Acute and sub-lethal toxicity in African mud catfish (*Clarias gariepinus*, Burchell, 1822) exposed to some pesticides

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Abstract

Pesticides from agricultural run-off pose a severe threat to non-target organisms, such as fishes. This study was carried out to evaluate acute toxicity and histological and genotoxic effects (erythrocytic nuclear abnormalities) of lethal and sub-lethal concentrations of three commonly used pesticides: Atrazine, Butachlor and Glyphosate on the African mud catfish (*Clarias gariepinus*). Fishes were exposed to the pesticides for 96h periods to determine their LC_{50} and the sub-lethal effect at various concentrations ($1/10^{th}$, $1/100^{th}$, $1/1000^{th}$ 96h LC_{50}) over 28 days. The 96h LC_{50} values were 7.63mg/l, 0.7mg/l and 15.97mg/l for atrazine, butachlor and glyphosate, respectively. Histological sections of the liver of *C. gariepinus* exposed to the three pesticides showed mild vascular congestion but no necrosis in the tissue. There was a significant ($p<0.5$) dose-dependent increase in micronuclei and nuclear abnormalities in the erythrocytes of exposed *C. gariepinus* compared to the control by 28 days. The study confirmed that *C. gariepinus* are at risk of adverse effects from exposure to pesticides. Discharge of agricultural run-off around water bodies should be prevented or prohibited to avoid adverse effects on aquatic life.

Keywords: African mud catfish, agrochemical runoff, herbicides, pesticides, priority pollutants, sub-lethal exposure

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Introduction

The rise in human population accompanied by the ever-increasing urbanisation and industrial development have significantly contributed to water pollution, especially in the agricultural sector. This has led to one of the major environmental and public health challenges (Ayoola 2008a). Water soluble toxicants from agricultural run-off are rapidly finding their way into natural water bodies, where they ultimately decompose, volatilise or sometimes form insoluble salts, which precipitate and get incorporated into the sediment (Ezemonye and Tongo 2009). Pollution from agricultural pesticides have gained much attention globally. It has now been classified as a priority pollutant based on its ability to bioaccumulate and deleterious impacts on the environment, animals and humans (Ullah *et al* 2016). High concentrations of herbicides are known to reduce fish survival, growth and reproduction (Rahman *et al* 2002).

Toxicity testing of pesticides on animals has been used for a long time to determine the potential harm pesticides pose to humans (Rahman *et al* 2002). It has been the cornerstone of programmes on environmental health and herbicide safety (Oshode *et al* 2008). Aquatic bioassays are necessary for water pollution control to determine whether a potential toxicant or noxious substance is dangerous to aquatic life and to find the relationship between toxicant concentration and its effect on aquatic organisms (Olaifa *et al* 2003).

Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamine)-S-triazine) is an herbicide commonly used in many countries to control weeds of broadleaves and grasses in intensive agricultural farms. Atrazine acts by inhibiting electron transport in Photosystem II, which disrupts the plant's ability to photosynthesize and causes starvation in broad-leaf plants and eventual death (Giddings *et al* 2004). It has been shown to have diverse effects on organisms such as amphibians and fish that develop and live in freshwater (Rohr 2018). Low concentrations of atrazine (1 µg/l) have been found to alter olfactory-mediated endocrine function in male Atlantic salmon (*Salmo salar*) (Moore and Lower, 2001). *In vitro* studies in fish have shown that atrazine may affect the secretion of cortisol, involved in osmoregulation and stress response (Bisson and Hontela 2002).

Butachlor (2-chloro-N-(2,6-diethyl phenyl acetamide) is an herbicide widely used by farmers to control perennial grasses and weeds with broad leaves (Geng *et al* 2005). It is found in effluents from farmlands causing contamination of the aquatic ecosystem they are deposited into (Ateeq *et al* 2005).

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Studies on *Salmonella typhimurium* strain TA 100 have demonstrated the mutagenicity potentials of butachlor (Hsu *et al* 2005) and induction of micronuclei in catfish erythrocytes (Ateeq *et al* 2002). Glyphosate (N-phosphonomethyl glycine) is a non-selective herbicide used to control a wide variety of annual and perennial grasses and broadleaved weeds in commercial farmlands and household gardens. Glyphosate, known as Roundup®, is next to atrazine (Rohr 2018). These three herbicides are highly soluble in water. They may leach into water bodies after being sprayed on farmlands or directly applied to water systems to control macrophytes (Soso *et al* 2007). Due to their high solubility in water, they regularly contaminate terrestrial and aquatic ecosystems.

Fish are essential bioindicators for determining the conditions and state of the aquatic environments. Hence, studying and understanding fish's physiological, biochemical and histopathological responses to changes or stress in their ecosystem is necessary and vital. The red blood cells of fish are suitable biomarkers for monitoring of water quality of an aquatic environment (Popoola 2018). They are also helpful in evaluating the damage to organs and the consequent physiological, biochemical and behavioural disorders of non-target animals. In order to investigate further the exposure risk of these herbicides, induction of genetic damage in the DNA following acute and/or chronic exposure to xenobiotics can be evaluated using micronucleus assays (Ruiz de Arcaute *et al* 2016; Sogbanmu *et al* 2016).

African catfish (*Clarias gariepinus*) is a significant commercial fish and it is one of the most common and widely consumed freshwater fish in Nigeria (Olaifa *et al* 2002). It is a very good bioindicator to study responses to various pesticides. *Clarias gariepinus* exhibits structural and physiological changes to the presence of xenobiotics. This fish is highly cosmopolitan; apart from being found in African rivers, they are also found in temporary puddles in arid areas after a bit of rain. This study aims to determine the effect of the 96-h acute and sub-lethal exposure of African catfish (*Clarias gariepinus*) to these three pesticides.

Materials and methods

Test pesticides

Trademarked chemicals were used for this study. Astraforce (active agent 80% atrazine) was used as atrazine, retachlor (50% EC butachlor) used as butachlor, and Wynca Nwura wura (active ingredient 360g/l glyphosate) was used as glyphosate. All three are soluble in water. Retachlor and Wynca Nwura wura were in liquid form, while Astraforce was applied in powdered form. They were obtained from a commercial vendor in Lagos, Nigeria.

Test animal: collection and acclimatisation

A total of 200 fingerlings (body weight range: 6-12g; total length range: 4.0-6.0cm) and 50 juveniles (body weight range: 15.7g-35.8g; total length range: 15.5-20.1cm) of *C. gariepinus* (Chordata, Osteichthyes, Siluriformes, Clariidae) were purchased from a reputable farm at Bariga. The fingerlings of African catfish were used for the toxicity test, while the juveniles were used for the chronic test. This is due to the more sensitive nature of fingerlings and juveniles than adults for toxicity tests. Water from the tap at the laboratory was dechlorinated by standing it in the sun for 36 hours before use. The fish were allowed to acclimatize to laboratory conditions (temperature: 28±2 °C; relative humidity: 78±5%) for 72 hours prior to tests. During acclimatization, the fish were fed twice daily with a commercial fish feed (Coppens), and the water in the holding tanks was renewed daily (this was done by replenishing 50% of the water in the stock tank to avoid stress to the fish). Feeding was stopped 24 h prior to commencement of the bioassays (Qayoom *et al* 2016). The water's temperature, pH and dissolved oxygen parameters were monitored. After acclimatization, the fish were introduced to the test toxicants.

Bioassay for acute toxicity

A static renewal bioassay technique by American Society for Testing of Materials (ASTM) was used for this study (ASTM 1990). Preliminary screening was carried out to determine the appropriate concentration range for the test chemicals as described by Sogbanmu *et al* (2018). For each herbicide setup, five fish were exposed per concentration in duplicates over a duration of 96h. Based on this, five concentrations of each herbicide were prepared and tested on fish for the definitive test. After a range finding test, the fingerlings were exposed to varying concentrations; 2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L, 10 mg/L and control (dechlorinated water alone) for atrazine, 14.4 mg/L, 16.3 mg/L, 18.0 mg/L, 19.8 mg/L, 21.6 mg/L and control (dechlorinated water alone) for glyphosate and 4.7 mg/L, 9.4 mg/L, 1.441 mg/L, 1.88 mg/L, 2.35 mg/L and control (dechlorinated water alone) for butachlor. At the beginning of the tests and every 24 hours, number of fish mortality was counted, removed and disposed immediately to prevent contamination of the test media (Sogbanmu *et al* 2018). Fish were considered dead when there was no movement and the body was floating horizontally at the top or sinking at the bottom of the test media. The results of the median lethal concentration (LC_{50}) at 96h were evaluated and recorded. The physicochemical parameters of the test media, including temperature, conductivity, dissolved oxygen, pH, salinity and total dissolved oxygen, were measured and maintained with Hanna instruments (Sogbanmu *et al* 2018).

Acute Toxicity Tests

The acute toxicity tests were carried out using *C. gariepinus* fingerlings. The Organization for Economic Co-operation and Development's (OECD) guidelines (OECD 1992) for acute toxicity testing in fish was used. The studies were conducted using plastic tanks measuring 5 L covered with a mesh to prevent the fish from escaping. Five fingerlings were selected randomly into the test tanks for toxicity testing. After a range finding test, the fingerlings were exposed to varying

concentrations; 2mg/l, 4 mg/l, 6 mg/l, 8 mg/l, 10 mg/l and control (dechlorinated water alone) for atrazine; and 4.7mg/l, 9.4 mg/l, 1.441mg/l, 1.88mg/l, 2.35mg/l and control (dechlorinated water alone) for butachlor; 14.4mg/l, 16.3 mg/l, 18 mg/l, 19.8mg/l, 21.6mg/l and control (dechlorinated water alone) for glyphosate.

Mortality

Mortality was recorded at an interval of 24 hours over a period of 4 days (96 hours). Dead fish were removed and disposed of immediately to prevent contamination of the test media (Sogbanmu *et al* 2018).

Sub-lethal Toxicity Tests

The sub-lethal toxicity tests with atrazine, butachlor, and glyphosate on juveniles of *C. gariepinus* were carried out for 28 days period. The fish were kept in plastic containers measuring 5 litre covered with mesh to prevent the escape of the fish. Five juveniles were randomly selected for the sub-lethal studies. The $1/10^{th}$, $1/100^{th}$ and $1/1000^{th}$ of the 96h median lethal concentration (LC_{50}) values of atrazine, glyphosate and butachlor against the juveniles of *C. gariepinus* were used. The juveniles were exposed to 0.763mg/l ($1/10^{th}$ 96-h LC_{50}), 0.0763 mg/l ($1/100^{th}$ 96-h LC_{50}), 0.00763 mg/l ($1/1000^{th}$ 96-h LC_{50}) for atrazine; 0.07mg/l ($1/10^{th}$ 96-h LC_{50}), 0.007mg/l ($1/100^{th}$ 96-h LC_{50}), 0.0007mg/l ($1/1000^{th}$ 96-h LC_{50}) for butachlor; 1.597mg/l ($1/10^{th}$ 96-h LC_{50}), 0.159mg/l ($1/100^{th}$ 96-h LC_{50}), 0.016mg/l ($1/1000^{th}$ 96-h LC_{50}) for glyphosate, and control (dechlorinated water alone). A semi-static bioassay protocol was followed in which the test media was changed every 48 hours to prevent contamination of the test media with waste metabolites (OECD 1992; Sogbanmu *et al* 2018).

Micronucleus (MN) assay

The juveniles of *C. gariepinus* were exposed to sub-lethal concentrations ($1/10^{th}$ and 1/100th of 96h LC_{50} value) of the test herbicides in duplicates for 28 days. At 28-day post-exposure, blood samples were collected from two active fishes exposed to the test herbicides and the control for micronucleus assay (Obiakor *et al* 2014). The blood samples were collected with a 2ml syringe, smeared on a clean glass slide tilted at an angle of 45° and dried for 24 hours at room temperature. After 24 hours, the slides were fixed in absolute methanol for five minutes and air dried. Subsequent staining was conducted using the May-Grunwald Giemsa stain. The smears were observed under a light microscope (Leica® DM500, Wetzlar, Germany) with an objective lens of ×100 (Mumuni and Sogbanmu, 2018). For proper identification, each MN must have the same colour, the plane of focus, clearly separated and smaller than one-third of the main nucleus (Nwani *et al* 2014).

Histological studies on *C. gariepinus* exposed to atrazine, butachlor, and glyphosate

At 28-day post-exposure, two active juveniles from which blood was drawn (for micronucleus assay) were euthanised and dissected. The liver from each fish was harvested and prepared for histological studies. The liver was fixed in Bouin's fluid and subsequently dehydrated through a graded series of ethanol (Eseigbe *et al* 2013). They were embedded in paraffin and then manually sectioned with a microtome at 4-5µm. The sections were then dewaxed and stained with hematoxylin and eosin (H&E) and examined using a digital light microscope (Leica® DM500) (Sogbanmu *et al* 2018).

Statistical analysis

The toxicological dose-response data for acute toxicity studies were calculated using the probit analysis to derive the median lethal concentrations (concentration of test pesticides that caused 50% mortality of exposed test fishes) for 96-h (96-h LC_{50}). Toxicity Factor (TF) was calculated as the ratio of the least toxic pesticide to the most toxic pesticide. The micronucleus assay results were presented as mean ± standard error. One-way analysis of variance (ANOVA) and Fisher's least significance difference (LSD) test were employed to test for significant difference between treatment means and control at $p< 0.05$. All statistical analyses were performed using IBM SPSS, Version 20.0.

Results

Acute toxicity concentration-response of *C. gariepinus* to pesticides exposure

The acute toxicity of *C. gariepinus* to atrazine, butachlor and glyphosate is summarized in Table 1. The lowest (2.0mg/l) and highest (10.0mg/l) concentrations of exposure to atrazine resulted in 20% and 70% mortality of catfish, respectively after 96h, indicating an increase in mortality as concentration increases. The Control (0.00mg/l) had a 10% mortality.

Table 1: Concentration-response relationship of *C. gariepinus* exposed to atrazine, butachlor and glyphosate

Pesticide	Conc. (mg/l)	Log Conc.	96h Mortality	% Mortality
Atrazine	2	0.3	2	20
	4	0.6	1	10
	6	0.78	4	40
	8	0.9	5	50
	10	1	7	70
	0	0	1	10
Butachlor	0.47	-0.33	4	40
	0.94	-0.03	6	60
	1.41	0.15	7	70
	1.88	0.27	9	90
	2.35	0.37	10	100
	0	0	1	10
Glyphosate	14.4	1.16	4	40
	16.3	1.21	5	50
	18	1.26	6	60
	19.8	1.3	8	80
	21.6	1.33	10	100
	0	0	0	0

The lowest concentration (0.47mg/l) of butachlor pesticide caused 40% of mortality after 96hours, while the highest (2.35mg/l) concentration resulted in 100% mortality of *C. gariepinus* after 96hours. There was 10% mortality in the Control after 96 hours. The lowest (14.4mg/l) concentration of glyphosate caused 40% of

mortality in the fish after 96 hours, while the highest (21.6mg/l) concentration resulted in 100% mortality of *C. gariepinus* after 96 hours. There was no mortality in the control after 96 hours.

The overall toxicity after 24hours showed butachlor as the most toxic of the three pesticides with LC_{50} value of 4.48mg/l, while atrazine and glyphosate had 43.45mg/l and 114.67mg/l, respectively. Butachlor showed a toxicity that was 7 times more than glyphosate and 2.6 times more than atrazine (Table 2). The toxicity of the pesticides after 24 hours is summarized as follows: Butachlor>Atrazine >Glyphosate. After 48 hours, butachlor was still the most toxic of the three pesticides, showing toxicity of over 16 times more than glyphosate and 7.5 times more than atrazine. After 72 hours, butachlor showed toxicity of over 23 times more than glyphosate and 11.6 times more than atrazine. The overall toxicity after 96 hours showed that, butachlor was the most toxic of the three pesticides with an LC_{50} value of 0.67mg/l, while atrazine and glyphosate had 15.97mg/l and 7.63mg/l, respectively. Butachlor showed toxicity approximately 24 times more than glyphosate and 11.32 times more than atrazine. The toxicity of the pesticides after 96hours is summarized as follows: butachlor>atrazine >glyphosate.

Nuclear abnormalities of *C. gariepinus* erythrocytes exposed to the pesticides

The frequencies of occurrence of these abnormalities in the *C. gariepinus* juveniles exposed to sub-lethal concentrations of the selected pesticides are presented in Table 3. The abnormalities observed in the red blood cell nucleus of exposed catfishes were micronuclei (MN), binuclei (BN), bud-shaped nuclei, 8-shaped nuclei, notched nuclei and bleb shaped nuclei (Plates 1 and 2).

Table 2: Comparative 24h, 48h, 72h and 96h acute toxicity of the pesticides on fingerlings of *C. gariepinus*

Time	Pesticides	LC_5 (mg/l)	LC_{50} (mg/l)	LC_{95} (mg/l)	DF	SE	Probit equation	TF
24h	Atrazine	2.99	11.40	43.45	3.00	1.24	3.00x -3.20	2.55
	Butachlor	0.05	4.48	384.58	3.00	0.86	0.88x - 0.55	1.00
	Glyphosate	8.40	31.04	114.67	3.00	3.44	3.50x -4.93	6.94
48h	Atrazine	2.88	8.54	25.33	3.00	1.24	3.50x - 3.5	7.56
	Butachlor	0.07	1.13	18.62	3.00	0.75	6.00x - 7.5	1.00
	Glyphosate	9.95	19.00	36.26	3.00	3.00	1.50x + 0	16.81
72h	Atrazine	1.87	8.24	36.37	3.00	0.92	2.86x - 2.36	11.65
	Butachlor	0.10	0.71	5.20	3.00	0.78	2.50x + 0.50	1.00
	Glyphosate	9.96	16.90	28.69	3.00	2.90	10.00x - 12.00	23.90
96h	Atrazine	1.29	7.63	45.32	3.00	0.83	3.57x -2.57	11.32
	Butachlor	0.17	0.67	2.72	3.00	0.86	2.86x + 0.64	1.00
	Glyphosate	11.15	15.97	22.45	3.00	3.06	9.33x – 11.13	23.70

LC_5 = 5% Lethal Concentration, LC_{50} = 50% lethal concentration, LC_{95} = 95% lethal concentration, DF = degree of freedom at 95% confidence level, TF = toxicity factor, SE= standard error

Table 3: Frequencies of nuclear abnormalities in *C. gariepinus* juveniles exposed to sub-lethal concentrations of the three pesticides (n = 1000 erythrocytes per pesticide)

Pesticides	Concentration	MN	8-SHAPED	NT	BLB	BUD	BN	Total
Atrazine	$1/10^{th}$	4 (0.4)	4 (0.4)	4 (0.4)	8 (0.8)	4 (0.4)	12 (1.2)	36 (3.6)
	$1/100^{th}$	6 (0.6)				6 (0.6)	10 (1.0)	22 (2.2)
	$1/1000^{th}$	3 (0.3)	2 (0.2)	3 (0.3)	6 (0.6)	6 (0.6)	3 (0.3)	23 (2.3)
Butachlor	$1/10^{th}$	10 (1.0)	14 (1.4)		8 (0.8)	8 (0.8)		40 (4.0)
	$1/100^{th}$	10 (1.0)	2 (0.2)	4 (0.4)		16 (1.6)		32 (3.2)
	$1/1000^{th}$	12 (1.2)	4 (0.4)	4 (0.4)		12 (1.2)	8 (0.8)	40 (4.0)
Glyphosate	$1/10^{th}$	5 (0.5)	9 (0.9)		10 (1.0)	14 (1.4)		38 (3.8)
	$1/100^{th}$	3 (0.3)	10 (1.0)	5 (0.5)	5 (0.5)	10 (1.0)		33 (3.3)
	$1/1000^{th}$	5 (0.5)	10 (1.0)			7 (0.7)		22 (2.2)
Control	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

MN = Micronucleus; NT = Notched; BLB = Blebbed; BUD = Budded; BN = Binucleated

All the cells of the exposed catfishes displayed at least two nuclear abnormalities with the micronucleus being the most observed in the exposed groups. Butachlor $1/10^{th}$ and $1/1000^{th}$ induced the highest nuclear abnormalities of 33 abnormalities, representing 3.3% of the exposed cells, while the least abnormalities were induced by atrazine $1/100^{th}$ and glyphosate $1/1000^{th}$. Figure 1 shows the nuclear abnormalities caused by different concentrations of atrazine, butachlor and glyphosate, while Figure 2 shows the comparison of the three herbicides.

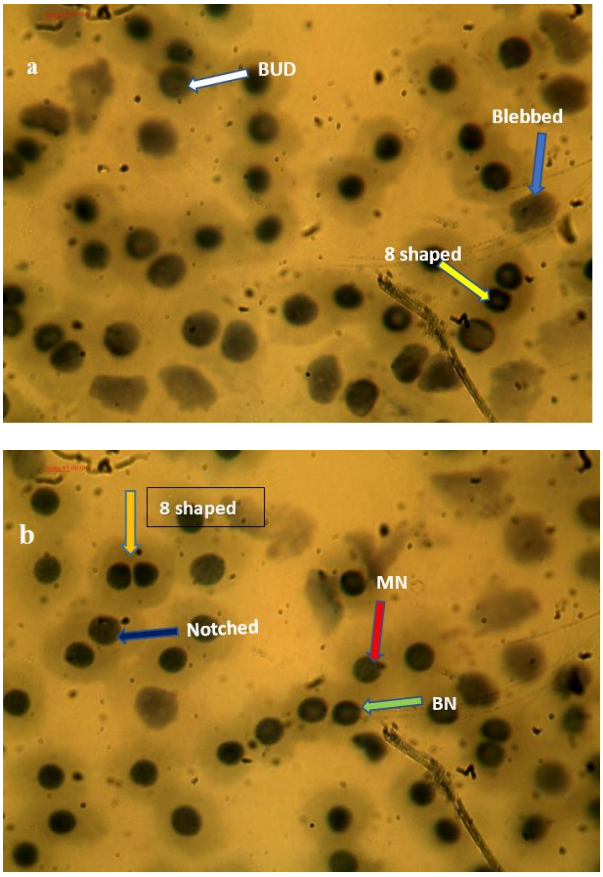

Plate 1. Nuclear abnormalities caused by (a) Atrazine and (b) butachlor

BUD = Budded, MN = Micronucleus; BN = Binucleated

Histology of the *C. gariepinus* liver exposed to the 1/10th, 1/100th and 1/1000th LC_{50} of Atrazine, Butachlor and Glyphosate

Plate 2a is the histologic section of *C. gariepinus* liver tissue exposed to 1/10th of the LC_{50} of atrazine. It showed parallel radially arranged plates of hepatocytes with central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. No abnormality was observed. Plate 2b is the histologic section of *C. gariepinus* liver tissue exposed to the 1/100th of the LC_{50} of atrazine. It showed parallel radially arranged plates of hepatocytes with central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. No abnormalities were seen. Plate 2c shows the histological section of *C. gariepinus* liver tissue exposed to the 1/1000th of the LC_{50} of atrazine. There were parallel radially arranged plates of hepatocytes with central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. No abnormality was seen.

The histologic section of *C. gariepinus* liver tissue exposed to the 1/10th of the LC_{50} of butachlor is shown in Plate 3a. It shows parallel radially arranged plates of hepatocytes with central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm with acinar cells. No abnormalities like necrosis were observed.

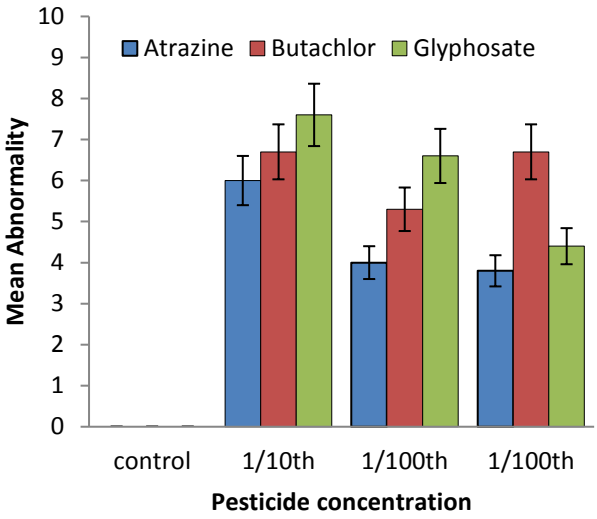

Figure 1. Mean nuclear abnormalities against sub-lethal concentration of atrazine, butachlor and glyphosate

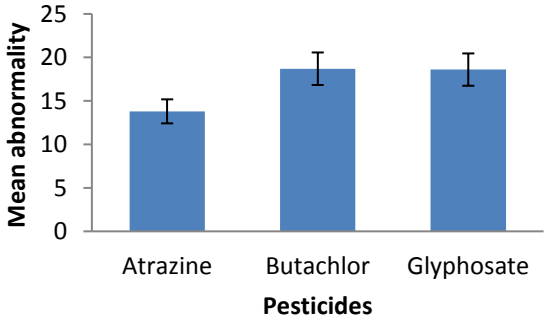

Figure 2. Mean nuclear abnormalities against all three pesticides

Plate 3b showed the histologic section of *C. gariepinus* liver tissue exposed to the 1/100th of the LC_{50} of butachlor. There were parallel and radially arranged plates of hepatocytes with central vein (CV) and portal vein (PV). The basophilic portion with nucleus and the acidophilic cytoplasm had acinar cells. No abnormalities were seen. Plate 3c showed the histologic section of *C. gariepinus* liver tissue exposed to the 1/1000th of the LC_{50} of butachlor. It had parallel and radially arranged plates of hepatocytes with a central vein (CV) and portal vein (PV). The background contained areas of aggregates of infiltrating red cells and congested blood vessels were observed.

The histologic section of *C. gariepinus* liver tissue exposed to 1/10th of the LC_{50} of glyphosate is shown in Plate 4a. It showed parallel radially arranged plates of hepatocytes with central vein (CV), portal vein (PV) and the basophilic portion with nucleus, and the acidophilic cytoplasm with acinar cells. No abnormality was observed. Plate 4b showed the histologic section *C. gariepinus* liver tissue exposed to the 1/100th of the LC_{50}

of glyphosate. The tissue had parallel and radially arranged plates of hepatocytes with central vein (CV) and portal vein (PV); the basophilic portion with nucleus and the acidophilic cytoplasm with acinar cells.

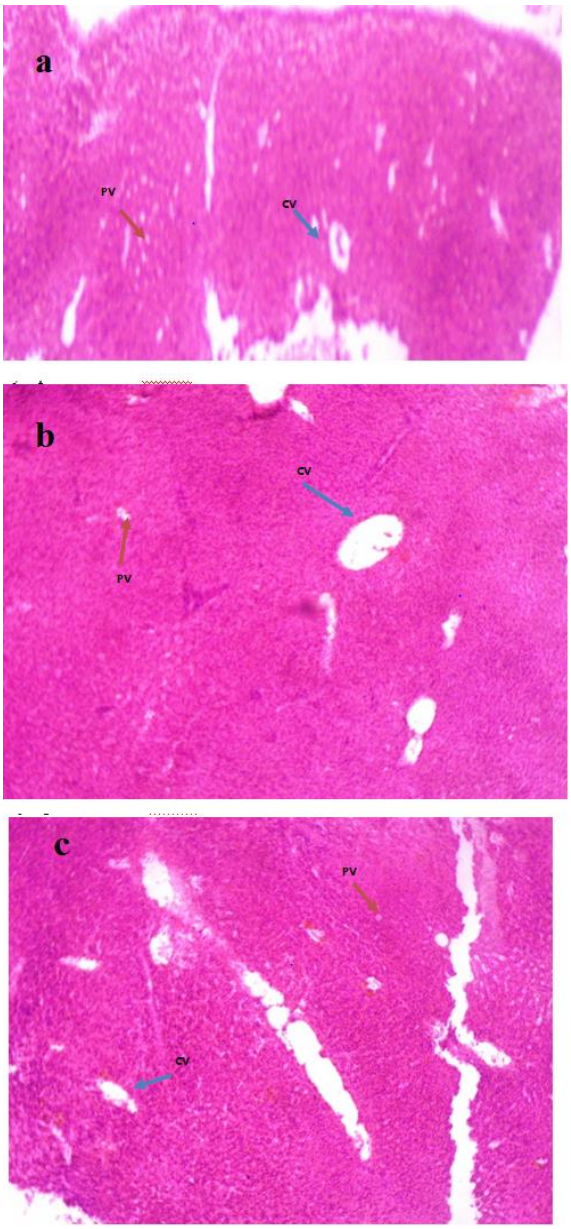

Plate 2. Section of the *C. gariepinus* liver exposed to (a) $1/10^{th}$ (b) $1/100^{th}$ and (c) $1/1000^{th}$ LC_{50} of atrazine

No abnormality was observed. Plate 4c showed the histologic section of *C. gariepinus* liver tissue exposed to the $1/1000^{th}$ of the LC_{50} of glyphosate. It had parallel and radially arranged plates of hepatocytes with central vein (CV) and portal vein (PV). The background had areas with aggregates of infiltrating red cells. Congested blood vessels were also observed.

Discussion

The result of this study showed that the three pesticides exhibited acute toxicity to the exposed catfishes for 96 hours and based on 50% lethal concentration; butachlor was the most toxic while glyphosate was the least toxic. The difference in the toxicity of the herbicides might be due to the differences in their chemical composition as well as their specific mechanism of action. For example,

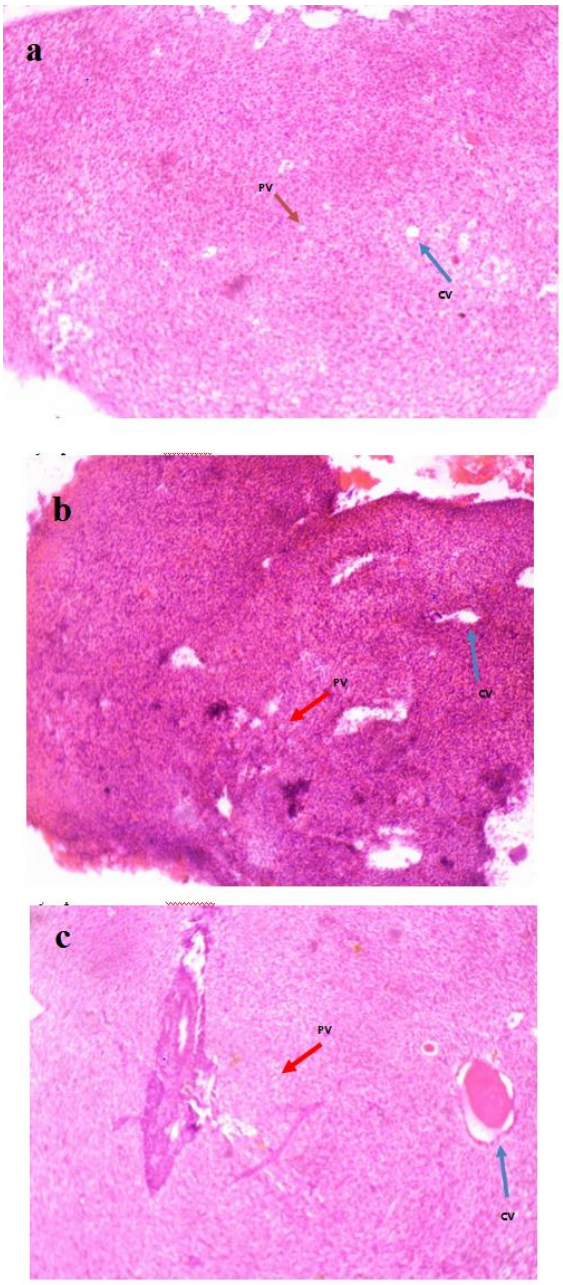

Plate 3. Section of the *C. gariepinus* liver exposed to (a) $1/10^{th}$ (b) $1/100^{th}$ and (c) $1/1000^{th}$ LC_{50} of butachlor

butachlor a chloroacetanilide compound acts by inhibiting the production of lipids, alcohols, fatty acids, proteins, isoprenoids and flavonoids (Heydens *et al* 2002). Atrazine is an endocrine disruptor that can cause hormone imbalance (EPA 2012); while glyphosate is a neurotoxin, which acts by inhibiting acetylcholinesterase (USEPA 2011). The observed LC_{50} values of the three pesticides varied with those from previous studies of Nwani *et al* (2020) who reported LC_{50} values of 4.84mg/l and 54.74mg/l for butachlor and atrazine pesticides

exposed to *C. gariepinus* and Ayoola (2008a) who reported LC_{50} value of 1.50mg/l for glyphosate exposed to Nile tilapia.

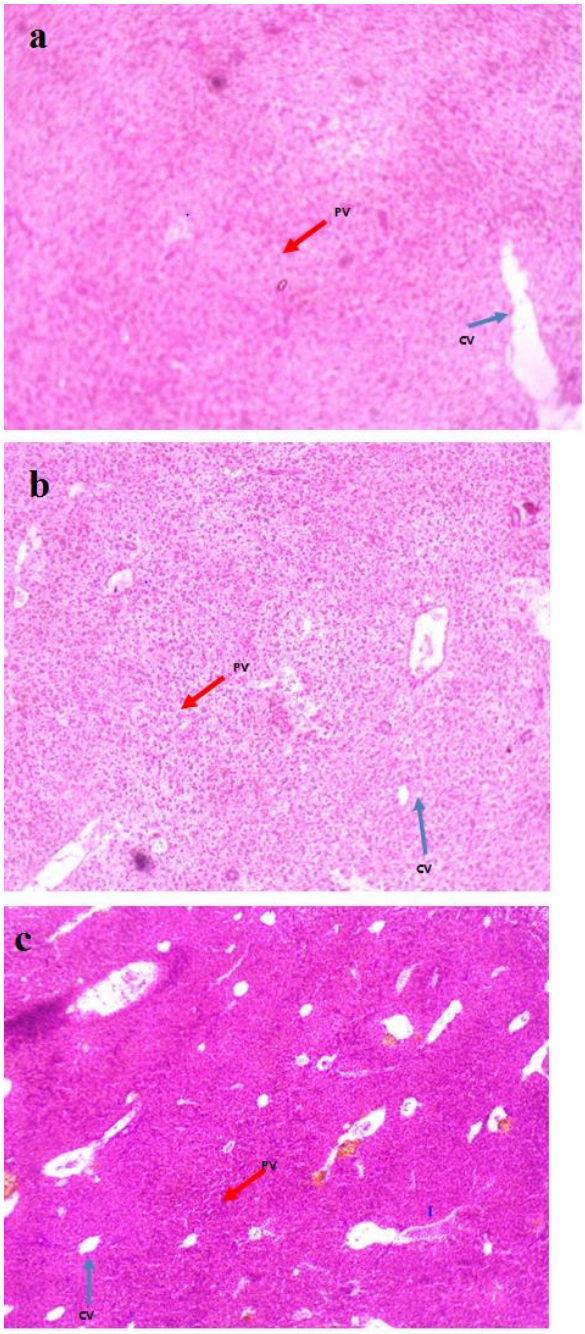

Plate 4. Section of the *C. gariepinus* liver exposed to (a) $1/10^{th}$ (b) $1/100^{th}$ and (c) $1/1000^{th}$ LC_{50} of glyphosate
CV = central vein; PV = portal vein

These differences in responses may be attributed to the formulation of the pesticides used in the studies, the age of species used, time and conditions of exposure. This study proved the fact that responses by organisms to pesticides are usually species dependent as well as pesticide specific. This implies that, it depended on organismal susceptibility even within same species, age, environmental conditions as well as the particular chemical characteristics of the pesticides rather than a universal toxicity response by organisms (Amaeze *et al* 2020).

Nuclear abnormalities and micronuclei induction in erythrocytes can be attributed to the elimination of amplified genetic materials from the cell (Fench 2011), they can also be from chromosomal breaks or losses that were not incorporated into the main nucleus during the cell division cycle (Renu and Saxena 2015). The findings of this study showed a correlation between exposure to pesticides and induction of nuclear abnormalities in the red blood cell of catfishes. Nuclear abnormalities especially micronucleus have been reported in several fish organs (Srivastava *et al* 2016), and their induction in fish has been associated with environmental stress (Arkhipchuk and Garanko 2005).

Sub-lethal concentrations of the three herbicides assessed in this study showed varying degrees of nuclear abnormalities. These abnormalities are induced in response to genotoxic agents being linked with exposure to heavy metals (Ergene *et al* 2007) and pesticides (Malla and Ganesh 2009) in fishes.

Results from this study showed a concentration and time-dependent increase in nuclear abnormalities in *C. gariepinus* exposed to atrazine, butachlor and glyphosate. It was similar to the findings of Nwani *et al* (2020) who reported an increase in micronucleus frequency in *C. gariepinus* exposed to three commercial herbicides. Cavas (2011) reported a significant increase in the frequencies of MN and DNA strand breaks in the erythrocytes of *Carassius auratus* exposed to sub-lethal concentrations of commercial atrazine formulation. Elevations of MN frequency have been reported in blood erythrocytes of *Cnesterodon decemmaculatus* (Vera-Candioti *et al* 2013) and *Rhamdia quelen* (Piancini *et al* 2015) exposed to the pirimicarb-based formulation and atrazine herbicide, respectively.

Fishes have a slower rate of repairing damaged DNA when compared to mammals especially humans, making them useful warning organisms i.e. sentinel species in assessing the impacts of genotoxic pollutants (Espina and Weis 1995). The increase in nuclear abnormality can be attributed to oxidative stress, resulting from the production of reactive oxygen species (ROS). This is buttressed by findings from Ullah *et al* (2016) and D'Costa *et al* (2018) who pointed to the fact that oxidative stress is a major precursor for DNA damage.

There are growing public health concerns of pesticide runoffs into freshwater bodies, the associated effects with the consumption of fishes, which have bioaccumulated these pesticides (Eqani *et al* 2013) as well as their usage as a source of portable drinking water (Sankhla *et al* 2018). Sankhla *et al* (2018) reported that pesticide exposures to humans are associated with reproductive and developmental defects as well as immunological abnormalities and haematopoietic cancers.

Morphological alterations in the organs of living organisms are usually more obvious to identify the physiological variations (Fanta *et al* 2003); hence are useful tools for early warning signs to the health of an

animal. Lanning *et al* (2002) describe histopathological study as a sensitive endpoint for evaluating the impacts of toxicants on organs of living organisms and capable of providing specific information on the acute and chronic effects of toxicants on targeted organs that may not be detected by functional biomarkers (Amacher *et al* 2006). In the present study, the exposed liver showed parallel radially arranged plates of hepatocytes with a central vein (CV), portal vein (PV) background containing an area of aggregates of infiltrating red cells and congested blood vessels as well as mild vascular congestion, none of the concentrations showed necrosis in the observed tissue. The histopathological abnormalities observed particularly in the higher sub-lethal concentrations of the pesticides agreed with the observations in the MN assay, which can serve as early warning indices for the evaluation of fish health in the aquatic environment.

Conclusion

The acute toxicity effects, induction of genotoxic effects in *C. gariepinus* as well as alteration of histological parameters observed for all pesticides assessed, show that non-target aquatic species are at risk of environmentally relevant concentrations (sub-lethal) of pesticides especially from non-point sources making it a serious health concern to aquatic organisms and man as well. In view of this, consistent monitoring of pesticide levels and sensitization of farmers on responsible use of pesticides should be carried out by environmental agencies to mitigate adverse ecological effects in aquatic ecosystems. More than ever, product control of pesticides, and the use of environmentally acceptable, target-specific and quick degrading chemicals in pesticide formulation should be encouraged in order to mitigate the potential negative consequences on fish and other non-target aquatic organisms. Furthermore, it is also important to locate farmlands far away from water bodies in order to minimize the risk of surface runoffs and atmospheric depositions into the aquatic ecosystem.

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