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Acute Toxicity and Histopathological Effects of 2,4-D Amine on Gill and Liver of *Clarias gariepinus* Juvenile

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Abstract: The acute toxicity of Checkmate®, a herbicide containing 720 g L^{-1} of 2,4-D amine (2,4-Dicholorophenoxyacetic acid) to juveniles of *Clarias gariepinus* of mean weight of 36.40±0.01 g and standard length of 17.91±0.21 cm were investigated under laboratory condition. In a static renewal bioassay, the fish were exposed to 0.00, 3.42, 3.76, 4.14, 4.50 and 4.86 mg L^{-1} of 2,4-D amine. The 96 hours lethal concentration (LC_{50}) of 2,4-D amine to juvenile of *Clarias gariepinus* was 3.95 mg L^{-1}. The fish exposed to 2,4-D amine showed toxicological signs of erratic swimming, loss of balance and air gulping before death during the acute bioassay. Histopathological changes in the gill architecture were characterized by lifting of epithelia layer, eroded gill filaments, necrosis of epithelial cells, hyperplasia and fusion of lamellar epithelial cells, hemorrhage and erosion of lamellae. There were evidence of ruptured hepatocytes, cellular infiltration and degeneration of the central vacoulation, haemorrhages, hypertrophy of hepatocytes, fibrosis and necrosis in the liver of fish exposed to 2,4-D amine. 2, 4-D amine is highly toxic and its use in or near aquatic environment should be monitored.

INTRODUCTION

Herbicides are widely used for the control of water plants which may impede the flow of aquatic life and may contribute long term effects in the environment (Annune *et al.*, 1994). The constant flow of agricultural effluents into fresh water often leads to a variety of pollutant accumulation which becomes apparent when considering toxic pollution (Mason, 1991). Pesticide surface runoff into rivers and streams can be highly lethal to aquatic life. Herbicides can accumulate in bodies of water to levels that kill off zooplankton, the main source of food for young fish.

Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Ward and Parrish, 1982). Aquatic

bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa *et al*., 2003). The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding and for the evaluation of the effects of noxious compounds (Ayoola, 2008).

2, 4-D is a broadleaf herbicides recommended for use in both terrestrial and aquatic environment. 2, 4-D exists in the form as amine, acid but oxyethyl ester or several salts which vary in their chemical properties, environmental behaviour and toxicological characteristic. The chemical has a half-life of 15 days and was reported by Tomlin (2006) to be highly toxic to aquatic organisms. Regardless of the brand purchased, the active ingredient for all 2,4-D products is the same with the molecular formula: $C_8 H_6 Cl_2 O_3$ (Tomlin, 2006). The LC50 of 2,4-D amine to fish and aquatic invertebrate ranges from 80-2244 mg acid equivalents per litre. The objective of this study is to investigate the lethal concentration and the acute effect of 2,4-D amine on gill and liver of the fish by histopathology.

MATERIALS AND METHODS

Source, experimental site and maintenance of test organisms: *Clarias gariepinus* juveniles from same parent stock with standard length 17.91±0.21cm and mean weight 36.40±0.01 g were obtained locally from tidoo fish farm makurdi. The experimental fish were conveyed in open-top plastic jerrycans to the Fisheries Department Laboratory of the Benue State Ministry of Agriculture and Natural Resources where the experiment was conducted. They were held in large water bowls and acclimated with River Benue water for 14 days to laboratory conditions. The top of the water bowls was covered with netted material to prevent jumping out of the fish. A slit was made at the middle of the net to allow for feeding fish and cleaning of the bowls. Feeding commenced 24 h after the arrival and stopped twenty-four hours before the commencement of the experiment. During acclimation, fish were fed twice daily (09:00 and 16:00 h) with commercial fish feed (Coppens) at 5% body weight. The fishes were accepted as well as adapted to laboratory conditions when <5% death was recorded for the 14 days. The water in the bowl was changed daily and uneaten feed and faecal matters were siphoned out using hose. Dead fish were also removed to minimize contamination of water.

Source of herbicide: Test chemical 2,4-D amine with the trade name Checkmate® a herbicide containing 720 g L^{-1} of 2,4-D amine (2,4-Dicholorophenoxyacetic acid) was obtained commercially from Franken Agrochemical store, Makurdi and was used for the study.

Acute toxicity test: Acute 96 h static renewal bioassays were conducted in the laboratory as described by Sprague (1975) and APHA, AWWA, WPCF (1980) to investigate the toxicity of 2,4-D amine to *C. gariepinus* after the range test was carried out. In the bioassay test carried out, six bowls of size 50 L were used for the study, five containing different concentration of the toxicant and the 6th bowl had no toxicant (control). The bioassay was conducted in triplicate. The desired 2,4-D amine concentrations were measured and introduced into 20 L of River Benue water in the bowls. The nominal concentrations for 2, 4-D amine used for *C. gariepinus* were 0.00, 3.42, 3.78, 4.14, 4.50 and 4.86 mg L^{-1}. The mixture was allowed to stand for 30 min before introducing test organisms (*C. gariepinus*). Thereafter, a total of 180 juveniles of *Clarias gariepinus* were sorted randomly and stocked at 10 fish per bowl for the experimental run. The 96 h LC_{50} was estimated using the probit analysis.

Histopathology studies: After the 96 hours experiment, the gill and liver of the survived fish from each treatment were exercised and preserved in 10% formalin. The samples were processed for histological examination using standard histological techniques (Avwioro, 2002).

Observation: During the period of acute toxicity test, mortality was observed and recorded for 6, 12, 24, 48, 72 and 96 h, respectively. During each observation time, fish were prodded gently to see if there was any response; the cessations of opercula movement also were used as indices of dead fish. Fish that did not respond was presumed dead. Dead fish were recorded and removed immediately from the test solution to avoid fouling the media. Behavioral and morphological changes were examined by visual observation. Physiochemical parameters of the test water were also monitored every 24 h during the period of acute test.

RESULTS AND DISCUSSION

Acute toxicity: The symptoms of toxicosis observed in fish exposed to lethal concentrations of 2,4-D amine were uncoordinated movement, vertical swimming, gasping for oxygen, restlessness, loss of equilibrium, white spots on the body, skin haemorrhage and death. Mucus accumulation was observed on the body surfaces and gill filaments of dead fish.

Table 1 shows the physico-chemical parameters of test solution in the acute bioassay while Fig. 1 shows the 96 h LC_{50} of 2,4-D amine calculated as 3.95 mg L^{-1}. The expression, R^2 value in the figure indicates that, mortality

Table 1: Mean water quality parameters obtained during exposure of *Clarias gariepinus* juveniles to acute toxicity of 2, 4-D amine

Concentration (mg L^{-1})	Dissolved oxygen (mg L^{-1})	pH	Temperature (°C)	Total dissolved solid (mg L^{-1})	Electrical conductivity (µS cm^{-1})
0.00	5.23±0.45^{e}	9.31±0.33^{a}	26.00±0.32^{a}	167.80±2.44^{a}	322.80±11.94^{a}
3.42	4.60±0.01^{d}	9.33±0.32^{a}	26.14±0.05^{a}	185.80±8.01^{ab}	389.80±9.23^{b}
3.78	4.50±0.03^{d}	10.07±0.14^{b}	26.08±0.04^{a}	194.00±7.36^{b}	394.90±11.98^{b}
4.14	4.08±0.04^{c}	10.17±0.01^{b}	26.10±0.03^{a}	208.20±9.24^{b}	438.60±11.42^{c}
4.50	3.22±0.04^{b}	10.18±0.01^{b}	26.04±0.02^{a}	234.00±11.79^{c}	511.80±7.94^{d}
4.86	3.04±0.05^{a}	10.22±0.00^{b}	26.06±0.02^{a}	284.60±7.83^{d}	587.80±8.33^{e}
p-value	<0.05	0.003	0.983	<0.05	<0.05

*means in the same column with different superscripts differ significantly

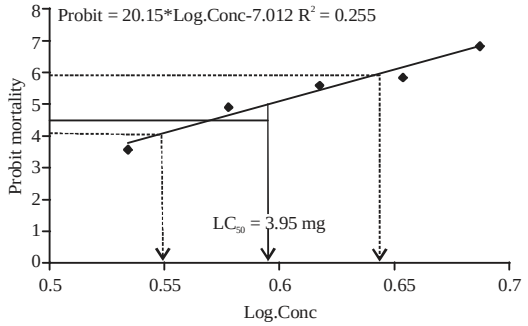

Fig. 1: Linear Relationship between log. Concentration of 2,4-D amine and the probit kill of *clarias gariepinus* Juveniles.

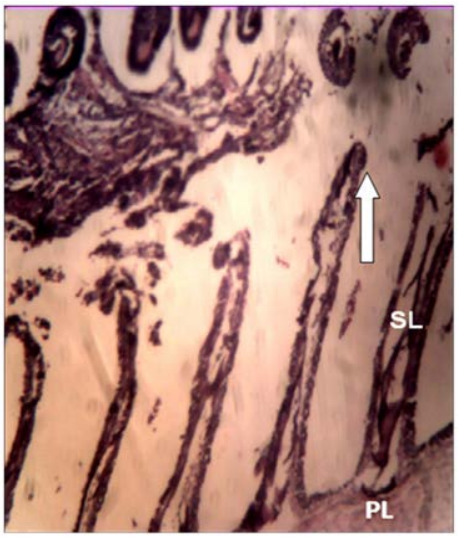

Fig. 2: Photomicrograph of gill cells of *Clarias gariepinus* juvenile at control (0.0 mg L^{-1}) showing normal pillar cell (white arrow), Primary Lamella (PL) and Secondary Lamella (SL). Mag×100

rate of fish increased with increase in concentration of 2,4-D amine. Appendix 1 shows the mortality record of *Clarias gariepinus* juveniles exposed to different concentrations of 2, 4-D amine for 96 h.

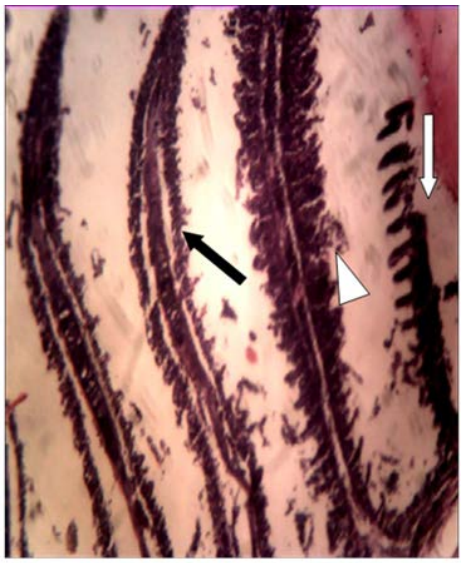

Fig. 3: Photomicrograph of gill cells of *Clarias gariepinus* juvenile exposed to 3.42 mg L^{-1} of 2,4-D amine showing degeneration of the gill filaments (black arrow), deformed secondary lamellae (white arrow) and gill filaments being eroded (arrow head). Mag×100

Histopathological effects: The results of histopathology of *clarias gariepinus* gills exposed to acute concentrations of 2,4-D amine is shown in Fig. 2-6 while that of the liver is shown in Fig. 7-11.

Several abnormal behaviours observed with *Clarias gariepinus* exposed to acute concentration of 2,4-D amine in this study were incessant jumping and gulping of air, restlessness, loss of equilibrium increased opercular activities, surface to bottom movement, sudden quick movement and resting at the bottom which were similar to the observations of Omoniyi *et al.* (2002), Ayuba and Ofojekwu (2011) and Okomoda and Ataguba (2011) who subjected *Claria gariepinus* to varying toxicants. The stressful and erratic behaviour of *C. gariepinus* juveniles in the experiment indicates respiratory impairment, probably due to the effect of the toxicant 2,4-D amine on the gills. This is similar to the report by Ezemonye and

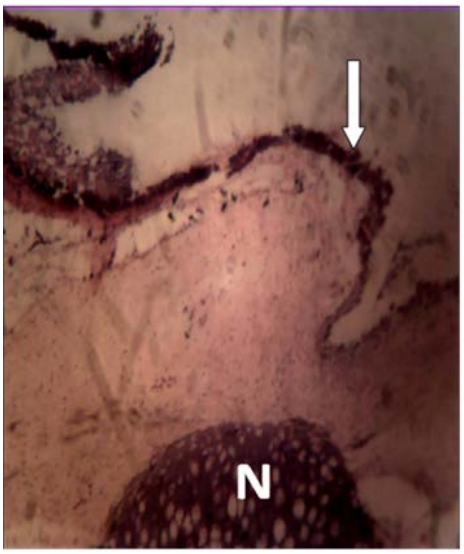

Fig. 4: Photomicrograph of gill cells of *Clarias gariepinus* juvenile exposed to 3.78 mg L^{-1} of 2,4-D amine showing intense cellular necrosis and blood congestion (N), deformed secondary lamellae (white arrow) and thick coating of mucus covering the entire gill filaments and lamellae (black arrow). Mag×100

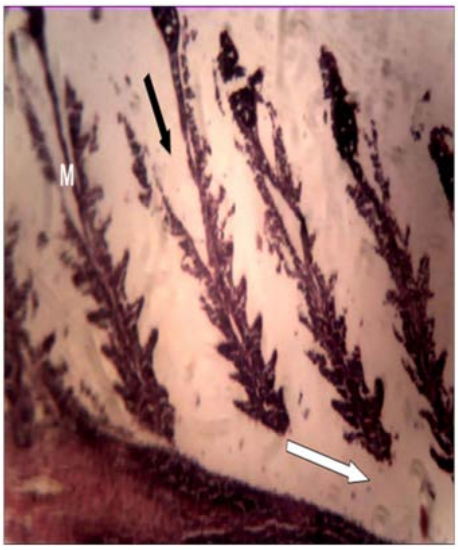

Fig. 6: Photomicrograph of gill cells of *Clarias gariepinus* juvenile exposed to 4.50 mg L^{-1} of 2,4-D amine showing distortion of gill filaments as some have blanketed and eroded (black arrow), malformed secondary lamellae (M) and epithelial rapture with haemorrhage (white arrow). Mag×100

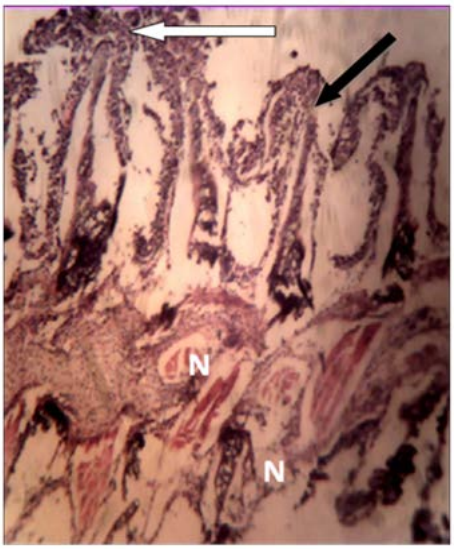

Fig. 5: Photomicrograph of gill cells of *Clarias gariepinus* juvenile exposed to 4.14 mg L^{-1} of 2,4-D amine showing intense cellular necrosis (N), erosion of secondary lamellae (black arrow) and hyperplasia and fusion of lamellar epithelium cells (white arrow). Mag×100

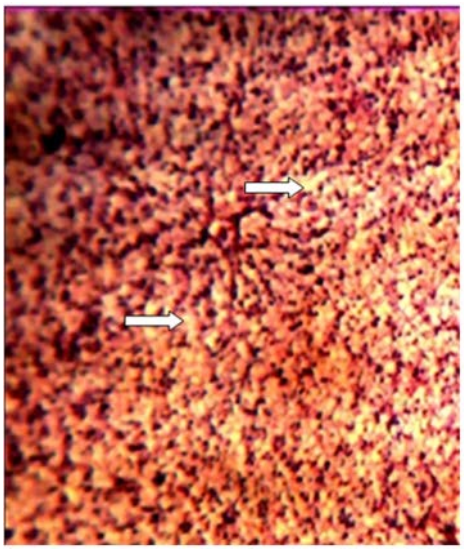

Fig. 7: Photomicrograph of liver cells of *Clarias gariepinus* juvenile at control (0.00 mg L^{-1}) showing hepatocytes (white arrows). Mag×250

Ogbomida *et al*. (2010). The stressful behaviour of respiratory impairment due to the toxic effect of 2,4-D amine on the gills was similar with the report of Omitoyin *et al*. (2006) and Aguigwo (2002) that pesticide impairs respiratory organs.

It was also observed in this study that the higher the concentration of 2,4-D amine, the higher the mortality rate

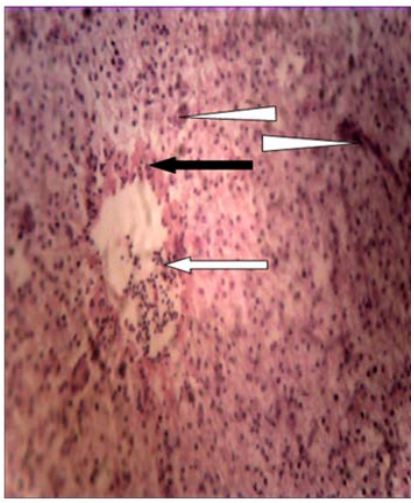

Fig. 8: Photomicrograph of liver cells of *Clarias gariepinus* juvenile exposed to 3.42 mg L^{-1} of 2,4-D amine showing ruptured hepatocytes (arrow heads), Cellular infiltration and degeneration of the central vacoulation (white arrow) and haemorrhages (black arrow). Mag×250

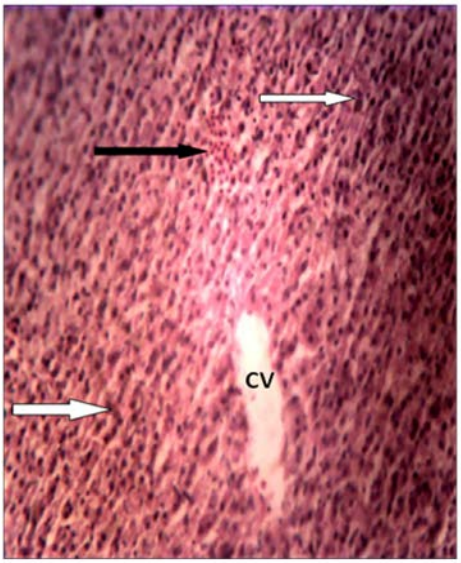

Fig. 9: Photomicrograph of liver cells of *Clarias gariepinus* juvenile exposed to 3.78 mg L^{-1} of 2,4-D amine showing deformed central vacoulation (cv), haemorrhages (black arrow) and hypertrophy of hepatocytes (white arrows). Mag ×250

which is similar to the observation of Fryer that in all toxicant, a threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is

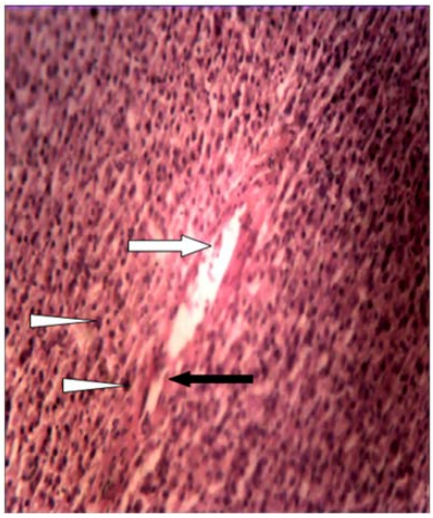

Fig. 10: Photomicrograph of liver cells of *Clarias gariepinus* juvenile exposed to 4.14 mg L^{-1} of 2,4-D amine showing Hypertrophy of the herpatocytes (arrow heads), cellular infiltration and degeneration of the central vacoulation (white arrow), cellular degeneration (black arrow). Mag×250

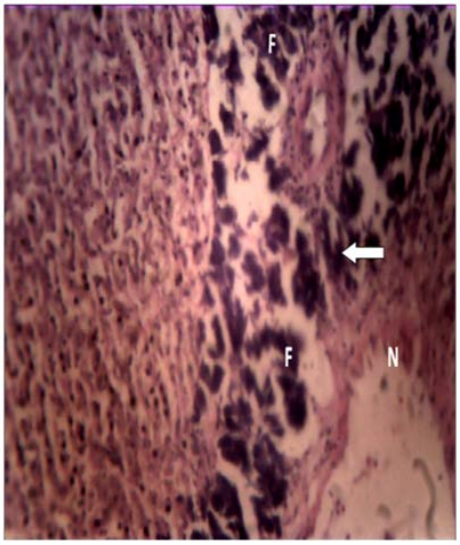

Fig. 11: Photomicrograph of liver cells of *Clarias gariepinus* juvenile exposed to 4.50 mg L^{-1} of 2,4-D amine showing Fibrosis (F), Cellular infiltration and degeneration of the central vacoulation (white arrow) and Necrosis (N). Mag×250

in a tolerance zone, above the tolerance zone is the zone of resistance. The time of toxicity disappearance and

mortality was observed from the record of the relative mortality time in different concentrations of 2,4-D amine for 96 h.

The first death was recorded 32 h after the introduction of *Clarias gariepinus* in the bowl with the highest concentration of 2,4-D amine (4.86 mg L^{-1}). This is in conformity with Guedenon *et al*. (2012) who recorded the first death after 30 h while treating *Clarias gariepinus* with 120 mg L^{-1} of cadmium sulphate.

Datta and Kaviraj (2003), Fafioye *et al.* (2004) and Okomoda *et al.*(2010) recorded their first death 36 h after the exposure to acute toxicity treatment of *Clarias gariepinus* with *synthetic pyrethyroid Deltamethrin, Raphia Vinifera* extracts and formalin respectively.

The duration of resistance of *Clarias gariepinus* in the present study agrees with the findings of Datta and Kaviraj (2003), Fafioye *et al.* (2004) and Okomoda *et al.* (2010) who reported that *Clarias gariepinus* is tolerant to pollutants.

Okeke (2004) reported 96 hrs LC_{50} of *Oreochromis niloticus* when exposed to paraquat to be 3.56 mg L^{-1}. The LC_{50} found in this investigation is 3.95 mg L^{-1} which is in variance with that of Shallangwa (2011) who worked on toxicity of 2,4-Dichlorophenoxyacetic acid on African mud fish *Clarias gariepinus* (Teugals). However, the LC_{50} value obtained in this study fell within the range of 80-2244 mg L^{-1} reported by Gervais *et al.* for fish and aquatic invertebrate. Makinde *et al.* (2015) reported LC_{50} of 0.20 g L^{-1} of 2, 4-D amine to *Clarias gariepinus* juvenile. While Gabriel *et al.*, (2010) reported LC_{50} of 0.17 g L^{-1} when fingerlings of *Clarias gariepinus* were exposed to amine salt of 2, 4-D.

While that of *Clarias gariepinus* exposed to diazinon was 6.03 mg L^{-1}. Aguigwo (2002) studied the toxic effects of cymbush pesticide on growth and survival levels of *Clarias gariepinus* fingerlings and reported the 96 h LC_{50} to be 4.17 mg L^{-1}. Toxicity of fish to toxicant depends on size and species (Noga, 2012; Craig, 2006). Kousar and Javed (2012) reported an age dependent increase in the tolerance of *Labeo rohita, Cirrhina mrigala, Catla catla* and *Ctenopharyngodon idella* to acute toxicity of copper.

The difference in the LC_{50} found in this investigation in addition to the above mentioned might be due to the various substances and compound used in the experiment and also the distinct environmental conditions.

The physico-chemical parameters of the test water used for the acute bioassay decreased in dissolved oxygen and increased slightly in pH with increase in concentration of the toxicant. Total dissolved solids and electrical conductivity varied significantly. This might be due to the effects of 2,4-D amine on the water quality. This is similar to Ayuba *et al*. (2012) and Ayuba and Ofojekwu (2011) who worked on *Datura innoxia* root extracts. Even though Total Dissolved Solids (TDS) and Electrical Conductivity (EC) values differed significantly ($p<0.05$) as compared to the control values, they were still within acceptable limits according to Mackereth. Oxygen concentration reduced with increased concentration of 2,4-D amine. Generally toxicity increased with reduced oxygen concentration. This is similar with reports by Adigun (2005), Kolo *et al.* (2008, 2009) and Ayuba *et al*. (2012) who worked on various toxicants. This result agrees with the report by Carozzi (1997), who reported that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration which will impair respiration leading to asphyxiation. Increased temperature and other physiological state of the fish can be attributed to the death in acute as reported by Boyd (1979), Rahman *et al.* (2002) and Olaifa *et al.* (2003).

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to toxicants. Thophon *et al*. (2003). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows for examining specific target organs including gills, liver and kidney that are responsible for vital functions, such as respiration, excretion, accumulation and biotransformation of Xenobiotics in the fish.

Gills are the first target organ of several pollutants because of their very large interface area between external and internal fish environment, performing vital functions such as gas exchange and ion osmoregulation, gills are partially sensitive to adverse environmental conditions (Ogundiran *et al*., 2009). The gill surfaces have a direct contact with aquatic toxicants (Simonato *et al*., 2008) and have been shown to display various structural alterations in the branchial respiratory epithelia surface upon exposure (Ferguson, 2006).

Alterations like epithelial lifting, hyperplasia and hypertrophy of epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanism, since, in general, these results in increase of the distance between the external environment and the blood and thus serves as barriers to the entrance of contaminant (Peleksic and Mitrovic-Tutundlic, 1994; Fernandes and Mazon, 2003).

In this investigation, the alterations occurred in acute concentrations of 2,4-D amine to gills of *Clarias gariepinus* juveniles were eroded gill filaments, degeneration and necrosis of epithelial cells, deformed lamellae, hyperplasia and fusion of lamellar epithelial cells, hemorrhage in the primary and secondary gill lamellae, distortion of the secondary lamellae and gill filaments, disruption of epithelial cells from pillar cells, thick coating of mucus covering the entire gill filaments and lamellae and erosion of secondary lamellae were all noticed during exposure of acute concentration which is similar to the alterations recorded by Olufayo and Alade (2012) when *Heterobranchus bidorsalis* was exposed to

acute toxicity of Cypermethrin; Babu etc in exposure of *Cirrhinus mrigala* to dichlorvos; Makinde *et al.* (2015) and Shallangwa (2011) in the exposure of *Clarias gariepinus* juveniles to acute concentrations of 2,4-D amine.

Severe alterations were observed in fish exposed to the highest concentration of 2, 4-D amine as compared to lesser concentrations. The damage to the gills could be responsible for the hyper respiratory and osmoregulatory activities of the fish which led to fatique and finally death of some of the fish. These findings are similar to that reported by Omoregie and Ufodike (1991). The alterations in gill architecture which are adaptive are necessary to reduce the rate of absorption of toxic substances. Epithelia hypertrophy increases the water-blood distance thereby reducing the rate of absorption of xenobiotics (Agamy, 2013). However, epithelia hypertrophy decreases the respiratory surface area thus, reducing the effectiveness of gas exchange and also leading to osmoregulatory dysfunction (Sakuragui *et al.*, 2003).

The organ most associated with the detoxification and accumulation process is the liver and due to its functions, position and blood supply; it is also one of the organs most affected by contaminants in water (Camargo and Martinez, 2007). It also plays a prominent role in fish physiology both anabolism and catabolism and its acts as storage center for many substance mainly glycogen.

The histological section of the liver of *Clarias gariepinus* juveniles exposed to acute toxicity of 2, 4-D amine in this study show alterations when compared with the control groups. There was evidence of ruptured hepatocytes, cellular infiltration and deformed or degeneration of the central vacoulation, haemorrhages, hypertrophy of hepatocytes, fibrosis and necrosis. This result conforms with the reports by Ezemonye and Ogbomida (2010) when *clarias gariepinus* were exposed to Gammalin 20; Babu etc when *Cirrhinus mrigala* were exposed to dichlorvos and Doherty *et al*. (2011) when *C. gariepinus* was exposed to 96 hours acute toxicity of paraquat dichloride.

The vacuolated cells observed in the liver of the exposed fish shows evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification as reported by Rahman *et al.* (2002).

CONCLUSION

The toxic effects of 2,4- D amine on *Clarias gariepinus* juveniles were assessed through this investigation and it could be concluded that, the environmental contamination can induce several histopathological alterations in the tissues of *C. gariepinus* which could lead to their death. Therefore, monitoring and prohibition of the use of Checkmate® (720 g L^{-1} of 2, 4-D amine) in agricultural fields is therefore advocated to protect aquatic organisms from this kind of toxic chemical.

APPENDIX

Appendix 1: Mortality Record of *Clarias gariepinus* juveniles exposed to different concentrations of 2, 4-D amine for 96 h

Concentration (mg L^{-1})	Log_{10}Conc	Total No. of test fish	Mortality rate after 96 h	Percentage mortality	Probit mortality value
0.00	-	30	0	0.00	-
3.42	0.5340	30	2	6.67	3.52
3.78	0.5775	30	14	46.67	4.92
4.14	0.6170	30	22	73.33	5.61
4.5	0.6532	30	24	80.00	5.84
4.86	0.6866	30	29	96.66	6.88

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