

Immunosuppressive Action of ridof (dichlorovos) in two Life Stages of *Clarias gariepinus* - Evaluation of Effect on Selected Enzymes, Haematology and Condition Factor

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ABSTRACT

*The sensitivity of fish to a range of agrochemicals, including Ridoff (organophosphate insecticide) has been thoroughly examined. These chemicals can reach waterways through both deliberate discharge and approved agricultural practices. The toxic effect of Ridoff was studied on fingerlings and post fingerlings of *Clarias gariepinus* for thirty days using the following concentrations: 0.025mg/l, 0.020mg/l, 0.015mg/l, 0.010mg/l, 0.00Mg/l (Control). The Fingerlings and Post fingerlings had a mean weight of 15±00g, 69.5±00g respectively and the mean length for the fingerling was 8.65±00cm and that of Post fingerling was 9.67±00cm. The sub-lethal exposure showed a decrease in weight of fish with increase in concentration. The Biochemical analysis showed a significant effect on some enzyme indices such as AST (Aspartate aminotransferase), ALP (Alkaline phosphatase) and ALT (Alanine aminotransferase). The Haematological study also showed a significant effect on the White blood Cell (WBC), Packed Cell Volume (PCV) as well as the Haemoglobin (HB). In all the parameters measured – enzymes, blood and condition factor, catfish fingerlings were less impacted by toxicant when compared with post fingerlings. Ridoff (Organophosphate insecticide) had a severe effect on the two life stages of the exposed fish. So, the use of Ridoff (Organophosphate insecticide) near fish farms or in areas close to aquatic environment should be discouraged.*

1. INTRODUCTION

Clariasgariiepinus, also known as the African mud catfish, exists in the wild but it is also cultivated in ponds, cages, and pens and is of great commercial importance. This is an omnivorous fish with a preference for a planktonic diet. It also feeds on other types of food items such as insects, insect larvae, pupae, fish, and fish remains. It also has a propensity for being carnivorous. Propagation of these fishes is widely practiced in the tropics. Catfish are a diverse group of ray-fined fish. They are named for their prominent barbells which resembles a cat's whiskers. *Clariids* catfish of the genus *Clarias* are important aquaculture species cultivated worldwide due to its hardiness and adaptable features, facilitated by its adequate air-breathing abilities. *Clarias gariiepinus* (catfish) is a species of high importance in Nigeria and it is widely cultured owing to its high market price, fast growth rate, and ability to withstand adverse environmental conditions such as low dissolved Oxygen. Its major attributes for aquaculture includes; high fecundity, efficient utilization of a wide variety of food items, habitat instability and tolerance to poor water quality (Uedeme-Naa and Nwafili, 2017).

Fishes are widely used to evaluate the health of aquatic ecosystem and physiological changes serve as biomarkers of environmental pollution (Knock *et al.*, 1996). *Clarias gariiepinus* most widely cultivated in Nigeria water bodies, hence used as biological indicators of ecological studies. *Clarias gariiepinus* lives and survives in captivity (ponds and reservoirs) with other fishes without disturbance. It feeds on natural and artificial diet, grows at a faster rate and attains marketable size in short span of time. *Clarias gariiepinus* also breeds successfully and prolifically in confinement at maturity, Hardy and able to tolerate climatic as well as environmental ecological changes in culturable waters. It resists parasites and diseases, palatable and highly nutritive (Gupta and Gupta, 2008). The genus *Clarias* is unique in its ability to survive a wide variety of environmental extremes (Gorman, 2000). Fishes in this group have a high efficient air-breathing organ which allows them to survive in Oxygen depleted water (Gui, 2003). They migrate over and for long distances as long as 1.2km and aestivate in damp burrows (Chandy, 1992). Juveniles of *Clarias* species are able to survive excessive crowding, feeds on natural and compounded feed for fast growth and rigours of transport (Si-fa and Chenghong, 2003). They also have proved to be a serious threat to native populations in areas where they have been introduced because of their adaptability (Uedeme-Naa and Nwafili, 2017).). *Clarias gariiepinus* has fast growth rate in the natural and cultured environment and has proved to be successful aquaculture species (Paul, 2007). Since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is very important for human beings. Fish like other aquatic organisms may be exposed to a great range of insecticides during the course of their life cycle. In fish, different insecticides can be absorbed through gills, skin or alimentary ducts (Schlenk, 2005; Banaee *et al.*, 2011; Banaee, 2012). Fishes are particularly sensitive to environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (Banaee *et al.*, 2011). So, the effects of insecticides on fishes are of great concern.

Insecticides are any toxic substance that is used to kill insects. Such substances are used primarily to control pests that infest cultivated plants or to eliminate disease-carrying insects in specific

areas. They are pesticides that are formulated to kill, harm, repel or mitigate one or more species of insect. Insecticides work in different ways. Some insecticides disrupt the nervous system, whereas others may damage their exoskeletons, repel them or control them by some other means. Pesticides otherwise referred to as Plant Protection Products (PPPs), by deliberate use and applications in pest control and crop protection have recorded elevated occurrence in the environment. The rapid expansion of various types of integrated farming systems for better income and livelihood of farmers is also gaining importance across several developing climes. Though integrated systems require fewer pesticides than the conventional ones, they are used lavishly in co-culture farming techniques, the eventual contamination of aquatic ecosystems with PPPs either due to spray-drift, leaching, runoff, and/or accidental spills and from aquacultural applications could culminate into risks of debilitating effects and mass mortalities of non-target species. In addition, a risk of sustained toxicity to local biota due to their persistence and high retention within environment is also a concern.

There is no pure specific mode of action by which pesticides can affect animals and humans. For instance, organophosphate pesticides are reported to affect acetylcholine esterase (AChE), an enzyme that hydrolyzes the neurotransmitter acetylcholine at neuromuscular junctions and brain cholinergic synapses. Because of that, organophosphates are extremely neurotoxic. On the other hand, they are claimed to damage hormonal balance, provoke immune disorders, and cause oxidative damage to lipids, proteins, and DNA. In terms of pesticide-induced toxicity in fish, recent papers have shown that biological effects comprise oxidative stress as the most common outcome of pesticide effects, in addition to neurological disorders, endocrine disorders, developmental toxicity, and metabolic changes.

Ridof is an organophosphate derivative used widely as an agricultural and household insecticide. Although it is inactivated by photochemical oxidation and expected not to be persistent, it has an estimated moderate to high toxicity to freshwater fish, estuarine, and marine fish and potential high toxicity to birds. Its high toxicity to bees and other beneficial insects portends risks of ecological effects, including invertebrate taxa loss and trophic cascades, which could also impact the survival of local fish populations. The toxic potential of this pesticide has been demonstrated using a number of biomarkers, including hematological changes (blood glucose, lipid, and serum enzyme profiles) and histopathology. The studies on cell lines suggest that it causes oxidative stress through free-radical generation and promotes DNA fragmentation. Thyroid disruption has been implicated in organophosphate exposures and growth inhibition due to Ridof exposures has been observed.

Recently, many studies have been conducted to determine the mechanisms of insecticides' damage in fishes, with the ultimate goal of monitoring, controlling and possibly intervening in xenobiotics exposure and its effects on the aquatic ecosystem.

2. MATERIALS AND METHODS

2.1 Study Site

This study was carried out at the Fisheries unit of the University of Port Harcourt Demonstration Farm, Choba Campus. The duration of this experiment was for thirty days. One hundred *Clarias*

gariapienus fingerlings and one hundred *Clarias gariapienus* post fingerlings used for this work were obtained from the University of Port Harcourt Demonstration farm (Fisheries unit).

2.2 Acclimation and Feeding of Fish

Respectively, one hundred fingerlings and post fingerlings of *Clarias gariapienus* were acclimated in fifteen rectangular plastic aquaria, containing twenty litres of water each for seven days. The top of the aquaria were covered with plastic lids to prevent the escape of fish. The water was changed daily and the treatments were applied daily. Prior to the acclimation of the fish, the aquaria were washed with clean running water and sponge. The fish were fed twice daily with 2mm coppers feed at 6% body weight..

2.3 Experimental Design

The experimental design was a completely randomized design (CRD) with five treatments levels and a control with each level having three replicates.

2.4 Test Solution

An uncommon insecticide (Ridof–Organophosphate insecticide) was purchased off shelf from a reputable chemical dealer in Port Harcourt, Rivers State.

2.5 Experimental Procedure

A range finding test (trial test) was carried out using Ridof Organophosphate insecticide obtained from Port Harcourt. The solution of the toxicant for trial and definitive test were prepared by serial dilution. During the trial test five *Clarias gariapienus* fingerlings and post fingerlings were exposed to various concentration of Ridof after which a definitive point was achieved for the two sizes. It was observed that concentration within 0.025mg/l was suitable for the fish survival. More so, after thorough observation and monitoring was made and the decision on the definitive test of 0.025, 0.020, 0.015 and 0.010 mg/l was made in agreement with Santanu (2013). The preparations were thoroughly mixed to avoid hot spot in three replicates per treatment. To avoid injuries or bruises on experimental fish, a scoop net was used to transfer fish to another container (bucket) until aquaria with solution was ready. The fish were exposed to Ridof solution 30days.

3.0 Physico-Chemical Assessment of Experimental Tank

3.1 pH: The effluent was collected in clean glass bottle. The pH of the samples was measured on getting to the laboratory, using model 291 Mk 2 pH meter produced by Hannah INC. LTD, China. The pH meter was first calibrated using standard buffer solutions of pH7. This was carried out by pouring small amount of the buffer, pH 7 into a clean beaker and a magnetic stirrer bar dropped into it and the beaker placed on magnetic stirrer to get a homogenous mixture. The pH meter electrode was lowered into the beaker, so that the tip was immersed in the buffer solution and the magnetic stirrer started. The meter was adjusted to read the pH value of the buffer. The electrode was removed, and washed with distilled water and dried with soft tissue paper.

3.2 Ammonia - Nitrogen (NH₃ - N)

The phenate method of ammonia determination (APHA, 1998) was adopted for the study. To 10.0ml sample in a 50ml beaker, one drop $MnSO_4$ solution was added. This was then placed on a magnetic stirrer and 0.5ml hypochlorous acid reagent was added. Thus reagent was added without delay using a burette. The set up was stirred vigorously during addition of reagent. The procedure was carried out for blank and standard, followed by measurement of absorbance at 630nm using reagent blank to zero the spectrophotometer.

3.3 Dissolved Oxygen (DO)

The Winkler's method (APHA, 1998) was used in detecting the dissolved oxygen in each of the aquarium. Water samples for dissolved oxygen were collected and to these were added, 1ml of potassium fluoride solution, 2ml alkaline iodine solution, 2ml manganese sulphate solution and 2ml concentrated sulphuric acid. Then 200ml of the treated sample was used in the titrimetry and the liberated iodine in the treated sample was titrated with a prepared standard of 0.025N sodium through sulphate titrant. Two milliliters starch indicator was added to the sample, when its colour turned pale straw in the process of titration, the value was recorded and used in the calculation.

Calculation

Average volume of 0.025N used = 5.65ml

Volume of treated sample = 200ml

$$\text{Collected sample aliquot} = \frac{200 \times 300}{300 - T}$$

The 200ml sample aliquot for the reagents was corrected to 300ml.

T = total volume of reagents added to the sample bottle

$$\text{Thus DO} = \frac{A \times N \times 8000}{B} \quad (\text{APHA 1998})$$

Where; A = Millimeters of titration sample

B = Millimeters of corrected sample aliquot

C = Normality of sodium thiosulphate

3.4 Temperature

Temperature measurements were made with a mercury-filled Celsius thermometer. Sufficient time was allowed for the thermometer to reach a constant reading. The thermometer readings were recorded in 0c for the samples.

3.5 Nitrite

The nitrite test was conducted by using a nitrite reagent spoon to collect a levelled spoon full of the reagent. This was put into a 5mls water sample calibrated test tube then stirred. At this point, there was a color change which matched with the reagent color chart

4.0 FULTON'S CONDITION FACTOR

Values of Fulton's condition factor were calculated using the formula:

$$\text{Fulton condition factor (K)} = \frac{\text{Weight of Fish}}{l^3} \times 100 \quad (\text{Fang et al., 2009}).$$

5.0. RESULTS

The mean values for water quality variables - pH, Ammonia, Nitrite, DO obtained in the experimental tanks during the exposure of fingerlings and post fingerlings of *C gariepinus* to graded levels of Ridof insecticide are presented in table 1. Ammonia, temperature, and pH were within the same range with no significant difference ($P>0.05$) in aquaria. Dissolved oxygen decreased with increase in concentration.

Table 1: Effect of treatments on physicochemical parameters

Variables	0.025mg/l	0.020mg/l	0.015mg/l	0.010mg/l	0.00mg/l
PH	6.80±0.11	6.80±0.11	6.80±0.11	6.60±0.11	6.6±0.11
Ammonia	1.50±0.11	1.50±0.11	1.50±0.11	1.50±0.11	1.5±0.11
Nitrate	0.02±0.00	0.02±0.00	0.016667±0.00	0.02±0.00	0.01±0.00
Do	3.00±0.011 ^a	3.00±0.01 ^a	2.00±0.011 ^b	1.00±0.011 ^c	0.01±0.01
Temp	27.00±1.15	26.80±0.11	26.60±0.11	26.60±0.11	26.43±0.06

^{abcd}: means across rows with different superscripts are significantly different ($p\leq 0.05$).

Ridof insecticide caused the fluctuation in AST activities at all the varied concentration levels and peaked at 0.020mg/l (4.10±0.06) when compared with control. At **0.025** (17.33±1.45) ALP was seen to be much higher when compared with control and other concentrations while ALT was within the same range when compared with control except at 0.015 and 0.010mg/l (Table 2). The effect of toxicant on the AST, ALP and ALT of post fingerlings was higher when compared with fingerlings (Figs. 1, 2 and 3).

Table 2: Effect of treatment on enzyme indices of fingerlings

Variables	0.025mg/l	0.020mg/l	0.015mg/l	0.010mg/l	0.00mg/l
AST	3.66±0.24 ^{ab}	4.1±0.05 ^a	3.23±0.14 ^{bc}	3.66±0.09 ^{ab}	2.8±0.24 ^c
ALP	17.33±1.45 ^a	13.66±0.33 ^{ab}	16.00±2.08 ^{ab}	12.00±1.15 ^b	16.6667±1.15 ^a
ALT	4.13±0.37 ^{abc}	4.36±0.06 ^{ab}	3.46±0.14 ^c	3.80±0.17 ^{bc}	4.82±0.20 ^a

^{abcd}: means with different superscripts across rows are significantly different ($p \leq 0.05$)
Packed cell volume, Haemoglobin count, Red blood cell count, White blood cell count, Platelet count, Neutrophils, Lymphocytes, Eosinophils, and Monocytes of *C. gariepinus* fingerlings were all raised with increase in toxicant concentration when compared with control (Table 3). PCV, Hb, Plt, N, L, E, M, in *C. gariepinus* fingerlings were less impacted by toxicant when compared with that of post fingerlings except in Rbc whereas Wbc and N were within the same range (Fig. 4 -12).

Table 3::Effect of treatment on haematological indices of fingerlings

Variables	0.025mg/l	0.020mg/l	0.015mg/l	0.010mg/l	0.00mg/l
PCV	21.00±2.08 ^a	17±0.58 ^{ab}	13±0.58 ^b	13.67±0.88 ^b	15.56±1.60 ^b
HB	7.00±0.68 ^a	5.67±0.20 ^{ab}	4.33±0.20 ^b	4.57±0.29 ^b	5.18±0.52 ^b
RBC	3.46±0.27 ^a	3±0.12 ^{ab}	2.6±0.06 ^b	2.77±0.15 ^b	2.42±0.29 ^b
WBC	4.46±0.20 ^{ab}	5.63±0.72 ^a	4.13±0.20 ^b	4.97±0.55 ^{ab}	4.31±0.10 ^{ab}
PLT	163.33±5.23 ^a	135.67±5.78 ^{bc}	145.67±5.81 ^{ab}	143±9.45 ^{abc}	123.56±2.93 ^c
N	36.00±2.65 ^{ab}	41.00±2.08 ^a	32.33±1.45 ^{bc}	26.66±3.17 ^c	15.33±1.01 ^d
L	58.67±1.86 ^{ab}	53±2.89 ^{bc}	60±2.89 ^{ab}	65.33±3.18 ^a	47.33±1.38 ^c
E	2.00±0.00 ^{ab}	2.00±0.00 ^{ab}	2.67±0.33 ^a	2.67±0.33 ^a	1.56±0.22 ^b
M	3.33±0.88 ^{ab}	4.00±1.00 ^{ab}	3.33±0.88 ^{ab}	5.33±0.33 ^a	2.44±0.59 ^c

^{abcd}: means with different superscripts across rows are significantly different ($p \leq 0.05$)

PCV: Packed cell volume, HB: Haemoglobin count, RBC: Red blood cell count, WBC: White blood cell count, PLT: Platelet count, N: Neutrophils, L: Lymphocytes, E: Eosinophils, M: Monocytes.

Table 4 shows that toxicant increased all the enzymatic activities with increase in concentration when compared with control. However, at 0.020mg/l (2.93±0.06) a sharp drop in enzyme activities was observed when compared with other concentrations.

Table 4: Effect of treatment on enzyme indices of post-fingerlings

Variables	0.025	0.020	0.015	0.010	0.00
AST	4.2±0.36056 ^a	2.93±0.06 ^b	3.1333±0.088 19 ^b	3.5±0.17321 ^b 25.3333±1.85	2.9±0.1 ^b
ALP	25±1.73 ^a	27.6667±0.33 ^a	27.3333±1.45 ^a	5 ^a	19.3333±0.66 ^b
ALT	7.2333±0.296	5.4667±0.185	5.4667±0.433	4.1333±0.202	2.9667±0.266
	27 ^a	59 ^b	33 ^b	76 ^c	67 ^d

^{abcd}: means with different superscripts across rows are significantly different ($p \leq 0.05$)

AST: Aspartate amino transferase, ALP: Alkaline phosphatase, ALT: Alanine amino transferase

Table 5, Figure 8, 12 shows the effect of Ridof insecticide on the haematological indices of the post fingerlings. PCV, Hb, RBC, WBC, PLT, N, L and M were all raised with increase in toxicant concentration while E and L decreased with increase in concentration when compared with control.

Table 5: Effect of treatment on haematological indices of post-fingerlings

Variables	0.025mg/l	0.020mg/l	0.015mg/l	0.010mg/l	0.00mg/l
PCV	23.33±2.40 ^{ab}	18±1.1547 ^b	21.66±2.33 ^{ab}	20.3333±1.45 ^{ab}	24.66±1.45 ^a
HB	7.76±0.70 ^{ab}	6±0.40 ^{ab}	5.56±0.97 ^b	7±0.68 ^{ab}	8.2±0.49 ^a
RBC	3.63±0.43	3.1±0.27	3.56±0.34	3.46±0.36	4.06±0.29
WBC	6.46±0.14 ^a	4.5667±0.18 ^b	4.26±0.39 ^b	5.2±0.68 ^{ab}	6.13±0.633 ^a
PLT	185.33±4.40 ^{ab}	189.67±1.45 ^{ab}	176.67±12.41 ^b	175.33±7.26 ^b	205.67±9.76 ^a
N	23±1.52 ^b	25.33±2.60 ^b	29.66±3.92 ^{bc}	39.66±5.23 ^{ab}	43.33±2.40 ^a
L	71±2.08 ^a	67.33±3.71 ^a	61.66±4.41 ^{ab}	53.33±6.00 ^b	49.33±2.33 ^b
E	2.33±0.33	3±1.00	3.33±0.33	2.66±0.66	2.33±0.33
M	3.66±0.88	4.33±1.20	5.33±0.33	4.33±0.66	5.00±0.00

^{abcd}: means with different superscripts across rows are significantly different ($p \leq 0.05$)

Comparatively, enzyme activities in post fingerlings were significantly higher ($p \leq 0.05$) than that of fingerlings as the two were exposed to the same toxicant concentration (Table 6)

Table 6: Comparative influence of toxicant on enzymes in the two life stages

Variables	Fingerlings	Post fingerlings
AST	3.32±0.07	3.33±0.07
ALP	14.38±0.58 ^b	24.93±0.58 ^a
ALT	3.55±0.11 ^b	5.05±0.11 ^a

^{abcd}: means across rows with different superscripts are significantly different ($p \leq 0.05$)

All the haematological variables of post fingerlings were significantly different ($p \leq 0.05$) from that of the fingerlings (Table 7, Figures 4, 5, 6)

Table 7: Influence of growth stage on haematological indices

Variables	Fingerlings	Post fingerlings
PCV	16.22±0.68 ^b	21.60±0.68 ^a
HB	5.41±0.25 ^b	6.91±0.25 ^a
RBC	2.91±0.12 ^b	3.57±0.12 ^a
WBC	4.658±0.20 ^b	5.31±0.20 ^a
PLT	144.95±3.31 ^b	186.53±3.31 ^a
N	32.97±1.29	32.20±1.29
L	53.97±1.49 ^b	60.53±1.49 ^a

E	2.17±0.20	2.73±0.20
M	3.86±0.33	4.53±0.33

^{abcd}: means across rows with different superscripts are significantly different ($p \leq 0.05$)

Table 8, Figures 1, 2, 3 shows that enzyme activities in ALP and ALT between fingerlings and post fingerlings were size related in that all the enzyme activities in post fingerlings were significantly different ($p \leq 0.05$) from that of fingerlings. At 0.020mg/l(fingerling);0.020mg/l(post fingerling);0.010mg/l(fingerling) AST activities in fingerlings were significantly ($p \leq 0.05$) higher than that of post fingerlings.

Table 8: Effect of interaction between treatments and life stage on enzyme indices

TREATMENTS	AST	ALP	ALT
0.025mg/L(fingerlings)	3.66±0.16 ^b	17.33±1.31 ^b	4.13±0.25 ^b
0.025mg/L(post fingerling)	4.2±0.16 ^a	25.00±1.31 ^a	7.23±0.25 ^c
0.020mg/L(fingerling)	4.1±0.16 ^a	13.66±1.31 ^b	4.36±0.25 ^b
0.020mg/L(post fingerling)	2.93±0.16 ^c	27.66±1.31 ^a	5.46±0.25 ^a
0.015mg/L(fingerling)	3.23±0.16 ^b	16±1.31 ^b	3.46±0.25 ^c
0.015mg/L(post fingerling)	3.13±0.16 ^b	27.33±1.31 ^a	5.46±0.25 ^a
0.010mg/L(fingerling)	3.66±0.16 ^b	12±1.31 ^b	3.8±0.25 ^c
0.010mg/L(post fingerling)	3.50±0.16 ^b	25.33±1.31 ^a	4.13±0.25 ^b
Control-fingerling	1.93±0.16 ^d	12.88±1.31 ^b	1.97±0.25 ^d
Control-post fingerling	2.90±0.16 ^c	19.33±1.31 ^b	2.96±0.25 ^d

^{abcd}: means along columns with different superscripts are significantly different ($p \leq 0.05$)

In the interaction between haematological variables, all the post fingerlings variables were significantly higher than that of the fingerlings except in WBC at 0.020mg/l; N at 0.015, 0.020 and 0.025mg/l; L at 0.010mg/l and E at 0.010mg/l (Table 4.9, Figures 4.7, 4.9, 4.10, 4.11)

Table 9: Effect of interaction between treatments and life stage on haematological parameters

mg/l	PCV	HB	RBC	WBC	PLT	N	L	E	M
0.025 (Fingerling)	21±1.53	7.00±0.56	3.46±0.27	4.46±0.46 ^c	163.33±7.41 ^b	36±2.89 ^b	58.66±3.34 ^c	2.00±0.45	3.33±0.73
0.025(Post)	23.33±1.53	7.76±0.56	3.63±0.27	6.46±0.46 ^a	185.33±7.41 ^a	23±2.89 ^c	71±3.34 ^a	2.33±0.45	3.66±0.73
0.020 (Fingerling)	17±1.53	5.66±0.56	3.00±0.27	5.63±0.46 ^b	135.66±7.41 ^c	41±2.89 ^a	53±3.34 ^c	2.00±0.45	4±0.73
0.020 (Post)	18±1.53	6±0.56	3.1±0.27	4.56±0.46 ^c	189.66±7.41 ^a	25.33±2.90 ^c	67.33±3.34 ^b	3.00±0.45	4.33±0.73
0.015 (Fingerling)	13±1.53	4.33±0.56	2.6±0.27	4.13±0.46 ^c	145.66±7.41 ^c	32.33±2.90 ^b	60±3.34 ^b	2.66±0.45	3.33±0.73
0.015(Post)	21.66±1.33	5.56±0.56	3.56±0.27	4.26±0.46 ^c	176.66±7.41 ^b	29.66±2.90 ^b	61.66±3.34 ^b	3.33±0.45	5.33±0.73
0.010 (Fingerling)	13.66±1.53	4.56±0.56	2.76±0.27	4.96±0.46 ^c	143±7.41 ^c	26.66±2.90 ^c	65.33±3.34 ^b	2.66±0.45	5.33±0.73
0.010 (Post)	20.33±1.53	7±0.56	3.46±0.27	5.2±0.46 ^b	175.33±7.41 ^b	39.66±2.90 ^a	53.33±3.34 ^c	2.66±0.45	4.33±0.73
Control(Fingerling)	16.44±1.53	5.46±0.56	2.71±0.27	4.08±0.46 ^c	137.11±7.41 ^c	28.88±2.90 ^c	32.88±3.34 ^d	1.55±0.45	3.33±0.73
Control (Post)	24.66±1.53	8.2±0.56	4.06±0.27	6.13±0.46 ^a	205.66±7.41 ^a	43.33±2.90 ^a	49.33±3.34 ^{cd}	2.33±0.45	5±0.73

^{abcd}: means along columns with different superscripts are significantly different (p≤0.05)

Tables 4, 5, 6, Figures 1 and 2 shows that the wellbeing of fish was size dependent as fingerlings felt better generally when compared with post fingerlings

Table 10: Effect of treatment on the condition factor (CF)

Variables	0.025	0.020	0.015	0.010	0.00
Initial CF1	3.30±0.00 ^c	3.26±0.00 ^d	3.62±0.00 ^b	2.77±0.00 ^e	5.21±0.00 ^a
Final CF2	1.28±0.00 ^e	3.16±0.00 ^b	3±0.00 ^d	3.02±0.00 ^c	4.33±0.00 ^a

^{abcd.}: means across rows with different superscripts are significantly different (p≤0.05)

Table 11: Effect of toxicant on condition factor (CF) for the two life stages.

Variables	Fingerlings	Post fingerlings
Initial CF1	3.98±0.00	3.28±0.00
Final CF2	2.91±0.00	2.99±0.00

^{abcd.}: means across rows with different superscripts are significantly different (p≤0.05)

Table 12: Effect of interaction between treatment and life stages on condition factor (CF)

TREATMENT	CF1	CF2
C1F	3.85±0.01 ^{bc}	2.53±0.01 ^{bc}
C1P	2.76±0.01 ^{cd}	0.03±0.01 ^d
C2F	2.08±0.01 ^d	1.59±0.01 ^c
C2P	4.45±0.01 ^b	4.73±0.01 ^a
C3F	4.07±0.01 ^b	2.53±0.01 ^{bc}
C3P	3.18±0.01 ^c	3.47±0.01 ^b
C4F	2.47±0.01 ^d	2.86±0.01 ^{bc}
C4P	3.07±0.01 ^c	3.18±0.01 ^{bc}
ControlF	7.45±0.01 ^a	5.08±0.01 ^a
ControlP	2.98±0.01 ^{cd}	3.58±0.01 ^b

^{abcd.}: means along columns with different superscripts are significantly different (p≤0.05)

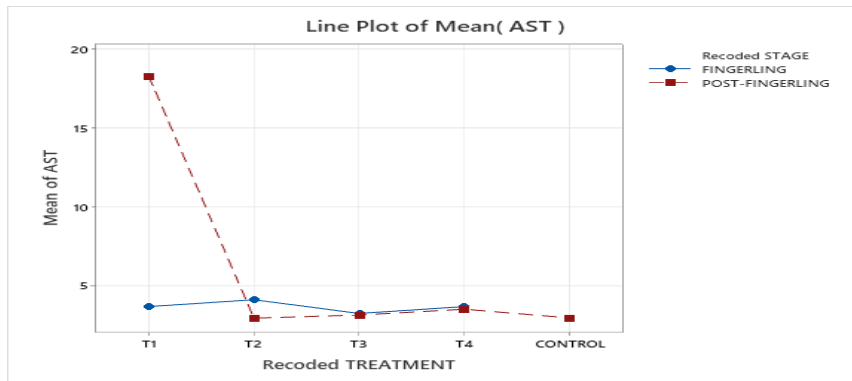


Figure 1: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings AST

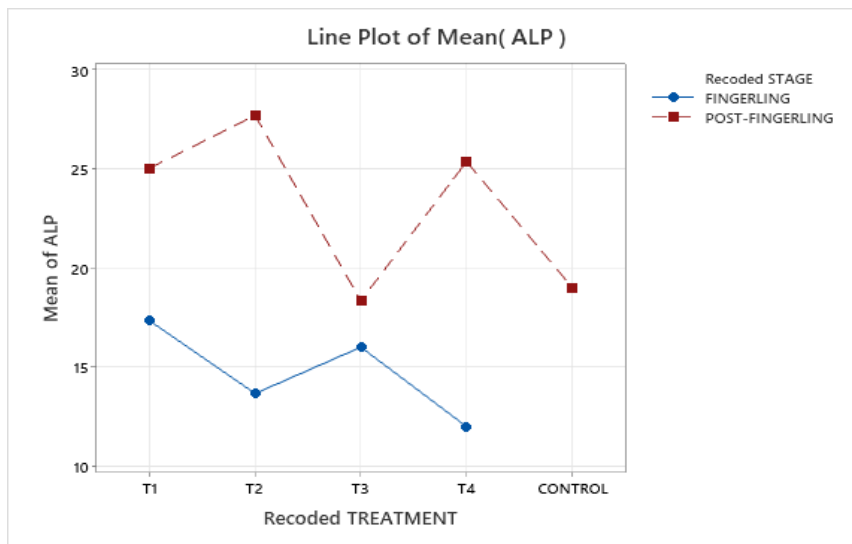


Figure 2: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings ALP

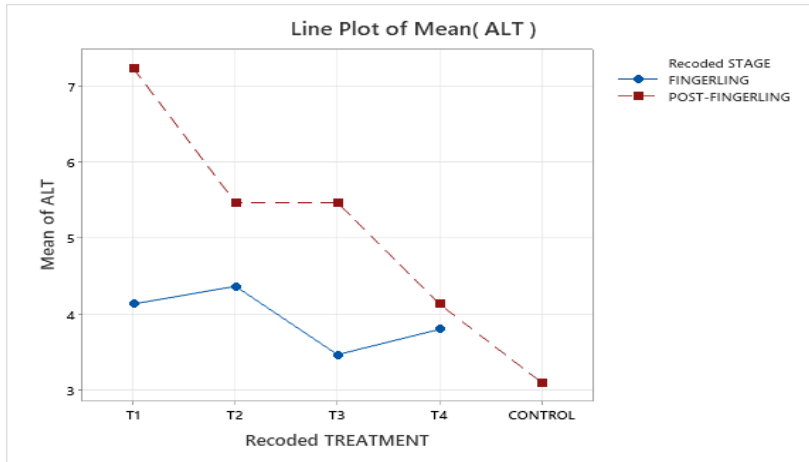


Figure 3: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings ALT

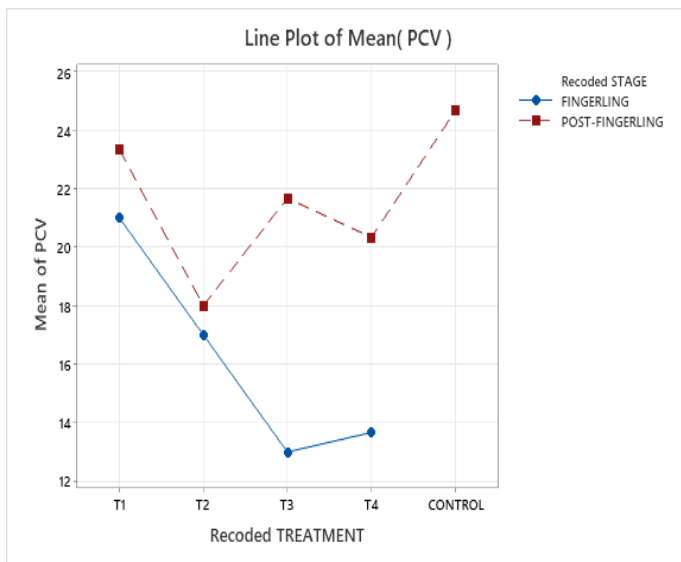


Figure 4: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings PCV

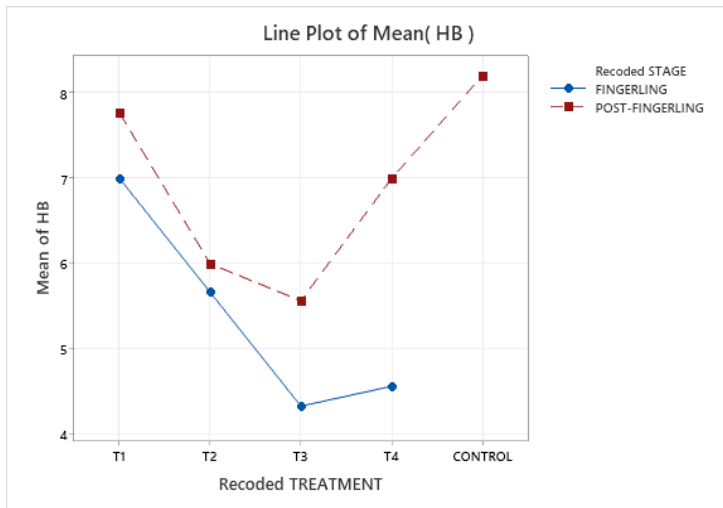


Figure 5: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings Hb

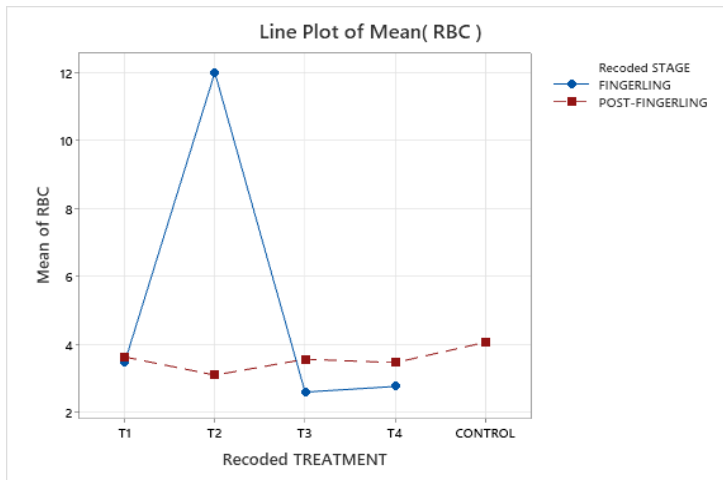


Figure 6: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings RBC

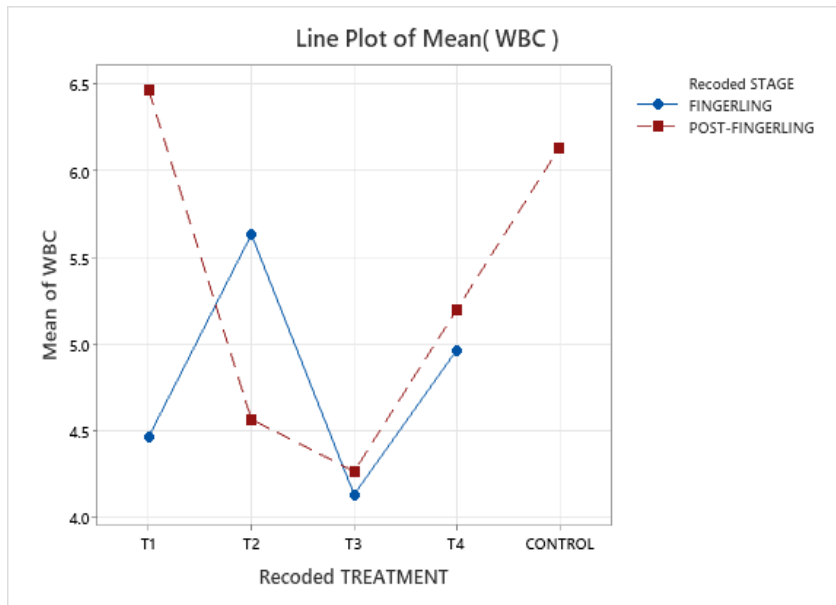


Figure 7: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings WBC

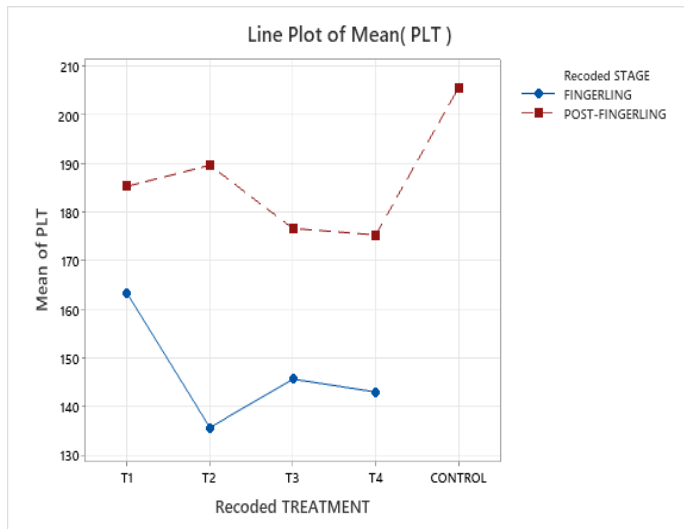


Figure 8: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings PLT

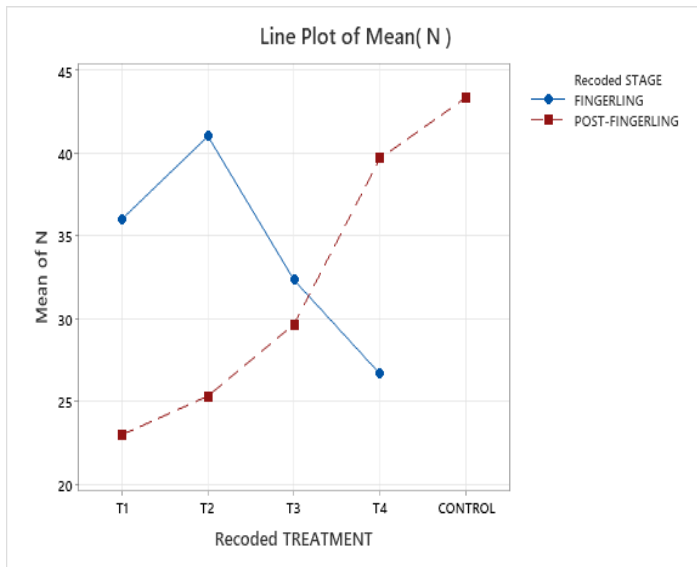


Figure 9: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings N

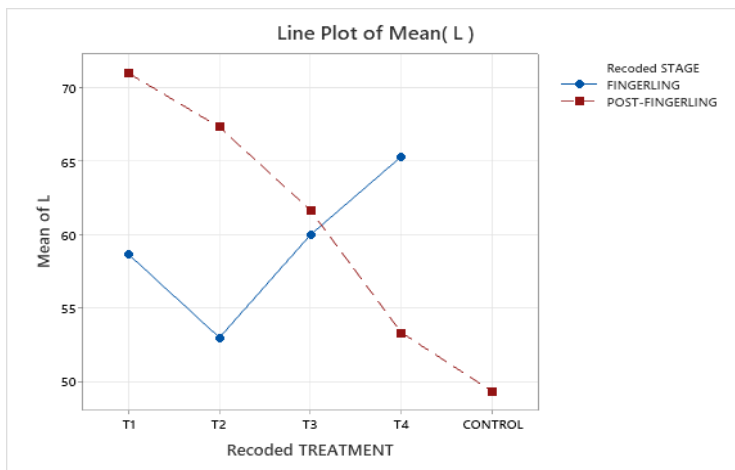


Figure 10: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings L

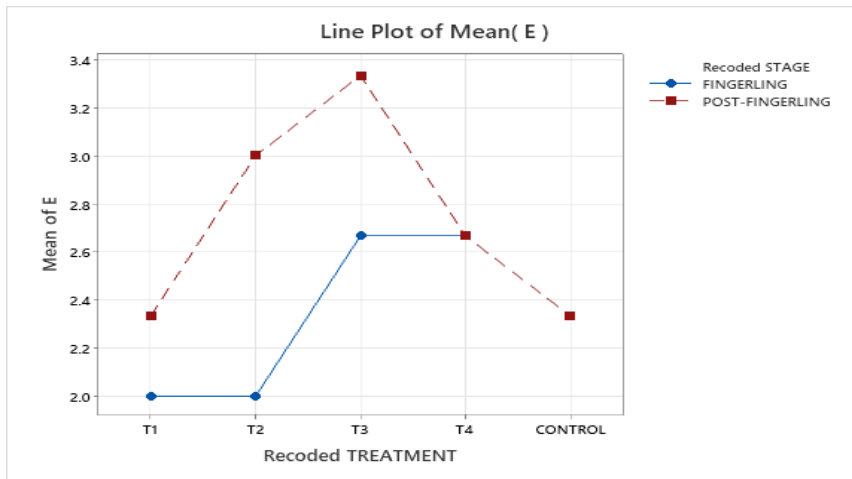


Figure 11: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings E

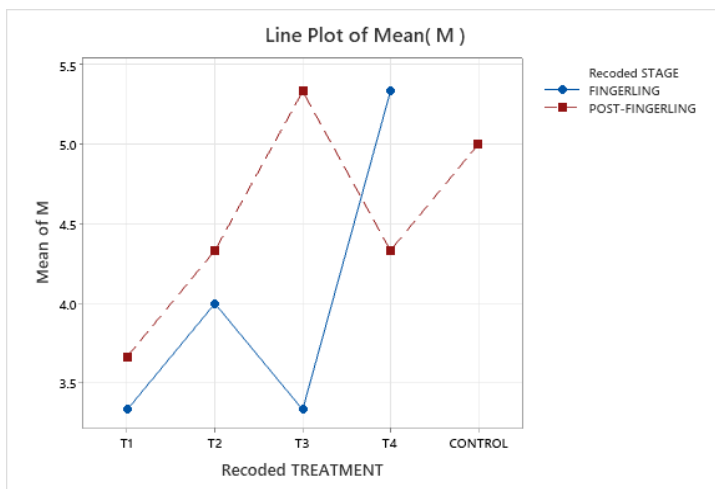


Figure 12: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings M

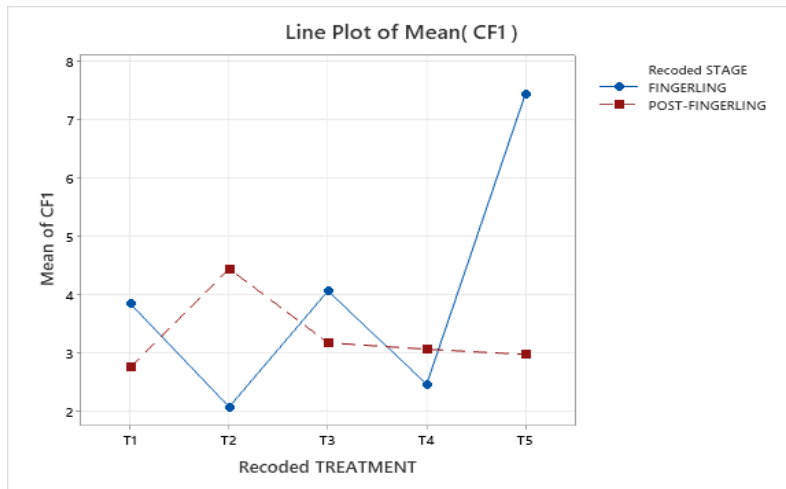


Figure 13: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings Condition factor (CF)

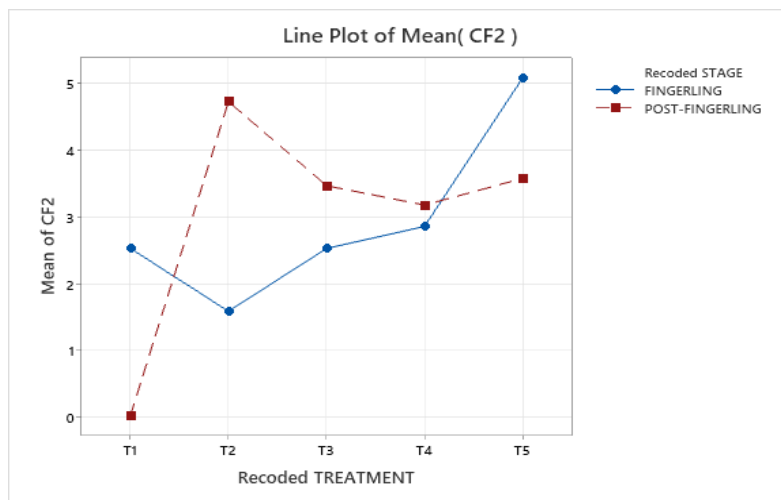


Figure 14: Comparative relationship between effects of Ridoff on fingerlings and post fingerlings CF2

6.0 DISCUSSION

In this work, the parameters – pH, ammonia, nitrite, dissolved oxygen and temperature were all respectively within the same range when compared with control. This is in agreement with the work of Onusiriuka and Ufodike (1994) who exposed *C. gariepinus* to Akee apple and sausage plants extracts and reported no significant difference ($P>0.05$) in the water quality parameters analyzed. This could be as a result of the type of toxicant and concentration levels. All the values

were within the suggested tolerance ranges for warm water fish species (EPA, 1976; EIFAC, 1977; APHA, 1999). Enzyme activities are seen as sensitive bio-chemical indicators and mostly used to assess the health of the organisms in aquatic toxicology (Gul *et al.*, 2004). Several soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage. Aspartate amino transferase (AST) and alanine amino transferase (ALT) are widely used in detecting tissue damage caused by toxicants (Martins *et al.*, 2006). In fish, the liver is the major organ for toxicant toxicity and plays a major role in uptake, accumulation, biotransformation and excretion of toxic substances (Isube *et al.*, 2011). In this work, ridof respectively influenced the activities of AST in fingerlings as against that of post fingerlings at 0.010, as: 20.95%: 21.01%; at 0.015 as 18.47%: 18.79%; at 0.020 as 23.44%: 17.59% and at 0.025 as 20.93%:25.21% when compared with control (0.00) of 16.00%: 17.41%. ALP in the two life stages were influenced as follows: at 0.010 as 15.86%: 16.34%; at 0.015 as 22.02%: 21.61%; at 0.020 as 18.05%: 21.61%; at 0.025 as 22.92%: 28.17%. ALT – at 0.010mg/l as 18.47%: 16.34%; at 0.015 as 16.82%: 21.61%; at 0.020 as 21.20%: 21.61% and at 0.025 as 20.08%: 28.17%. This is in line with the report of Uedeme-Naa and George (2019), who noted that variations in metabolic enzyme activities in fish are directly proportional to the concentration of the toxicant. Uedeme-Naa and Gabriel (2017) also noted that Alanine amino transferase (ALT) is frequently used in the diagnosis of damage caused by xenobiotics in various tissues of fish. Acid phosphatase hydrolyzes large variety of organic phosphatase esters with the formation of an alcohol and a phosphate ion. The decreased profile of this enzyme estimated in this study is attributed to adverse effect of ridof on cell and its organelles (Jana *et al.*, 1985).

ALP is basically a membrane bound enzyme and hence, any perturbation in the membrane property as a result of interaction with detergent could lead to alteration in ALP activity (Hedayati *et al.*, 2010). The increased activity of ALP observed in this study may be attributed to increased synthesis and reduced biliary excretion (Kaplan, 1986). Ogochukwu and Joseph, (2009) also reported increased enzymatic activity of alkaline phosphatase in the fish *C. gariepinus* under the stress of Ariel detergent and suggested extensive damage of liver cells and rupture of blood vessels as possible reasons of increased ALP activity for compensatory action of physiological stress. Alanine phosphatase (ALP) was altered in the muscles of the two life stages due to the influence of ridof concentration. This is in line with the findings of Giboney, (2005), who observed that Phosphates (ALP and ACP) and transferases (AST and ALT) tests are part of standard laboratory tests to detect health abnormalities in animals. Alterations in these enzymes (protein that regulate the rate of a chemical reaction in the body) activities of fish resulting from toxicant or contaminant effects in various organs of fish have been reported (Begun, 2004). Such biochemical changes in fish are aimed at maintaining equilibrium in the presence of these toxicants, which are known to disrupt physiological and biological processes (Wedemeyer and Mcleay, 1981). The transaminases are a group of enzyme catalyzing interconversion of amino acids and α - ketoacids by transfer of amino groups and elevated activity of these tissues – specific enzyme has been used to diagnose damage to liver. The exposure of gill, kidney, muscle, serum plasma and liver AST, ALT, ACP and ALP enzymes of both sizes of fish to detergent resulted in alterations in their activities. This could be why Ozer *et al.*, 2008, noted that when hepatocytes are damaged, enzymes normally located in cytosol are liberated into the extra cellular space and enter the circulation due to

membrane defects causing increased permeability. Detergent induced alterations in aspartate aminotransferase and alkaline phosphates activities have been reported in fish and this elevation was directly attributed to toxic action of detergent on liver (Agrahari *et al.*, 2007). Following chronic carbofuran treatment, in monosex *O. niloticus* and *C. gariepinus* initial increase and then sharp decrease in ALT, AST, and ALP enzyme activities were observed and these findings were confirmed by severe hepatic necrosis preceded by exposure period (Soufy *et al.*, 2007 and Zeni *et al.*, 2002). Ololade and Oginni (2010) opined that the most common haematological variables measured during stress includes Red, White blood cells count, haemoglobin content, and haematocrit value and red blood cells indices (Suganthi *et al.*, 2015). In their independent research works, Abdulkareem and Owolabi (2014) and Nwani *et al.* (2015) reported significant changes in the white blood cell (WBC), red blood cell (RBC) and other haematological parameters of *Clarias gariepinus* exposed to various pesticides and this is in line with the Haematological analysis carried out as it showed a significant change ($P < 0.05$) WBC, PCV, HB, L and E across the treatments of *Clarias gariepinus* aquarium medium. This signified that RIDOFF (Organophosphate insecticide) had toxic effects on *C. gariepinus* fingerlings and post fingerlings. This is in line with study of Audu *et al.* (2014) who reported that the sub-lethal concentration of Agave Americana leaf dust caused deleterious effects on the haematological indices of *C. gariepinus*. Blood is the most essential and abundant body fluid and is a vehicle for quickly mobilizing defence against trauma and ill health (Adewumi *et al.*, 2018). In assessing the toxic effects of chemicals in aquatic organisms, the use of haematological techniques has become more relevant in recent times, because of the relevance of blood in maintaining homeostasis and life functions of fishes (Musa and Omoregie, 1999; Gabriel *et al.*, 2006). Studies have revealed that when the aquatic quality is affected by contaminants, any physiological variations will be revealed in values of one or more haematological parameters of aquatic animals (Akinrotimi *et al.*, 2007; Adewumi *et al.*, 2018). Samprath *et al.* (1993) observed that the relevance of haematological studies of fish, lies in the possibility that the blood will reveal anomalies within the body of the fish long before there is any outward manifestation of symptoms of disease or effects of unfavourable environmental factors. To this end, many laboratory studies have also elucidated effects of toxicants on the haematology of *Clarias gariepinus* including exposure to chlorpyrifos and DDforce (Adewumi *et al.*, 2018), cypermethrin (Akinrotimi *et al.*, 2012), as well as dichlorvos (Ezike, 2017), thereby supporting earlier assertions. The toxicity test carried out showed a significant change ($P < 0.05$) WBC, PCV, HB, L and E across the treatments of *Clarias gariepinus* aquarium medium. This signified that Ridof (Organophosphate insecticide) had toxic effects on *C. gariepinus* fingerlings and post fingerlings. This is in line with study of Lakshmaiah (2017) who reported that the sub-lethal concentration of toxicant caused deleterious effects on the haematological indices of fish. The WBC in concentrations of 0.025mg/L and 0.020mg/L significantly reduced compared to the control group ($p < 0.05$). The reduction in values as reported was in agreement with the findings of Uedeme-Naa *et al.*, 2023 in *Oreochromis niloticus* exposed to herbicide but contrary to that of Uedeme-Naa and George (2019) in which there was steady increase in the WBC with increasing level of the toxicant. This might be due to degree of the potency of the toxicant. No definite pattern was observed in the values of PCV, HB, L and E across the treatment relative to the insecticide concentrations. This was in agreement with the findings of

Pal *et al.*, (2012).in *Clarias gariepinus* exposed to toxicant. The PCV, Hb and RBC are good indicators of oxygen transportation capacity of the fish (Palma *et al.*, 2008).

Toxicant exposure to organisms inhibits growth performance in aquatic animals (George *et al.*, 2020 and Singh and Singh (2007). reported a significant reduction of growth performance of rainbow trout, *Oncorhynchus mykiss*, exposed to arsenic. In this study, the results indicated that fish exposed to sub lethal concentrations of Ridoff (organophosphate insecticide) had reduced weight gain when compared to the control and the reduction of growth performance may be caused by the demand for energy to detoxicate the organophosphate which caused the drop in the energy. Also, the poor condition factors of fingerlings and post fingerlings were affected negatively by the slight increase in temperature with the treatments that had higher concentration of Ridof. Wang *et al.*, 2005 reported that high temperature could cause severe accumulation in fish exposed to toxicant, which may need more energy for detoxification. This is also in accordance with report of Tripathi *et al.*, (2011). who observed that exposure to formalin at therapeutic levels for eight weeks reduced the growth of common carp fry. Similarly, Ayanda *et al.*, (2017).had observed depressed weight in Nile Tilapia (*Oreochromis niloticus*) exposed to sub lethal concentrations of formalin.

7.0 CONCLUSION

This study have shown that the presence of commonly used organophosphate pesticides in freshwater reservoirs could cause harmful effects on the earlier life stages of some fresh water fish, this also shows the threat pesticides might possess to other delicate species in the wild. Their physiological alterations may potentially decrease their survival rate in the nature. Therefore, measures should be taken to mitigate the possible contamination of the aquatic ecosystem by such toxic chemicals, and to strengthen the current findings, further continuation of research should be made. Additionally, more studies for their potential residual effects are required to be performed for completely understanding their hazardous impacts on aquatic ecosystems, with the requirements of using environmentally safe agricultural pesticides.

This study has shown that the use of organophosphate pesticides such as RIDOF is highly toxic to the two life stages of *Clarias gariepinus*.

RECOMMENDATION

I recommend that there should be strong legislation by relevant authorities on the uncontrolled use of pesticides as it affects the aquatic environment. This would go a long way to reduce it's negative Impact on the aquatic flora and fauna.

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