophils, Lymphocytes and Monocytes; Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin concentration (MCHC).

Hematocrit (Ht)

The blood sample was taking by capillary attraction into the capillary tubes up to three-quarter of the capillary tubes. The other end of the capillary tube was sealed up with plasticine and the capillary tubes were placed in a micro hematocrit centrifuge with the sealed end outward. The samples were centrifuged at 3000rpm for 5minutes and the capillary tubes were removed and placed in the groove of a hematocrit reader for the estimation of the Ht/PCV.

Haemoglobin (Hb)

The dilution tube of the haemometer was filled to the 10 marks with freshly prepared 0.1M HCl and the blood sample was collected from the EDTA bottle with the haemometer pipette up to the 20mm mark. The tip of the pipette was wiped clean to prevent any excess blood sample, then the 20mm blood sample inside the pipette was gently blown the into the acid in the haemometer dilution tube. The pipette was carefully sucked up and down twice to mix thoroughly and allow all the red cells to haemolyze and form the acid haematin. The mixture was allowed to stand for exactly 5 minutes and distilled water was added drop-wise, while stirring with a glass rod and the colour of the haemolyzed blood was compared against that of the haemometer standard in bright diffuse day light with a sheet of white paper as background. This was done continually until the dilution colour is slightly darker than the haemometer standard, then the reading of the meniscus level (X_1%) was obtained. The addition of water is continued until the dilution was slightly lighter than the haemometer standard. The reading of the meniscus level (X_2%) was also obtained. The mean of the readings (X%) was calculated. Since 100% in the Sahli scale represents a hemoglobin concentration of 14g/100ml of blood, the mean (X%) is converted to g/100ml by simple proportion.

Red Blood Cell Count (RBC)

The blood sample was drawn exactly to the 0.5 mark on the pipette using gentle suction on the mouthpiece. The excess blood from the outside of the pipette was wiped to avoid transfer of cells to the diluting fluid. The diluting fluid was then sucked up to the 101 mark. While rotating the pipette between the thumb and the forefinger to mix the sample and diluent. The mixing is continued for about 2minutes to ensure the blood and the diluting fluid were mixed properly and the cells were evenly distributed. The pipette was then kept horizontally for another 2 to 3minutes. A clean haemocytometer cover slip was placed on the counting chamber by moistening the shoulders of the counting chamber which the cover slip rests on (these aids in keeping the cover slip stuck to the counting chamber). Then 2 to 3 drops of the undiluted fluid are discarded from the pipette and then the pipette is placed about 45^0 angle and the tip of the pipette is placed on the counting chamber to allow the mixture flow in-between the cover slip and the counting chamber. The cells were allowed to settle for about 3 minutes in the counting chamber and then it was viewed under the microscope with magnification of X40. The cells in five small squares were counted and the result is as follow:

$$\text{RBC Count} = \frac{N \times DF \times 10^6}{A \times D}$$

Where: N = Number of cells counted; DF= The dilution factor (201); 10^6 = Converts to cells per liter; A= Area of chamber counted ($0.04mm^2 \times 5 = 0.2mm^2$); D = Depth of chamber (0.1mm)

White Blood Cell Count (WBCC)

The blood sample was drawn exactly to the 0.5 mark on the pipette using gentle suction on the mouthpiece. The excess blood from the outside of the pipette was wiped to avoid transfer of cells to the diluting fluid. The diluting fluid was then sucked up to the 11 mark. While rotating the

pipette between the thumb and the forefinger to mix the sample and diluent. The mixing is continued for about 2minutes to ensure the blood and the diluting fluid were mixed properly and the cells were evenly distributed. The pipette was then kept horizontally for another 2 to 3minutes. A clean haemocytometer cover slip was placed on the counting chamber by moistening the shoulders of the counting chamber which the cover slip rests on (these aids in keeping the cover slip stuck to the counting chamber). Then 2 to 3 drops of the undiluted fluid are discarded from the pipette and then the pipette is placed about 45^0 angle and the tip of the pipette is placed on the counting chamber to allow the mixture flow in-between the cover slip and the counting chamber. The cells were allowed to settle for about 3 minutes in the counting chamber and then it was viewed under the microscope with magnification of X10. The cells in four large squares were counted and the result is as follows:

$$\text{WBC Count} = \frac{N \times DF \times 106}{A \times D}$$

Where:
N = Number of cells counted; DF= The dilution factor (20); 10^6 = Converts to cells per liter
A= Area of chamber counted ($1mm^2$ x 5 =5 mm^2); D = Depth of chamber (0.1mm)

White Blood Cell Differential Count (WBDCC)

A small drop of the blood sample was placed at one end of a clean and dry microscope slide and was gently spread the drop of blood towards the other end of the slide. The procedure was done without pressing the slides together, as this would damage the cells. The blood sample was dried by waving it repeatedly in the air. The slide was viewed under low power microscope to ensure that the blood cells were evenly spread. Then the film was stained with Leishman's satin and then left for about 2minutes. Buffered distilled water was added via a pipette (twice the volume of stain). The slide was rocked gently several times for proper mixing, then left for about 10minutes. The mixture was poured off and the film was gently washed with water then allowed to dry. The slide was afterwards viewed under high-power microscope of X100 magnification with oil immersion. As each cell type was identified in each field, a total of 100 cells were counted and was represented in percentage of each cell in the sample.

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 of SPSS® version 2.1.0.

Blood Performance

Fish growth is closely related to its health status. A fish with a higher growth rate is more likely to be a healthy one (Esmaeili, 2021). Blood Performance is a formula that contains five parameters: red blood cells count (RBC), white blood cells count (WBC), haemoglobin (Hb), haematocrit (Ht), and total protein (TP), and sums up the natural logarithm of these parameters. Under different stressful situations, from pollution to thermal stress, fish under stress has a lower BP than the

control. BP can thus be a reliable indicator of fish health and growth when it is compared between groups in the same experiment. There is a strong link between BP and stress (negative); the high value of BP is a sign of better health (Esmaeili, 2021).
The Blood Performance (BP) formula adapted after Esmaeli (2021) was as follows:
Blood performance (BP) = Ln (Hb (g/dL) + Ln Ht (%) + Ln RBC ($*10^5/mm^3$) + Ln WBC ($*10^3/mm^3$) + Ln TP (g/L)
Since in this study, total protein (TP) was not analyzed for as there was no feeding during the experiment it was modified to:
BP = Ln Hb (g/dL) + Ln Ht (%) + Ln RBC ($*10^5/mm^3$) + Ln WBC ($*10^3/mm^3$)

Growth Performance
The lengths and weights of the survivors were taken post-exposure and transferred into clean water (of acceptable quality based on exposure concentrations) to monitor for growth- length and weight. They were fed with Coppen fish feed (0.8-1.2mm and then 1.2- 1.5mm) at 5% body weight twice daily to achieve maximum growth under experimental conditions. The growth patterns were measured weekly and compared. The survivor fish were regularly observed for any disease condition or abnormalities. During growth period, feeding was done in segments based on body weight (feed to be applied = 5% body weight) which was adjusted weekly during the growth period. The feeding segments were to; reduce food wastage and to also reduce toxic build-up due to unused or waste food materials, increased biological and chemical oxygen demand (BOD and COD), to prevent fouling and opportunistic bacterial build-up. This % ration was shared into two, applied twice daily at 9am and 4pm respectively at a particular corner of the container (the feeding point) so as to condition them.

The mean weight gain, RGR and SGR were determined using the formulae by Bagenal and Tesch (1978) and Blackwell *et al*. (2000) as follows:

For the growth perfomance;
RGR (%) = $(W_2 - W_1)/ W_1 \times 100$
Where; RGR = Relative Growth Rate, W_1 = Initial weight of fish (g), W_2 = Final weight of fish (g)

SGR (%) = $Log_e W_2 - Log_e W_1/ T_2 - T_1 \times 100$
Where; SGR = Specific Growth Rate, Loge= Natural Logarithm of base e, W_1 = Initial weight of fish (g) in T_1 days; W_2 = Final weight of fish (g) in T_2 days

Fulton's Condition Factor (K) was determined using the formula of Pauly (1993):
$K = W \times 100/ L^3$
Where; W= Weight of fish (g); L= Total length of fish

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 values with exposure concentrations; with the 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 (from 133.76±3.20 to 159.36±3.20mg/L while 151.50±11.37mg/L in the control).

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

Haematological Parameters

Table 2 shows the mean values ± standard deviation of the determined haematological parameters: Haematocrit (%), Haemoglobin (g/100ml), Red Blood Cell Count (cells $*10^{6}/mm^{3}$), White Blood Cell Count (g/dL), Mean Corposular Haematological Count-MCHC: Eosinophils (%), Basophils (%), Lymphocytes (%), Monocytes (%); Mean Corpuscular Haemoglobin (pg/cell), and Mean Corpuscular Haemoglobin Concentration (g/dL) of the fingerlings of *Clarias gariepinus* exposed to the different concentrations of the 'Tri-Pyrethroid insecticide', and the control (0ml/L) after 96hrs.

Table 2: Mean ± Standard Deviation of the Haematological Parameters of *Clarias gariepinus* Fingerlings Exposed to Different Concentrations of the 'Tri-Pyrethroid Insecticide' after 96hrs

	Exposure Concentrations (ml/L)					
Haematological Parameters	**0.0**	**0.0045**	**0.0085**	**0.0110**	**0.0125**	**0.0150**
Haematocrit (%)	25.53± 0.251^{b}	11.50± 0.458^{b}	17.57± 0.513^{d}	13.80± 0.361^{c}	13.90± 1.153^{d}	9.13± 0.288^{b}
Haemoglobin (g/100ml)	28.47± 0.362^{b}	5.80± 0.781^{ab}	5.47± 0.351^{c}	5.57± 0.802^{b}	4.87± 0.416^{c}	4.27± 0.152^{a}
Red Blood Cell Count (cells *10^{6}/mm^{3})	2.41± 0.174^{a}	2.11± 0.148^{a}	1.90± 0.085^{ab}	2.08± 0.145^{ab}	2.07± 0.090^{ab}	2.17± 0.108^{a}
White Blood Cell Count (g/dL)	2.50± 0.100^{a}	3.80± 0.100^{a}	2.83± 0.057^{b}	2.73± 0.058^{ab}	2.40± 0.100^{b}	2.20± 0.100^{a}
Eosinophils (%)	1.00± 0.000^{a}	3.33± 0.577^{a}	2.33± 0.573^{ab}	2.33± 0.577^{ab}	1.33± 0.577^{ab}	1.00± 0.00^{a}
Basophils (%)	0.00± 0.00^{a}	1.00± 0.00^{a}	1.00± 0.000^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}	1.00± 0.00^{a}
Lymphocytes (%)	54.00± 1.00^{c}	75.67± 8.020^{e}	65.67± 1.527^{g}	62.67± 1.527^{f}	61.00± 1.00^{g}	59.00± 1.00^{e}
Monocytes (%)	1.00± 0.00^{a}	6.33±0. 577^{ab}	5.33± 0.577^{c}	4.00± 0.000^{ab}	4.00± 0.000^{c}	3.33± 0.577^{a}
Mean Corpuscular Haemoglobin (pg/cell)	3.25± 0.430^{a}	2.87± 0.384^{a}	2.96± 0.102^{b}	2.78± 0.220^{ab}	2.45± 0.327^{b}	2.03± 0.115^{a}
Mean Corpuscular Haemoglobin Concentration (g/dL)	29.66± 1.642^{f}	49.11± 7.077^{d}	29.80± 2.327^{f}	39.12± 7.229^{e}	35.66± 0.992^{f}	32.73± 0.630^{d}

Key: Means with the same superscripts across the columns are not statistically different at P<0.05

Generally, the Ht, Hb, RBCC and MCH values reduced with increase in concentration while the control had higher values. The MCHC values conversely increased as the exposure concentrations increased with significant (P<0.05) fluctuations while lower values were determined in the control. For the WBCC, Eosinophils, Lymphocytes and Monocytes, the values reduced with increased concentration but with fluctuation and were higher than the control. The Basophils showed no significant difference in the values with concentration. The haematocrit values ranged from

9.13±0.288 - 17.57±0.513%; Haemoglobin was 4.27±0.152 - 5.80± 0.781g/100ml; Red Blood Cell Count was from 1.90± 0.085 - 2.17±0.10 x 10^6/mm^3; White Blood Cell Count ranged from 2.20±0.100 - 3.80±0.100g/dL. MCH values were found to be 2.03±0.115 - 2.96±0.102pg/cell, reduced with increase in concentration and were lower than the control (3.25±0.430pg/cell) while the MCHC ranged from 29.80±2.327 - 49.11±7.077g/dL, but had converse values to the MCH and were higher than the control (29.66±1.642g/dL). The values of the parameters were affected by the exposure concentrations when compared with the control with negative consequences on their functions.

Blood Performance (BP)

Table 3 shows the blood performance (BP) variations (without the total protein) with respect to exposure concentrations against the control.

Table 3: Blood Performance (BP) of the *Clarias gariepinus* Fingerlings Exposed to Different Concentrations of a Tri-Pyrethroid Insecticide after 96hrs

Parameters	**Exposure Concentrations (ml/L)**					
	0.0	**0.0045**	**0.0085**	**0.0110**	**0.0125**	**0.0150**
Haematocrit (%)	25.53	11.50	17.57	13.80	13.90	9.13
Haemoglobin (g/100ml)	28.47	5.80	5.47	5.57	4.87	4.27
Red Blood Cell Count (cells *10^6/mm^3)	2.41	2.11	1.90	2.08	2.07	2.17
White Blood Cell Count (g/dL)	2.50	3.80	2.83	2.73	2.40	2.20
Blood Performance (BP)	1743.0	534.8	516.8	439.7	313.8	147.9

The BP values indicated the concentration-dependent effects of the insecticide on the health of fish as the BP values reduced with increase in exposure concentration, 534.8 and 147.9 for the lowest (0.0045ml/L) and highest (0.0150ml/L) concentrations respectively, which were by far lower than the control (1743.0).

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Growth Response

The mean lengths and weights of *Clarias gariepinus* fingerlings from the different exposure concentrations were evaluated for two weeks to assess growth response/performance of the fish post-exposure based on; the mean weight gain (WG), specific growth rate-SGR (which is the measure of the daily growth rate), relative growth rate-RGR (which is the increase in growth of the fish during the growth phase) and the Fulton's condition factor- K (which is the well-being of the fish) from the different concentrations during the two weeks growth period were determined (Figure 1 and Table 4). The results of the mean weight gain (Figure 1) indicated that the three highest concentrations had higher mean weight gain (4.92±0.957, 4.52±3.304 and 6.02±4.820g) than the control (2.29±2.772g) which was similar for the growth parameters analyzed. Interestingly, negative growth (reduction in weight) was observed in the survivor fish from the 0.0085ml/L concentration, attributed to intrinsic factors.

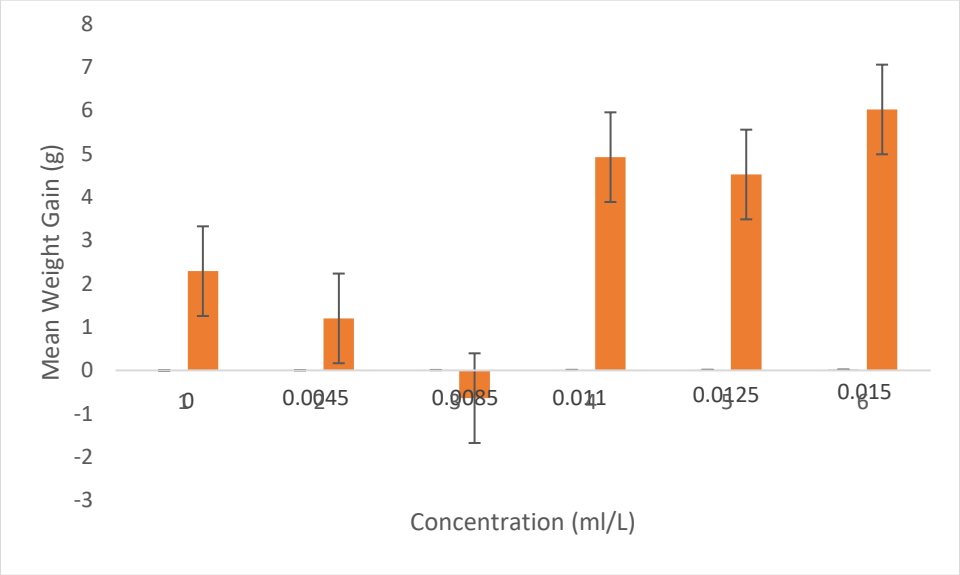

Figure 1: Two Weeks Mean Weight Gain (g) of *Clarias gariepinus* Fingerlings after Exposure to the 'Tri-Pyrethroid Insecticide' (Number of fish per concentration = 30)

Table 4: Growth Parameters- Mean Weight Gain (g), Relative Growth Rate (%), Specific Growth Rate (%) and Condition Factor After Two-Week Growth Period

Exposure Concentrations (ml/L)	Post-Exposure (Initial)		Week 2 (Final)		Mean Weight gain (g)	RGR (%)	SGR (%)	K
	Length (cm)	Weight (g)	Length (cm)	Weight (g)				
0.000	8.26±0.957^{a}	4.98±1.517^{a}	12.00±1.095^{b}	8.85±0.602^{ab}	2.29±2.772^{abc}	109.56	14.96	0.50
0.0045	8.33±1.251^{a}	5.10±1.512^{a}	7.92±4.340^{a}	7.90±3.860^{ab}	1.20±2.580^{ab}	54.9	11.06	1.60
0.0085	9.00±2.096^{a}	6.65±3.404^{a}	8.92±1.281^{ab}	4.88±1.817^{a}	-0.64±0.957^{a}	-26.26	5.31	0.69
0.0110	8.15±2.134^{a}	4.78±2.486^{a}	11.83±2.380^{ab}	11.98±8.229^{b}	4.92±0.957^{bc}	150.63	17.61	0.72
0.0125	8.42±1.772^{a}	4.38±2.404^{a}	9.75±4.876^{ab}	10.70±2.243^{ab}	4.52±3.304^{bc}	144.29	17.49	1.15
0.0150	7.17±1.329^{a}	3.78±0.974^{a}	11.50±2.258^{ab}	12.00±6.713^{b}	6.02±4.820^{c}	217.46	22.68	0.79

Key: Insecticide Post-Exposure Phase (Initial measurements); Two-week Growth Period (Final measurements); RGR= Relative Growth Rate; SGR= Specific Growth Rate; K= Fulton's condition factor; Number of fish per concentration (N) = 30

The RGR and SGR (Table 4) also had higher mean values (150.63 - 217.46% and 11.06 – 22.68% respectively) in the three highest concentrations than the control (109.56% and 14.96% respectively) which also had higher value than the lowest concentration (0.0045ml/L). For the Fulton's Condition factor (K), fish from the exposure concentrations had K values (0.69 - 1.60) greater than the control (0.5) and were generally concentration-dependent with 0.0045 and 0.0125ml/L having the highest condition factors of 1.60 and 1.15 respectively.

DISCUSSION

The differences 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 insecticide concentrations. The DO and EC levels were observed to be higher than the WHO (2008) and USEPA (2011) guidelines for water hence, fish. These implied the increased levels of solutes in the test media which indicated increased physiological activities/stress of the fish. The parameters determined were affected by the insecticide and the variations were concentration-dependent with attendant negative implications for fish physiology evidenced in the TDS values (increased waste matter), salinity and EC values.

In fish, blood transports nutrients (glucose, amino acids, and fatty acids), hormones, minerals, immune components (immunological functions, coagulation and messenger functions), microorganisms, water, gases (supply of oxygen), toxins and waste products like carbon dioxide, urea and lactic acid (Ciesla, 2007). Rebl *et al*. (2021), reported that, studies on blood can provide

useful information about the welfare of fish with respect to health, oxygen transport capacity, immune/stress responses; short-term and long-term effects of "sub-optimal" environmental conditions/physicochemical parameters; potential disease outbreak and nutritional status. Quantitative evaluation of blood cells (haematological parameters) can be used in assessing the impacts of toxic substances in fish (Witeska *et al*., 2023). These blood indices are sensitive and quick biomarkers of the presence of various toxicants, and environmental impacts that have been shown to cause haematological changes in fish (Kanu *et al*., 2023; Rohani, 2023). Blood parameters reflect a wide range of physiological alterations, both adaptive and disruptive; and can provide wide information about various physiological functions in fish (Witeska *et al*., 2023). Toxicants in the blood show either destructive effects like, anemia and immunosuppression or compensatory effects like increase in blood oxygen transport capacity or inflammation, and non-specific stress response (Kanu *et al*., 2023; Rohani, 2023).

Generally, the red blood parameters- haematocrit (Ht), haemoglobin concentration (Hb), erythrocyte count (RBC), mean corpuscular hemoglobin (MCH) in the test fish decreased in the values with increase in concentration while the and mean corpuscular hemoglobin concentration (MCHC) increased. These may indicate the changes in oxygen transport capacity in the fish, impaired gas exchange which may affect the gill epithelium or activate fish metabolism like increasing detoxification pathways. These were the observations in *Brycon amazonicus* following acute exposure to Cypermethrin (Dias de Moraes *et al*., 2018). Decreased values as observed in this study may be due to damage to circulating erythrocytes, direct haemolysis or shortened erythrocytes life span which are anaemic responses. These were accompanied by decrease in MCH (while MCHC levels conversely increased) which may cause strong impairment of oxygen carrying capacity of the RBC in the test fish. Dawood *et al*. (2020), revealed similar observations in their findings on Deltamethrin on *Oreochromis niloticus*. Zhang *et al*. (2020), reported that haematological parameters of fish could be altered after exposure to pyrethroids.

According to Witeska *et al*. (2016) and Fazio *et al.* (2019), RBC values usually range from 0.5–1.5 × 10^6 /μL in less active fish species to 3.0–4.2 × 10^6/μL in more active ones. These values are affected by various environmental and biological factors. The 1.9-2.17 x 10^6/mm determined in the exposed fish as against the control (2.41 x 10^6/mm) in this study for *C. gariepinus* an active species may be attributable to the stage of the fish (fingerlings) and the toxicant. The haematocrit (Ht), a measure of the erythrocyte content in blood as a percentage of erythrocytes in blood volume for different fish species range from 9.4 to 33.53%, is also affected by various factors. The Ht values from this study were 9.13 - 11.50(%) and 25.53(%) from the exposure concentrations and the control respectively. Hb, the protein found in RBCs for aerobic metabolism which involves reaching oxygen, dissolving large amounts of gases, and transporting it to the tissues. These gases are used by tissues as the final receptor of electrons derived from oxidative catabolic reactions and ATP metabolism (Wells, 2009). Any stimulus, both internal and external, can influence metabolism, which in turn influences oxygen demand. As a result, the quantity and function of Hb play critical roles in basic metabolism and ultimately, fish growth and health (Wells, 2009). Hb in different fish species range from 4.70 to 16.6g/dL. In this study, Hb values ranged from 4. 27-

5.80g/100ml in the exposed fish while the control was 28.47g/100ml. Witeska *et al*. (2016), indicated that, for *C. carpio* MCH values range from 31.8–139.0pg, and MCHC from 150–446g/L. In this study, these were found to be 2.03±0.115 - 2.96±0.102pg/cell for MCH, which reduced with increase in concentration and were lower than the control (3.25±0.430pg/cell) while the MCHC at 29.80±2.327 - 49.11±7.077g/dL had converse values and were higher than the control (29.66±1.642g/dL). The analyses of these parameters are useful in diagnosis of some fish diseases like anaemia confirmed in the RBC values. The values from this study were attributable to the exposure to the insecticide, as well as, the species and stage of the test fish.

WBC is an important parameter in the assessment of the immune status of vertebrates. Similar to the red blood parameters, environmental factors (as well as, sex, season, feeding habits, stress, diseases) and presence of toxicants affect WBC count (Ahmed *et al*., 2020). There was general increase in the WBC count in this study for the exposed fish which were higher than the control. An increase in WBC (leukocytosis) is usually interpreted as activation of the immune response due to tissue damage and often, neutrophilia or/and monocytosis is/are observed, indicating an inflammatory response (Witeska *et al*., 2023). Increase in lymphocytes are interpreted as compensation action to the potential compromised immune functions (Zahran *et al*., 2018). The WBC and lymphocyte counts in this study were higher than the control suggestive of leukocytosis and compensation action for compromised immunity in the test fish.

Fazio *et al*. (2019), reported that, WBC in different fish species ranged from 9.41 to 829.33 × 10^3/μL. From this study, the count ranged from 2.2 – 3.80g/dL while the control was 2.50g/dL attributable to the toxicant, as well as, the species and stage of the test fish. For the lymphocytes, the exposure concentrations had values from 59.00 - 75.67% compared to the control (54.00%). Increase in lymphocytes in the immune system is attributable to stress factors and, for cell-mediated and cytotoxic adaptive immunity. According to Varol *et al*. (2015) and Witeska *et al*. (2016), eosinophils are effective in controlling parasitic infections; basophils, the least abundant leukocyte; and monocytes, immune response components for defense in fish have normal ranges of 0–1.87%, 0 - 5.0% and 0.1–13.6% respectively. From this study, these blood cells had values of 1.33- 3.33%; 1.0% and 3.33 - 6.33% compared to the control values of 1.00%, 0.0% and 1.00% for the eosinophils, basophils and monocytes respectively which were indicative of triggered immune responses.

Blood Performance, (BP) a formula that sums up the natural logarithm of the five common blood biochemistry parameters- red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (Ht), and total protein (TP) is considered a reliable and accurate means for monitoring fish health and growth under different stressful situations, from pollution to thermal stress; the fish under stress has a lower BP value than the control. BP can be a reliable indicator of fish health and growth when it is compared between groups in the same experiment (Esmaeili, 2021). Fish with a higher BP, indicates that, most of its blood variables were higher (Esmaeili, 2021). In this study, the BP value of the fish in the lowest concentration showed a better 'health status' (534.8) amongst

the exposure concentrations which was lower than the control BP value (1743.0) that indicated the 'health status' of the control.

The growth response/performance of the survivor fish after exposure was determined after a two-week growth period based on the growth parameters- mean weight gain (WG), specific growth rate- SGR (%) which is the measure of the daily growth rate, relative growth rate-RGR (%) which is the increase in growth of the fish during the growth period, and the condition factor (K) which an indication of the state of well-being of the fish. The values from the study revealed that the WG, SGR and RGR values were highest 6.02±4.820g, 22.68% and 217.46% from the highest exposure concentration (0.0150ml/L) when compared with the control at 2.29±2.772g, 14.96% and 109.56% respectively. Hence, better growth of the survivor fish after exposure to the insecticide. Hossain *et al*. (2022), on growth performances of *Oreochromis niloticus* exposed to Chlorpyrifos compared to the control showed contrary results to the findings of this study. WG and SGR reduced with increased Chlorpyrifos concentration. These differences were attributed to the differences in the toxicant, exposure concentrations, fish species and stage. The values of the condition factor (K) from this study ranged from 0.69 – 1.15 for the survivor fish from the exposure concentrations which were better than the control (0.50), indicative of a 'comparatively favourable state of well-being and good recovery' for the survivors compared to the control. Though, Bagenal and Tesch (1978), from their studies recommended K value range of 2.9-4.8 as suitable for 'mature' freshwater fish. Considering the stage of the fish (fingerlings) and their exposure to the Tri-pyrethroid insecticide', the WG and K values from this study could be seen as favourable and can be alluded to the recovery of the test fish after exposure to the toxicant. This can be explained by the 'phenomenon of sufficient challenge'- the dosage makes the poison (Ottoboni, 2011). Fish growth is closely related to its health status. A fish with a higher growth performance is more likely to be a healthy one (Esmaeili, 2021), which can be said to be the case with the survivor fish in this study. Implying that, with the removal of the toxicant, survivors will likely thrive.

CONCLUSION

Clarias gariepinus fingerlings exposed to 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L of a Cypermethrin, Deltamethrin and Cyhalothrin insecticide for 96hours resulted in haematological changes- the RBC, Ht, Hb, MCH counts reduced with increase in concentration while the WBC, lymphocytes, eosinophils and MCHC increased, the basophils and monocytes counts were also affected. These were validated by the blood performance (BP) values that reduced with increase in exposure concentration which indicated negative implications for the fish physiology and health status. The two-weeks growth response of the survivor fish post-exposure based on the exposure concentration determined using the growth parameters- mean weight gain (WG), specific growth rate (SGR%), relative growth rate (RGR%) and condition factor (K) gave values that were comparatively better than the fish in the control. These showed that, there was good recovery post-exposure and favourable growth of the survivor fish after their removal from the media with the 'Tri-pyrethroid insecticide'.

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