International Journal of Biological Sciences and Applications
2018; 5(1): 1-12
http://www.aascit.org/journal/ijbsa
ISSN: 2375-3811

AASCIT American Association for Science and Technology

Keywords
Acute,
Chronic,
Histological,
Behavioural,
C. gariepinus.
Chlorpyrifos,
DDforce

Received: November 28, 2017
Accepted: December 19, 2017
Published: January 11, 2018

Histological and Behavioural Changes of *Clarias gariepinus* Juveniles Exposed to Chlorpyrifos and DDforce

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Citation

Bamidele Adewumi, Michael Olufemi Ashamo, Damilohun Samuel Metibemu. Histological and Behavioural Changes of *Clarias gariepinus* Juveniles Exposed to Chlorpyrifos and DDforce. *International Journal of Biological Sciences and Applications.* Vol. 5, No. 1, 2018, pp. 1-12.

Abstract

The necessity to produce food in large quantities to provide for the ever-increasing human population in the developing parts of the world has led to increasing in the use of agrochemicals (fertilizer and pesticides). Attempt was made in assessing the histological alterations in the gill, liver and kidney of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of Chlorpyrifos and DDforce. The range-finding tests for Chlorpyrifos (0.40, 0.55, 0.70 and 0.85mg/l) and DDforce (0.15, 0.20, 0.25 and 0.30mg/l) was carried-out to determine the concentrations of the test solution for the definitive test. The 96h LC_{50} value was found to be 0.30mg/l and 0.18mg/l respectively. Subsequently, 1/10th and 1/100th of LC_{50} was determined and the experiment was continued for four weeks (28days). Standard histological procedures were adopted in the assessment of the tissues. The pH and dissolved oxygen monitored decreased significantly ($p<0.05$) from lowest concentration to the highest concentration while the temperature value increased with an increase in concentrations as compared to the control. Fish exposed to different concentrations of Chlorpyrifos and DDforce observed showed general body weakness, hyperventilation, skin discoloration, loss of reflex, hyperactive, erratic swimming, which are behavioural changes. Histological alterations observed in the gill include congestion of secondary gill lamellae, hypertrophy, haemorrhage and lifting of the epithelia. Liver alterations include degeneration of hepatocytes, necrosis, severe vacuolar degeneration, congestion of central tubular and sinusoids. Kidney alterations include haemorrhage, necrosis, degeneration of kidney tubule and collapsing of the glomeruli. These results suggest that Chlorpyrifos and DDforce are toxic and have the disruptive effect on the tissues of fish.

1. Introduction

Indiscriminate use of pesticides in agriculture, animal husbandry, and post-harvest technology is a risk to the natural water system, public health and well-being of mankind [37]. However, the unregulated discharge of agricultural chemicals especially pesticides into water bodies have caused ecological problems to all classes of animals in the aquatic habitat. The aquatic environment is faced with the threat of biodiversity loss due to

indiscriminate use of pesticides [31]. Widespread application of various pesticides has intensified the problem of contamination to aquatic environment. They cause a series of problems to aquatic animals [24]. Due to these synthetic chemicals, the environment has failed to keep its healthy characteristics.

Chlorpyrifos (0, 0-Diethyl – 0 – 3, 5, 6 – trichloro-2-pyridylphosphorothioate) and DDforce (2, 3-dichlorovinyl dimethyl phosphate) an organophosphate (OP) insecticides are highly toxic to fish and aquatic invertebrates. They kill insects and other target organisms. This is achieved by inhibition of the enzyme acetylcholinesterase (AchE) that breaks down acetycholine at the receptor site for partial uptake into the nerve terminal. Without functioning AchE, accumulation of acetylcholine results in depolarizing block of muscle membrane, producing rapid twitching of involuntary muscles, convulsions, paralysis and death [19].

The uses of these chemicals have an impact on non-target organisms and information on this is growing. The contaminants can be carried from one organism to another along a food chain. Their role in the degradation of the aquatic ecosystem cannot be overlooked [30]. They could, therefore, be stored in the tissues of these non-target organisms, thereby inducing their ability to adapt to the environment.

The African catfish (*C. gariepinus*) is a vital food fish in Nigeria, which is also good for research work [1]. *C. gariepinus* is the most notably farmed fish species in aquaculture sector in Nigeria and has also functioned as an experimental model of aquatic vertebrate for two decades [9]. *Clarias species* are generally strong fishes. They have accessory air breathing organs that enable them to bear opposing aquatic conditions where other cultivated fish species cannot endure [28].

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory [36] and field studies [35]. One of the great advantages of using histopathological biomarkers in environmental studies is that this category of biomarkers allows the examination of the gills, kidney and liver [13]. The gills and other accessory respiratory organs of fish [5] being constantly in contact with fish‘s external environment, are vulnerable to aquatic toxicants [1]. Studies have been conducted on histopathological changes in the gills, liver and kidney of fish exposed to various substances [3] including pesticide which have been reported to cause pathological alteration in the exposed African catfish (*Clariasgariepinus*) [3]. It is with this view that this study was carried out to investigate the potential toxic effects of Chlorpyrifos and DDforce on the histological parameters of *C. gariepinus* (Burchell, 1822) juveniles.

2. Materials and Methods

2.1. Sample Collection, Examination, and Preparation

Two hundred and fifty (250) healthy and active *C. gariepinus* juveniles (15-21cm in length; 58-75g in weight) were obtained from Federal Department of Fisheries, Alagbaka, Akure, Ondo State, Nigeria; and transported in a plastic container filled with pond water to the Environmental Biology and Public Health Laboratory of the Federal University of Technology, Akure, Ondo State, Nigeria. The health status of selected fish was assessed based on the presence or absence of physical injuries and other morphological deformations. The fish were certified healthy by assessment before the commencement of the study. They were acclimatized under laboratory conditions (27°C Temperature, 42% Relative Humidity) for three weeks (21 days) prior to the commencement of the experiment. They were fed to satiety daily (7:00 am and 7:00 pm) with Durante floating pellets containing 65% crude protein. Feeding was terminated 24h prior to the range-finding and toxicity test, to reduce ammonia content in the water.

2.1.1. Water Quality Parameter Measurements

Some water quality parameters were measured during and before the acute toxicity test. A hygrothermometer (HH439 model) was used to measure the water temperature (°C). pH and Dissolved oxygen (mg/l) level was measured using Hanna pH meter (HI 96107 model) and a dissolved oxygen meter (DO- 970 model) according to APHA (1992).

2.1.2. Test Chemicals

Chlorpyrifos 480EC (0, 0-Diethyl – 0 – 3, 5, 6 – trichloro-2- pyridy phosphorothioate), Batch No: 20140620, NAFDAC Reg: A5-0714 and DDforce 1000EC (2, 3-dichlorovinyl dimethyl phosphate), Batch No: 20141212, NAFDAC Reg: A5-0107 an organophosphate (OP) insecticide were purchased from an agrochemical shop in Akure, Ondo State, Nigeria; and stored at ambient temperature (27°C). The test concentrations were prepared as described by Food and Agricultural Organization (1977) manual of Aquatic Science Research.

2.2. Acute Toxicity Test

A toxicity assay to determine the 96h LC_{50} values of Chlorpyrifos and DDforce was conducted with a definitive test in a semi-static system in the laboratory following standard methods (APHA, 2005). A range-finding tests for Chlorpyrifos (0.40, 0.55, 0.70 and 0.85mg/l) and DDforce (0.15, 0.20, 0.25 and 0.30mg/l) was carried out to determine the concentrations of the test solution for the definitive test. The fish were starved for 24 hours prior to acute toxicity tests. The experiment was conducted in 40 x 20 x 20 cm plastic tank containing 25L of well water which was continuously aerated in the laboratory. Dead fish were immediately removed to avoid possible deterioration of the water quality. In the definitive test, a set of 10 fish specimens were randomly exposed to Chlorpyrifos and DDforce at 0.40, 0.55, 0.70, 0.85mg/l; and 0.15, 0.20, 0.25, 0.30mg/l concentrations in triplicates. Another set of 10 fish specimens was

simultaneously maintained in water, without test chemical, and considered as control. Behavioral changes in the fish during the test period were observed. Probit analysis was used to determine the concentration at which 50% mortality (LC_{50}) occurred using SPSS version 22.0. The temperature, pH, and dissolved oxygen were monitored during and before the experiment.

2.3. Sub-lethal Toxicity Test

For chronic toxicity study, the 96h LC_{50} value of Chlorpyrifos and DDforce on *C. gariepinus* was found to be 0.30mg/l and 0.18mg/l, 1/10th and 1/100th of LC_{50} was taken and the experiment was continued for four weeks (28days). Fish were fed once a day and water exchange was made daily with fresh test solutions in each experimental container. The fish were randomly divided into three groups without regard the sex. Fish in the first treatment group were not exposed to treatmet and served as control, while those in second and third groups were treated with 0.03, 0.003mg/l of Chlorpyrifos and 0.018, 0.0018 of DDforce respectively. Each treatment group was further randomized into three replicates of 10 fish per replicate in 25L (40 x 20 x 20 cm) plastic tanks.

(i) Tissues Collection and Processing

Samples of fish from both control and experimental groups were excised, rinsed in physiological saline solution and preserved in sample bottles containing formaldehyde, the samples were taking to the Animal Production and Health Laboratory of the Federal University of Technology Akure, Ondo State for analysis.

(ii) Histological Analysis

The gills, livers and kidneys preserved were subjected to micro techniques using the method of Bucke [8]. The tissues were dehydrated in an ethyl alcohol series of ascending concentrations; they were cleared in xylene and embedded in paraffin. Further the sagittal sections (5µ thickness) were cut using a rotary microtome and mounted on glass slides. Sections were deparaffinized in xylene, hydratated in ethanol and stained with haematoxylin and alcoholic eosin (H&E) for general histological evaluation. Photomicrographs of stained sections were made using photoelectron-microscope (XSZ-107BN, China). Photomicrographs of control groups were compared with those of exposed groups under the guidance of a pathologist.

(iii) Statistical Analysis

Data obtained for haematological parameters of *C. gariepinus* were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 22 to generate the mean and standard error. Mean generated were separated and compared by Duncan's New Multiple Range Test (DNMRT). The mean mortality percentage of *C. gariepinus* treated with different concentrations of Chlorpyrifos and DDforce for 96h were subjected to straight line graph using Microsoft Excel 2010.

3. Results

The 96h lethal concentrations obtained for *C. gariepinus* treated with different concentrations of Chlorpyrifos and DDforce are presented in Table 1 and 2. The lethal concentrations at different levels of percentages were obtained using probit analysis. The LC_{50} for Chlorpyrifos and DDforce was 0.30mg/l and 0.18mg/l; the lower and upper limit are 0.210 and 0.392; 0.115 and 0.224 respectively.

The mean values of physicochemical parameters monitored during and before the 96h exposure to Chlorpyrifos and DDforce are presented in Tables 3 and 4. The pH and dissolved oxygen monitored decreased significantly ($p<0.05$) from lowest concentration to the highest concentration while the temperature showed a slight increase with an increase in concentrations as compared to the control. There were significant differences ($p<0.05$) in the pH and dissolved oxygen measured during the 96h exposure except for temperature at 0.85mg/l of Chlorpyrifos and 0.18mg/l of DDforce (24h, 72h and 96h) that shows no significant difference ($p>0.05$) as compared to the control. It is important to stress that the initial temperature, dissolved oxygen and pH measured before the treatments exposure to Chlorpyrifos and DDforce showed no significant difference ($p>0.05$) except for the variations observed with the progress of the experiment when the exposed treatments were compared, even to the control.

The 96h (Acute toxicity test) percentage mortality of *C. gariepinus* treated with different concentrations of Chlorpyrifos and DDforce are presented in Figure 1 and 2. The percentage mortality was found increasing with an increase in the concentrations. The least and highest mortality rate responses were observed at 0.40mg/l (30%) and 0.85mg/l (85%) of Chlorpyrifos; 0.18mg/l (30%) and 0.30mg/l (90%) of DDforce respectively.

The behavioural responses of *C. gariepinus* at 96h exposure are presented in Table 5 and 6. In the control group, normal behavioural responses such as non-hyperactivity and normal swimming patterns were observed. Fish exposed to different concentrations of Chlorpyrifos and DDforce displayed behavioural responses such as hyperventilation, motionless State, increase opercular ventilation, general body weakness, skin discoloration, loss of reflex, erratic swimming, and movement in response to the test chemical. Skin discoloration was mostly observed in fish that were exposed to the highest concentration.

The histological alterations observed in the gill, liver and kidney of *C. gariepinus* juveniles exposed to Chlorpyrifos and DDforce are presented in Figure 3 to 6. Histological alterations in the gill, liver and kidney of *C. gariepinus* juveniles exposed to $1/10^{th}$ (0.03mg/l) of Chlorpyrifos are presented in Figure 3. Histological observation of gill in the control fish showed normal mucous cell (MC), Epithelia cell (EC), Secondary gill lamellae (SGL) and primary gill lamellae (PGL). Gill treated with $1/10^{th}$ (0.03mg/l) of Chlorpyrifos Showed Epithelial hyperplasia (HE), Haemorrhage in the Central venous of cartilaginous core

(HCV) and Telengiectasia (T). Liver in the control showed normal Hepatocyte (HP), Sinusoid vessels (SV) and Blood vessels (BV). Treated liver with $1/10^{th}$ (0.03mg/l) of Chlorpyrifos showed Pyknotic Nuclei (PC), and vacuolar degeneration (VD). Control kidney showed normal Blood vessels (BV), Glomerulus (G) and Bowman's capsule (BC). Kidney treated with $1/10^{th}$ (0.03mg/l) of Chlorpyrifos showed Hydrophobic swelling (HS), Desquamation (D) and damaged of the blood vessel (DBV).

Histological alterations in the gill, liver and kidney of *C. gariepinus* juveniles exposed to $1/100^{th}$ (0.003mg/l) of Chlorpyrifos are presented in Figure 4. Gill treated with $1/100^{th}$ (0.003mg/l) of Chlorpyrifos showed lifting of the epithelial (LE), desquamation (D) and hypertrophy (H). Liver showed vacuolar degeneration (VD) and congestion of sinusoids (CS). Kidney showed necrotic of the proximal tubules (NPT), and degeneration of epithelium (DE).

Histological alterations in the gill, liver and kidney of *C. gariepinus* juveniles exposed to $1/10^{th}$ (0.018mg/l) of DDforce are presented in Figure 5. Gill in the control fish showed normal mucous cell (MC), Epithelia cell (EC), Secondary gill lamellae (SGL) and primary gill lamellae (PGL). Gill treated with $1/10^{th}$ (0.018mg/l) of DDforce Showed epithelial hyperplasia (EH), Dilation of the Secondary Gill Lamellae (DSGL). Liver in the control showed normal Hepatocyte (HP), Sinusoid vessels (SV) and Blood vessels (BV). Liver treated with $1/10^{th}$ (0.018mg/l) of DDforce showed Haemosiderin (H) and necrosis (N). Control kidney showed normal Blood vessels (BV), Glomerulus (G) and Bowman's capsule (BC). Kidney treated with $1/10^{th}$ (0.018mg/l) of DDforce showed collapsing of the glomeruli (CG), necrotic of the proximal tubules (NPT) and degeneration of epithelium (DE). Histological alterations in the gill, liver and kidney of *C. gariepinus* juveniles exposed to $1/100^{th}$ (0.0018mg/l) of DDforce are presented in Figure 6. Gill treated with $1/100^{th}$ (0.0018mg/l) of DDforce Showed hypertrophy (H), congestion and deformed secondary gill lamellae (CDSGL). Liver showed vacuolar degeneration (VD). Kidney showed Necrosis (N).

Table 1. *Lethal concentrations for C. gariepinus treated with Chlorpyrifos.*

95% Confidence Limit			
Probit	**Concentrations**	**Lower limit**	**Upper limit**
LC_5	0.049	0.005	0.170
LC_{10}	0.133	0.012	0.196
LC_{20}	0.175	0.034	0.233
LC_{30}	0.204	0.071	0.269
LC_{40}	0.253	0.130	0.312
LC_{50}	0.297	0.210	0.392
LC_{60}	0.349	0.281	0.596
LC_{70}	0.414	0.333	1.070
LC_{80}	0.505	0.386	2.238
LC_{90}	0.667	0.461	6.392
LC_{95}	0.839	0.531	15.326

Table 2. *Lethal concentrations for C. gariepinus treated with DDforce.*

95% Confidence Limit			
Probit	**Concentrations**	**Lower limit**	**Upper limit**
LC_5	0.049	0.002	0.084
LC_{10}	0.063	0.005	0.103
LC_{20}	0.085	0.013	0.125
LC_{30}	0.106	0.026	0.144
LC_{40}	0.128	0.047	0.165
LC_{50}	0.178	0.115	0.224
LC_{60}	0.182	0.125	0.243
LC_{70}	0.219	0.172	0.366
LC_{80}	0.273	0.213	0.692
LC_{90}	0.371	0.266	1.815
LC_{95}	0.471	0.313	4.104

Table 3. *Physicochemical parameters (mean±SE) measured at 96h exposure to Chlorpyrifos.*

Time	**Parameters**	**0.00mg/l**	**0.40mg/l**	**0.55mg/l**	**0.70mg/l**	**0.85mg/l**
	Temp (0°C)	26.33 ± 0.27^{a}	25.83 ± 0.17^{a}	25.83 ± 0.17^{a}	26.17 ± 0.17^{a}	26.33 ± 0.17^{a}
Before the EP	pH	6.71 ± 0.00^{a}	6.77 ± 0.03^{ab}	6.70 ± 0.06^{a}	6.70 ± 0.06^{a}	6.67 ± 0.07^{a}
	DO_2 (mg/l)	5.97 ± 0.03^{a}	5.97 ± 0.03^{a}	5.90 ± 0.06^{a}	5.97 ± 0.03^{a}	5.87 ± 0.03^{a}
	Temp (0°C)	25.67 ± 0.33^{b}	25.00 ± 0.00^{a}	24.83 ± 0.17^{a}	26.12 ± 0.17^{c}	26.33 ± 0.17^{c}
24h	pH	6.87 ± 0.03^{d}	6.63 ± 0.07^{c}	6.57 ± 0.03^{bc}	6.43 ± 0.03^{ab}	6.30 ± 0.06^{a}
	DO_2 (mg/l)	5.93 ± 0.03^{e}	5.80 ± 0.00^{d}	5.63 ± 003^{c}	5.50 ± 0.00^{b}	5.37 ± 0.03^{a}
	Temp (0°C)	25.67 ± 0.33^{c}	24.17 ± 0.17^{a}	24.17 ± 0.17^{a}	24.67 ± 0.17^{ab}	25.24 ± 0.18^{b}
48h	pH	6.70 ± 0.00^{e}	6.43 ± 0.03^{d}	6.30 ± 0.00^{c}	5.93 ± 0.07^{b}	5.70 ± 0.00^{a}
	DO_2 (mg/l)	5.93 ± 0.03^{e}	5.80 ± 0.00^{d}	5.63 ± 003^{c}	5.50 ± 0.00^{b}	5.37 ± 0.03^{a}
	Temp (0°C)	24.67 ± 0.03^{b}	23.67 ± 0.33^{a}	23.50 ± 0.00^{a}	23.54 ± 0.00^{a}	24.67 ± 0.16^{b}
72h	pH	6.33 ± 0.07^{d}	6.03 ± 0.03^{c}	6.00 ± 0.00^{c}	5.70 ± 0.00^{b}	5.55 ± 0.03^{a}
	DO_2 (mg/l)	5.47 ± 0.03^{c}	5.10 ± 0.10^{d}	4.87 ± 0.03^{c}	4.63 ± 0.03^{b}	4.33 ± 0.03^{a}
	Temp (0°C)	24.17 ± 033^{b}	23.10 ± 0.5^{a}	23.00 ± 0.00^{a}	23.23 ± 0.17^{a}	24.00 ± 0.00^{b}
96h	pH	6.13 ± 0.07^{e}	5.77 ± 0.03^{d}	5.60 ± 0.00^{c}	5.40 ± 0.00^{b}	5.27 ± 0.03^{a}
	DO_2 (mg/l)	5.03 ± 0.03^{e}	4.70 ± 0.06^{d}	4.47 ± 0.03^{c}	4.23 ± 0.03^{b}	3.83 ± 0.03^{a}

Note: Mean values with the same superscript alphabets in the rows are not significantly different ($p<0.05$) from each other using Duncan's New Multiple Range Test (DNMRT).
Key: Ep= Experiment

Table 4. *Physicochemical parameters (mean±SE) measured at 96h exposure to DDforce.*

Time	Parameters	0.00mg/l	0.40mg/l	0.55mg/l	0.70mg/l	0.85mg/l
	Temp (0°C)	26.33±0.27^{a}	26.83±0.12^{a}	25.93±0.34^{a}	25.17±0.18^{a}	26.83±0.23^{a}
Before the EP	pH	6.71±0.00^{a}	6.73±0.03^{a}	6.45±0.09^{a}	6.76±0.08^{a}	6.68±0.04^{a}
	DO_2 (mg/l)	5.97±0.03^{a}	5.65±0.09^{a}	5.87±0.06^{a}	5.89±0.09^{a}	5.97±0.05^{a}
	Temp (0°C)	25.67±0.33^{c}	24.44±0.18^{a}	24.58±0.00^{ab}	25.05±0.02^{b}	26.51±0.11^{c}
24h	pH	6.87±0.03^{d}	6.68±0.08^{c}	6.56±0.08^{bc}	6.45±0.09^{ab}	6.30±0.05^{a}
	DO_2 (mg/l)	5.93±0.03^{e}	5.80±0.03^{d}	5.66±0.07^{c}	5.53±0.04^{b}	5.36±0.06^{a}
	Temp (0°C)	25.67±0.33^{c}	24.08±0.05^{a}	24.18±0.04^{a}	24.17±0.19^{a}	25.03±0.03^{b}
48h	pH	6.70±0.00^{e}	6.53±0.08^{d}	6.40±0.04^{c}	5.91±0.07^{b}	5.73±0.04^{a}
	DO_2 (mg/l)	5.67±0.03^{e}	5.54±0.06^{d}	5.38±0.03^{c}	5.15±0.13^{b}	4.83±0.05^{a}
	Temp (0°C)	24.67±0.03^{b}	23.56±0.18^{a}	23.46±0.04^{a}	23.69±0.20^{a}	24.38±0.19^{b}
72h	pH	6.33±0.07^{d}	6.08±0.11^{c}	6.10±0.13^{c}	5.78±0.10^{b}	5.59±0.13^{a}
	DO_2 (mg/l)	5.47±0.03^{c}	5.27±0.30^{d}	4.97±0.11^{b}	4.93±0.13^{b}	4.43±0.19^{a}
	Temp (0°C)	24.17±0.33^{b}	22.93±0.11^{a}	23.12±0.05^{a}	23.27±0.19^{a}	23.54±0.26^{b}
	pH	6.13±0.07^{e}	5.89±0.08^{d}	5.68±0.04^{c}	5.45±0.01^{b}	5.29±0.08^{a}
	DO_2 (mg/l)	5.03±0.03^{e}	4.80±0.06^{d}	4.49±0.06^{c}	4.22±0.05^{b}	3.87±0.09^{a}

Note: Mean values with the same superscript alphabets in the rows are not significantly different ($p<0.05$) from each other using Duncan's New Multiple Range Test (DNMRT).
Key: Ep= Experiment

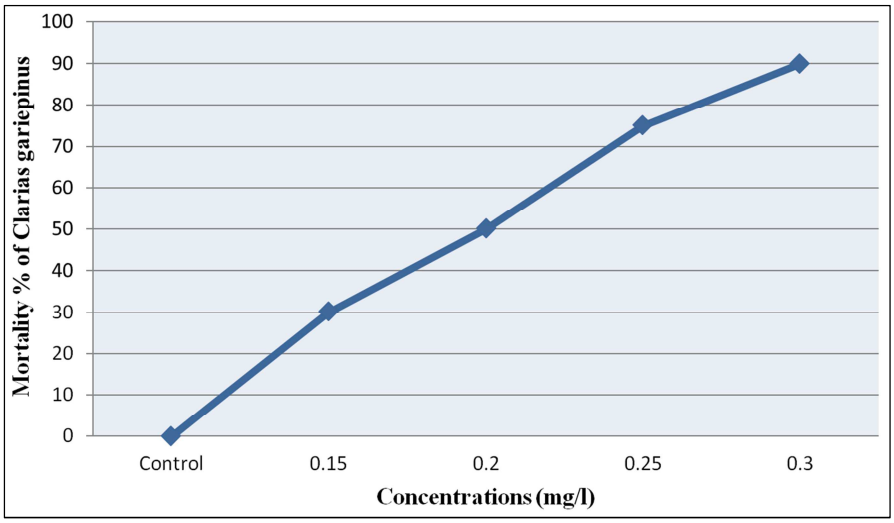


Figure 1. *Mean mortality (%) of C. gariepinus treated with different concentrations of DDforce.*

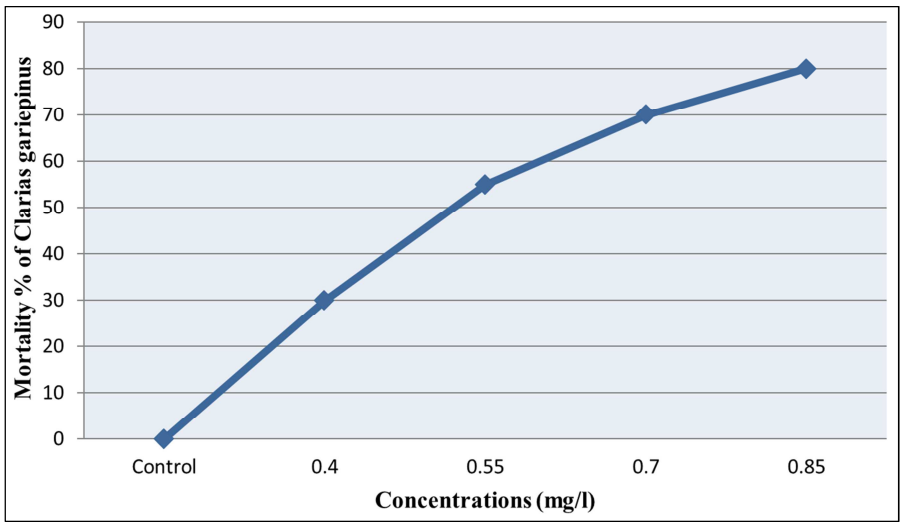


Figure 2. *Mean mortality (%) of C. gariepinus treated with different concentrations of Chlorpyrifos.*

Table 5. *Behavioural responses of Clarias gariepinus at 96h exposure to Chlorpyrifos.*

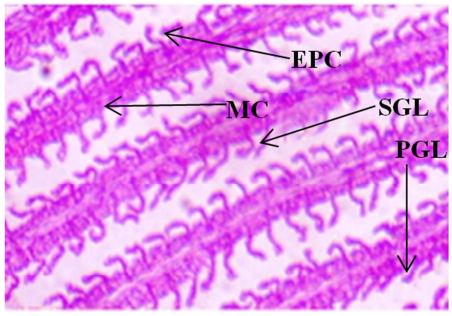
Concentration (mg/l)					
Behaviour	**Control**	**0.40**	**0.55**	**0.70**	**0.85**
Hyperactive	-	+	+	+	+
Hyperventilation	-	+	+	+	+
Erratic Swimming	-	+	+	+	+
Motionless State	-	+	+	+	+
Skin discolouration	-	+	+	+	+
Body Weakness	-	+	+	+	+
Loss of Reflex	-	+	+	+	+

KEY: - = absent, + = present

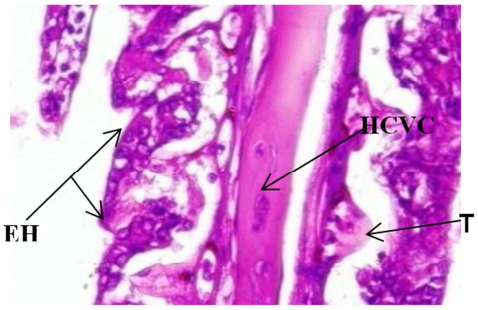
Table 6. *Behavioural responses of Clarias gariepinus at 96h exposure to DDforce.*

Concentration (mg/l)					
Behaviour	**Control**	**0.15**	**0.20**	**0.25**	**0.30**
Hyperactive	-	+	+	+	+
Hyperventilation	-	+	+	+	+
Erratic Swimming	-	+	+	+	+
Motionless State	-	+	+	+	+
Skin discolouration	-	+	+	+	+
Body Weakness	-	+	+	+	+
Loss of Reflex	-	+	+	+	+

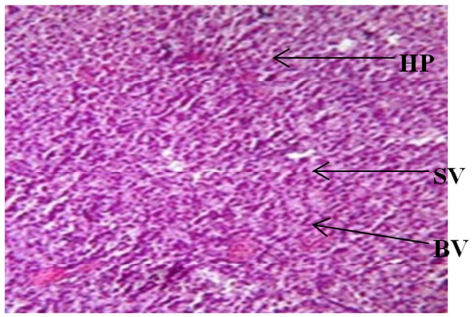
KEY: - = absent, + = present



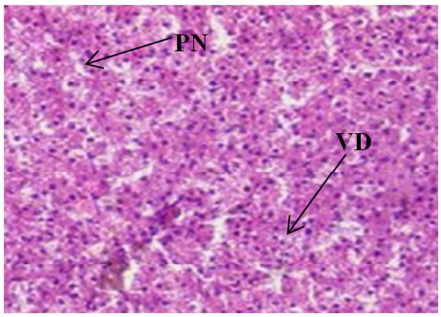
Control gill showing mucous cell (MC), Epithelia cell (EC), Secondary gill lamellae (SGL) and primary gill lamellae (PGL).



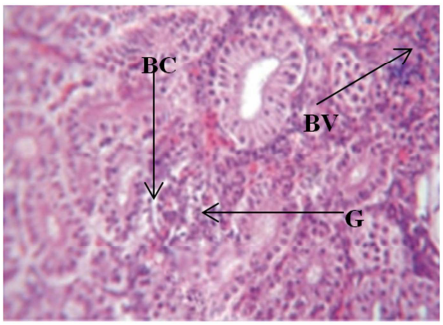
Gill treated with 1/10th (0.03mg/l) of Chlorpyrifos Showing Epithelial hyperplasia (HE), Haemorrhage in the Central venous of cartilaginous core (HCVC) and Telengiectasia (T).



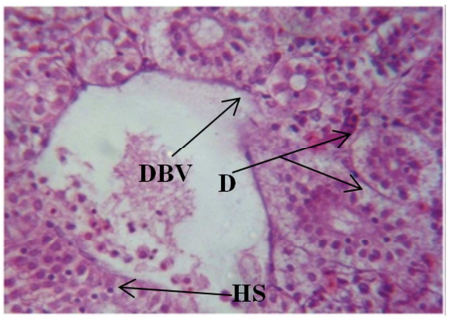
Control liver Showing Hepatocyte (HP), Sinusoid vessels (SV) and Blood vessels (BV).



Treated liver withb 1/10th (0.03mg/l) of Chlorpyrifos Showing Pycnotic Nuclei (PC), and vacuolar degeneration (VD).

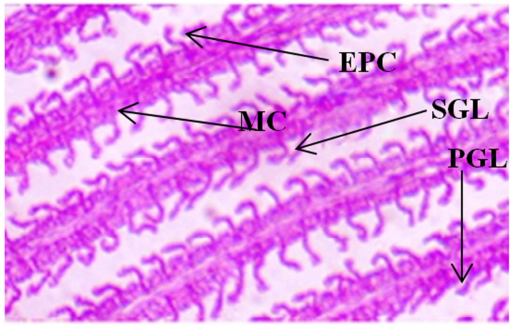


Control kidney showing Blood vessels (BV), Glomerulus (G) and Bowman's capsule (BC).

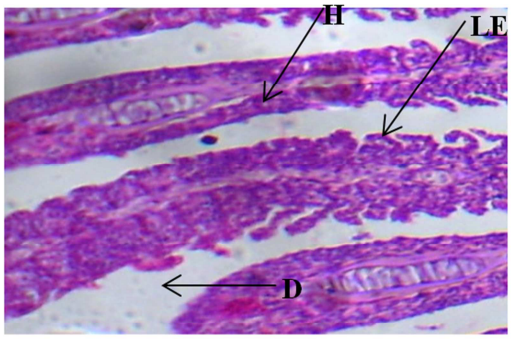


Liver treated with 1/10th (0.03mg/l) of Chlorpyrifos showing Hydrophobic swelling (HS), Desquamation (D) and damaged of the blood vessel (DBV).

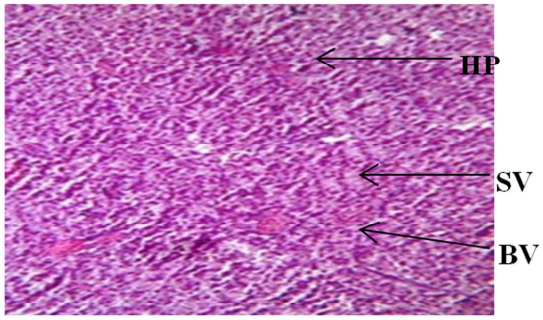
Figure 3. *Histological alterations in the gill, liver and kidney of Clarias gariepinus juveniles exposed to 1/10th (0.03mg/l) of Chlorpyrifos.*



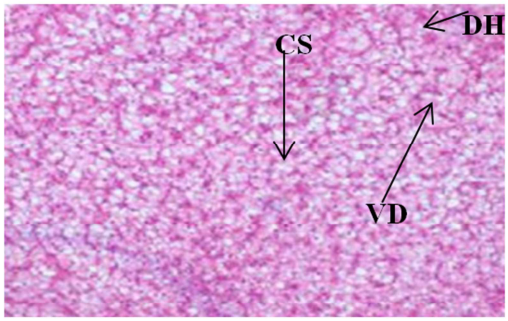
Control gill showing mucous cell (MC), Epithelia cell (EC), Secondary gill lamellae (SGL) and primary gill lamellae (PGL).



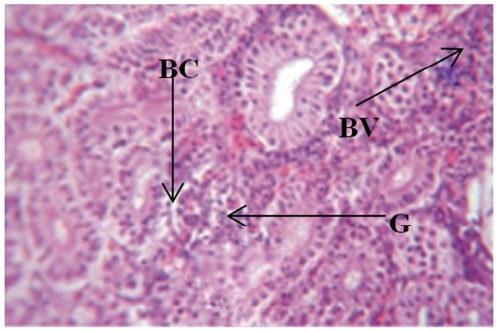
Gill treated with 1/100th (0.003mg/l) of Chlorpyrifos showing Lifting of the epithelial (LE), desquamation (D) and hypertrophy (H).



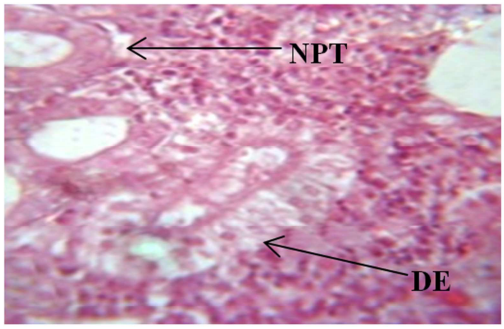
Control liver showing Hepatocyte (HP), Sinusoid vessels (SV) and Blood vessels (BV).



liver treated with 1/100th (0.003) of Chlorpyrifos showing vacuolar degeneration (VD), degeneration of hepatocyte (DH) and congestion of sinusoids (CS)

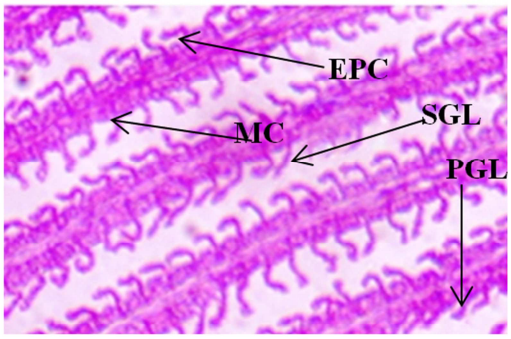


Control kidney showing Blood vessels (BV), Glomerulus (G) and Bowman's capsule (BC).

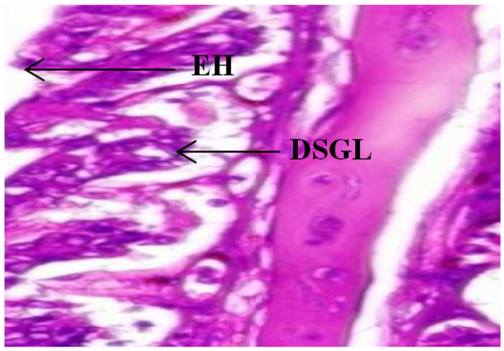


Kidney treated with 1/100th (0.003mg/l) of Chlorpyrifos Showing necrotic of the proximal tubules (NPT), and degeneration of epithelium (DE).

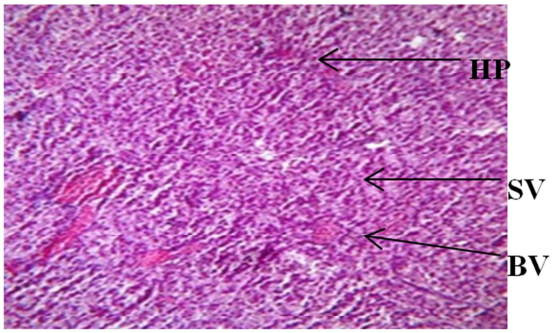
Figure 4. *Histological alterations in the gill, liver and kidney of Clarias gariepinus juveniles exposed to 1/100th (0.003mg/l) of Chlorpyrifos.*



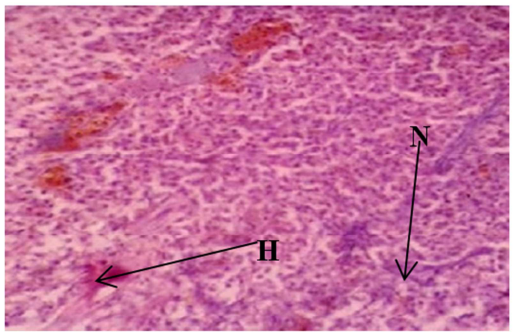
Control gill showing mucous cell (MC), Epithelia cell (EC), Secondary gill lamellae (SGL) and primary gill lamellae (PGL).



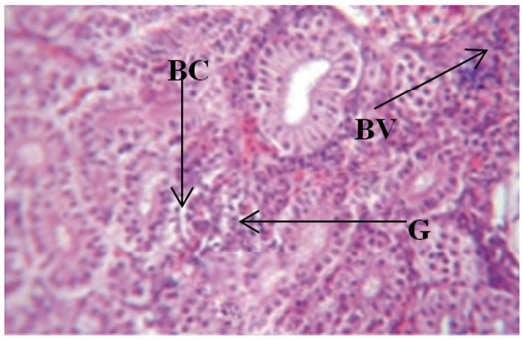
Gill treated with $1/10^{th}$ (0.018mg/l) of DDforce Showing Epithelial hyperplasia (EH), Dilation of the Secondary Gill Lamellae (DSGL).



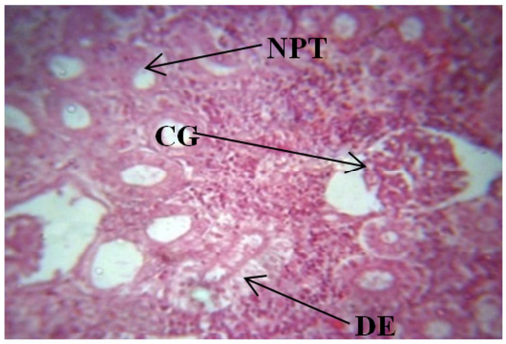
Control liver Showing Hepatocyte (HP), Sinusoid vessels (SV) and Blood vessels (BV).



Liver treated with $1/10^{th}$ (0.018mg/l) Showing Haemosiderin (H) and necrosis (H).

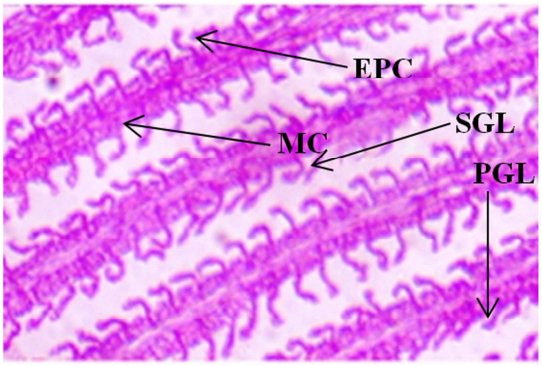


Control kidney showing Blood vessels (BV), Glomerulus (G) and Bowman's capsule (BC).

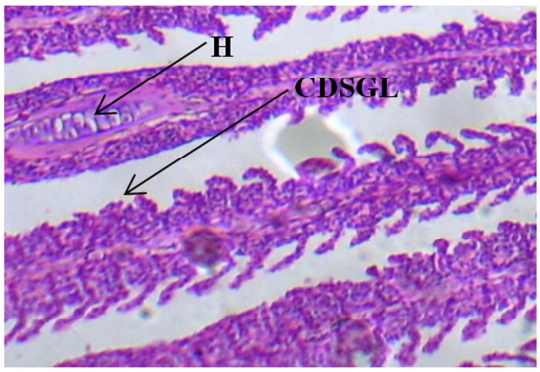


Kidney treated with $1/10^{th}$ (0.018mg/l) of DDforce collapsing of the glomeruli (CG), necrotic of the proximal tubules (NPT) and degeneration of epithelium (DE).

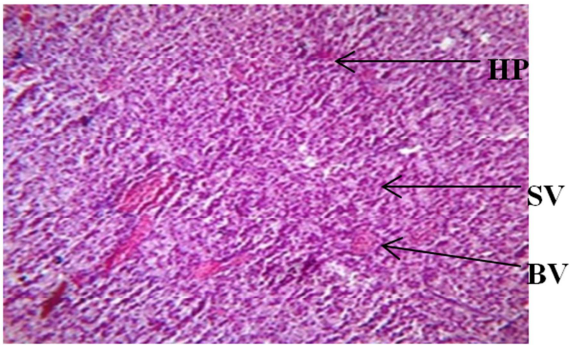
Figure 5. *Histological alterations in the gill, liver and kidney of Clarias gariepinus juveniles exposed to $1/10^{th}$ (0.018mg/l) of DDforce.*



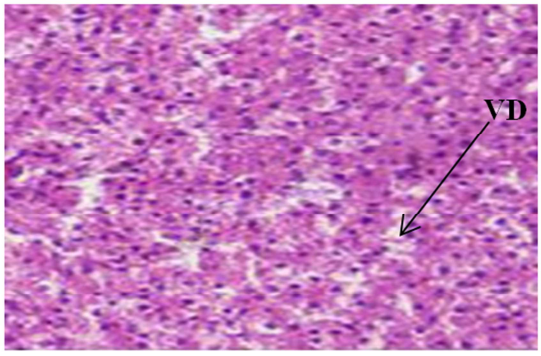
Control gill showing mucous cell (MC), Epithelia cell (EC), Secondary gill lamellae (SGL) and primary gill lamellae (PGL).



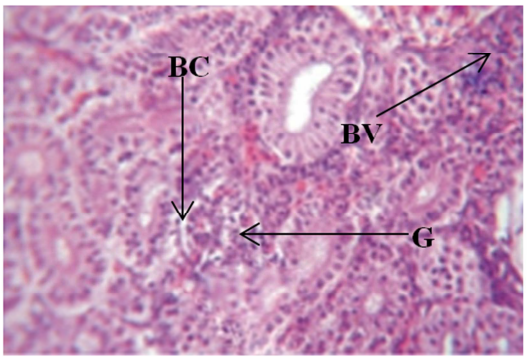
Gill treated with 1/100th (0.0018mg/l) of DDforce Showing hypertrophy (H), congestion and deformed secondary gill lamellae (CDSGL).



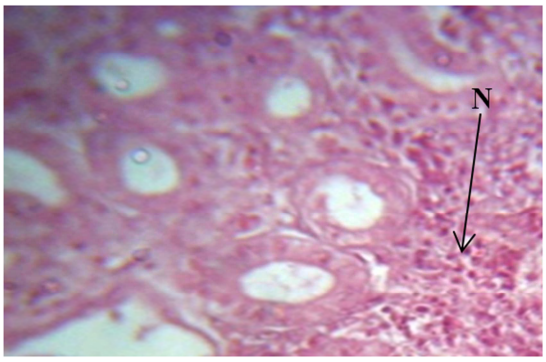
Control liver after 28 days showing Normal liver Showing Hepatocyte (HP), Sinusoid vessels (SV) and Blood vessels (BV).



Liver treated with 1/100th (0.0018mg/l) of DDforce showing vacuolar degeneration (VD).



Control kidney after 28 days showing Blood vessels (BV), Glomerulus (G) and Bowman's capsule (BC).



Kidney treated with 1/100th (0.0018mg/l) of DDforce showing Necrosis (N).

Figure 6. *Histological alterations in the gill, liver and kidney of Clarias gariepinus juveniles exposed to 1/100th (0.0015mg/l) of DDforce.*

4. Discussion

Acute and chronic toxicity tests are mostly used to assess the toxicity of chemicals on non-target animals [34]. The 96h LC_{50} is one of the most vital factors for assessing the toxic effects of contaminants. The 96h LC_{50} value of Chlorpyrifos and DDforce in this study was found to be 0.30mg/l and 0.18mg/l which suggest that the pesticides are toxic to fish.

Water quality characteristics are major factors that influence fish survival, reproduction, growth performance, and overall biological production [20]. They affect aquatic biotic integrity by directly causing mortality and or shifting the equilibrium among species due to subtle influences such as reduced reproductive rates or alternations in competitive ability.

The physicochemical parameters observed in this study appeared to be within ideal range for fish culture as reported by [30] and [27]. The pH and dissolved oxygen of the exposed fish decreased significantly ($p<0.05$) from lowest concentration to the highest concentration. The temperature also showed a slight increase with increasing concentration

compared to the control. This observation was in agreement with [26] who investigated the lethal effect of the elephant Blue detergent (R) on the Nile Tilapia and *Oreochromis niloticus*. [38] Had earlier reported that the introduction of a contaminant into an aquatic environment might reduce the dissolved oxygen concentration, which will impair respiration leading to suffocation. This was possibly why the fish were stressed progressively with time before death.

Behavioural alterations in fish are the most sensitive signs of potential lethal effects of pesticide exposure [4], [33]. The behavioural responses observed during *C. gariepinus* exposure to Chlorpyrifos and DDforce in the study is similar to observations by [2] on the responses of *Cyprinus carpio* exposed to different concentrations of fenthion. The abnormal behaviour displayed by fish in the experimental groups include; hyperactive, hyperventilation, general body weakness, skin discoloration, loss of reflex, erratic swimming, and motionless state which is due to inhibition of acetylcholinesterase activity leading to accumulation of acetylcholine in cholinergic synapses thus causing hyper stimulation of the toxicants and became exhausted owing to respiratory difficulty which made the exposed fish in their respective treatment tanks to settled down passively at the bottom and finally died. Thus, swimming performance is considered one of the measures which could serve as a possible sensitive sign of sub-lethal toxic exposure. It has been reported that under stress condition, fish become hyperactive, perhaps to get out of the stressful medium and would need an increased amount of oxygen to meet their energy demand [2].

A consistent trend was generally observed in the mortality rate of *C. gariepinus* which increases with an increase in concentrations. At the early stage of the toxicant introduction, all the fish survived the initial attack. This may be owing to their defensive adaptations as the respiratory mucosa on the inner walls of the air-sacs is thrown into folds and ridges for increasing the surface area for gas exchange. Under low oxygen level, *C. gariepinus* breathe through their skin and even use their air bladder as an emergency lung by gulping surface air. During the 48h, 72h, and 96h of exposure, the fish displayed physiological malfunctions which were noticeable particularly among some fish in the highest concentrations of Chlorpyrifos (0.85mg/l) and DDforce (0.30mg/l) in which 80% and 90% mortality was recorded. The physiological malfunctions are believed to weaken the organism's resistance to toxins and consequently resulting in the significant death of almost 50% at the highest concentration. With progressive exposure, deaths become inevitable even at a lower concentration. This could be due to stress and the cumulative impact of Chlorpyrifos and DDforce toxicity.

The mortality pattern recorded in this study agrees with that observed by Rand and [32] which stated that there should be less than 35% mortality in one of the concentrations and at least more than 65% mortality in the highest concentration. The mortality observed in the study was considered a result of stress-induced on the immune system of fish. Thus, slow toxic progress and long continuance can result into a chronic toxic response.

Histological changes have been used as important biomarkers in environmental monitoring that allows examining specific target organs. The histological results observed in all the tissues of *C. gariepinus* in this research indicate that sub-lethal concentrations of Chlorpyrifos and DDforce caused moderate to severe alteration in the gill, kidney and liver which are an important organs performing vital functions like detoxification, respiration, osmoregulation, acid base balance. Various alterations in gill were recorded in this study such as deformed and congestion of secondary gill lamellae, hypertrophy, epithelial hyperplasia, haemorrhage in the central venous of cartilaginous core, telengiectiasis, lifting of the epithelia, dilation of the secondary gill lamellae and desquamation. The different sub-lethal concentrations of Chlorpyrifos and DDforce used in the study as well as the different exposure periods showed different degrees of histological changes. Histological results indicated that gill was the primary target tissue affected by Chlorpyrifos and DDforce. Gills are generally considered good indicator of water quality, since the gills are the primary route for the entry of pesticide. In fish, gills are critical organs for their respiratory, osmoregulatory and excretory functions. Many investigators have reported the histological changes in gills of different fish species exposed to pesticides.

Epithelial hypertrophy and Hyperplasia observed in this study could be as a result of epithelial detachment as stated by [23] on exposure of *Metynnis roosevelti* to methyl parathrion. Epithelial lifting increases the distance through which the toxicant reaches the blood stream thereby causing impaired oxygen uptake [21] and could result in dysfunction or even non-functional gills and eventually suffocate the fish. The deformed, curve and congestion of secondary gill lamellae was probably due to increased capillary permeability [29]. Histological alterations observed in the gill tissues of *C. gariepinus* exposed to Chlorpyrifos and DDforce in the study are similar to reports in *Oreochromis niloticus* exposed to dimethoate [12], *Puntius gonionotus* exposed to paraquat [11], *Oncorhynchus mykiss* exposed to the fungicide captan [7] and *C. carpio* [6] exposed to atrazine.

The most organ associated with the detoxification and accumulation process is liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water, it also plays a prominent role in fish physiology, both in anabolism (protein, lipid, carbohydrate) and catabolism (glycogenolysis, detoxification) and it acts as storage centre for many substances, mainly glycogen. The liver of the fish exposed to sub-lethal concentrations of Chlorpyrifos and DDforce compared to the control showed varying degrees of alterations which includes, haemosiderin, necrosis, pyknotic nuclei, vacuolar degeneration, degeneration of hepatocyte, congestion of central tubular vein and congestion of sinusoids. Histological alterations observed in this study are in agreement with the findings of some authors who noticed different toxicological

changes in the liver of fish after exposing to different toxicants. Congestion of central vein in fish liver was reported by [18]. [15] studied the impact of different toxicants on fish liver and they found degeneration of many hepatocytes. Hepatocytes with pyknotic nuclei in liver were studied by [22] and [16] in *Labeo rohita.* The most frequent encountered alterations in the liver of fish exposed to Chlorpyrifos and DDforce are those of vacuolar degeneration and necrosis. The necrosis of the liver tissues and vacuolar degeneration in this study probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis and vacuolar degeneration. The degeneration of hepatocytes may be attributed to direct toxic effects of pollutants on hepatocyte as found in pesticide toxicity, because it is the site of detoxification of all type of toxins and chemicals [25].

The kidney is a vital organ of the body and proper kidney function is to maintain the homeostasis. It is not only involved in removal wastes from blood but it is also responsible for selective reabsorption, which helps in maintaining volume and pH of blood and body fluids and erythropoieses [17]. The alterations observed in the fish exposed to sub-lethal concentrations of Chlorpyrifos and DDforce in this study includes, necrosis, desquamation, damaged of blood vessels, necrotic of the proximal tubules, hydrophobic swelling, degeneration of epithelium, collapsing of the glomeruli. Alterations observed in this study are in accordance with the findings observed by [10]. [10] Reported degeneration in epithelia cell of renal tubules, pyknotic nuclei in the haematopietic tissue and degeneration of glomeruli of fish exposed to deltamethrin. [14] Also reported various histological changes such as degeneration of tubular epithelia and collapsing of glomerulus in the kidney of *Puntius conchonius* following exposure to cadmium.

5. Conclusion

The alterations reported in the histological and behavioural responses of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of Chlorpyrifos and DDforce in this study indicate that histological analysis maybe useful approach for monitoring the long-term effects of pesticides on cultured fish. This in turn will affect the growth and fitness, fecundity of the fish population and other non-targeted organisms such as man through the food chain. Therefore, the toxic hazard of Chlorpyrifos and DDforce should be taken into consideration during its use near the aquatic habitat.

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