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Histopathological Effects of Cypermethrin on Juvenile African Catfish (*Clarias gariepinus*)

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Summary

The acute toxicity of cypermethrin an insecticide, to juvenile African catfish, *C. gariepinus* was investigated with emphasis on histopathological effects. *Clarias gariepinus* (Burchell 1822) juvenile were exposed to 0, 1.9, 4.1, 9, 21, and 45mg/l of cypermethrin. The lethal concentration (LC_{50}) value of cypermethrin was 0.063mg/l for 96h of exposure. The mean mortality percentages were 0, 0, 70, 90, 100 and 100, in the order of concentration of 0, 1.9, 4.1, 9, 21 and 45 mg/L respectively.
Cypermethin concentration corresponding to the 96h LC_{50} value for juvenile *C. gariepinus* was used to study the effects of cypermethin exposures in inducing histopathological changes of gills, liver, kidney and brain. In the gills, filament cell proliferation, cellular infiltration, haemorrhage and epithelial lifting were observed. In the liver, there was vacuolation of hepatocytes and necrosis. In kidney there was exfoliation and swollen with pyknotic nuclei. The brain showed neuronal degeneration and spongiosis. These changes occurred predominantly in the 96h exposure. Respiratory stress, erratic swimming and instant death of fish were observed in exposed fish, which varies with the concentration of the toxicant and it's showed that mortality increased with increase in concentration. Cypermethrin is highly toxic to juvenile fish.

KEY WORDS: *Clarias gariepinus*, acute toxicity, histopathology, cypermethrin

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Introduction

The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding, and for aquatic system, fish have become indication for the evaluation of the effects of noxious compounds. (Evrnest, 2004). Pesticides occupy a unique position among many chemicals which are encountered daily by man. They are deliberately added to the environment for the purpose of killing, injuring or at times enhancing the development of some forms of life. Pesticides represent one group of agro-chemicals that are used in large quantities by agro-farmers.

Water pollution by pesticides is a serious problem to all aquatic fauna and flora . In aquatic environment, pesticides may also cause several physiological and biochemical defects in fishes (Vasanthi *et al*, 1989).

Nowadays contamination of water with these recalcitrant chemicals often results in bioaccumulation in fish and other biota, sometimes to biologically active levels. These chemicals have been suspected to be cancer-causing agents in fish and other aquatic organism (GESAMP, 1991). Residues of these toxic chemicals found in water, sediments, fish and other aquatic biota can pose a risk to organisms, to predators and to human being. Pesticides at high concentrations are known to reduce the survival, growth and reproduction of fish and produce many visible effects on fish. (Rahman *et al*, 2002). Water pollution also is recognized globally as a potential threat to both human and other animal population, which interact with the aquatic environment (Biney *et al* 1987; Svensson *et al*, 1995). Due to the residual effects of pesticides important organ are damaged (Rahman *et al* 2002).

Cypermethin is a synthetic pyrethroid with potent insecticide property (WHO, 1992). The cypermethin pesticide whose toxicity is to be investigated is widely used in Nigeria by agro-formers for the treatment of ectoparasitic disease and pests of maize, cotton, vegetables and sorghum. The usefulness of the pesticide has always masked its toxic effects on the aquatic environment (Osibanjo and Bambose 1990, Olaruntuyi *et al* 1992).

Thus, the objective of this study was to investigate the acute toxic effects of cypermethrin in African catfish (*C. gariepinus*) with emphasis in the histopathological changes in the gills, livers, kidney and brains.

Materials and Methods

Five hundred species of *C. gariepinus* with the mean weight of 10.0±0.3g and standard length mean length of 6.0±0.1cm were used for the experiment. This is due to the more sensitive nature of juveniles than adult for toxicity test. (Solbe 1995, Odiette 1999, Reish and Oshida 1987). They were purchased from a reputable fish farm in Oyo State, Nigeria. The fish were acclimatized in laboratory conditions for four weeks during which they were fed with commercial floating pellets at 10% of their body weight.

Unconsumed feed and faecal were removed and water replenished regularly as recommended by Oyelese and Faturoti (1995).

Dechlorinated tap water of temperature = 26.0 ±0.8 ^{0}C, p^{H} = 7.0, and dissolved oxygen 6.3±0.1mg/l were used. 2

Acute Toxicity tests

A static renewal bioassay techniqu(ASTM 729-90(1990)) was adopted in which the test media was renewed at the same concentration once every 24hours. Preliminary screening was carried out to determine the appropriate concentration range for testing chemical as describe by Solbe (1995). The following concentration in weight per volume of cypermethin were used 0.0,0.01, 0.1, 1, 10, 100, 1000mg/L. Seven *C. gariepinus* juvenile per concentration of toxicant were used with 3 replicates each for 96h. Based on this, five concentrations (0.0, 1.9, 4.1, 9, 21, and 45mg/l) of the insecticide were prepared and tested on the *C. gariepinus* juveniles for the definitive test. Ten acclimated fish were used in each aquarium containing different concentrations of cypermethrin as well as in the control as describe by Solbe (1995) and Rahman *et al* (2002).

At the beginning of the tests and every 30 minutes behavioural changes and the number of dead fish were recorded. Other external changes in the body of the fish were observed accordingly. Dead fishes were promptly removed and preserved in 10% formaldehyde. The organs (gills, livers, kidneys and brains) were removed and prepared for histopathological observation. They were fixed in Bouin's fluid for 24hours, and washed with 70% ethanol and dehydrated through a graded series of ethanol (Schalm *et al* 1995, Kelly 1979). They were embedded in paraffin, sectioned at 4-5µm; thickness stained with hematoxylin and eosin and examined using light microscope and photomicrography (Keneko, 1989). The mean physico-chemical parameters of the test concentrations (cypermethrin) on *Clarias gariepinus* were observed. The median lathal concentration (LC_{50}) at 96h was computed using the probit, logit analysis and ANOVA.

Results

The mean physico- chemical parameters of the test concentrations (cypermethrin) on *C. gariepinus* are presented in Table 1 while the effect of different concentrations and exposure time of cypermethrin on *C. gariepinus* a juveniles are presented in Table 2 There were significant relationship ($p<0.05$) between the temp, p^{H}, and dissolved oxygen with cypermethrin concentration.

Toxicity of cypermethrin

The LC_{50} value based on probit analysis was found to be 0.063mg/l for 96h and is presented in Fig. 1. No adverse behavioral changes or any mortality was recorded in the control fish throughout the period of the bioassay. The behaviour of the control fishes and their colour were normal. Symptoms of toxicosis observed in fish behaviour with cypermethrin include lack of balance, agitated or erratic swimming, air gulping, restlessness, sudden quick movement, excessive section of mucus, rolling movement and swimming on the back were observed. The fish became very weak, settled at the bottom and died. The colour of the skin of *C. gariepinus* changes from normal darkly pigmentation in the dorsal and lateral parts to very light pigmentation in the dorsal and lateral part.

Histopathological studies

Summary of histopathological changes observed in the gill, liver, kidney and brain of *C. gariepinus* subjected to different concentration of cypermethrin for 96h is presented in table 3.

3

Gills

No recognisable changes were observed in the gills of the control fish. Each gill consisted of a primary filament and secondary lamellae (Plate 1a). At different concentrations of cypermethrin there were cellular infiltration, swollen tip of the gill filament, congestion, severe gill damaged and hererophilic infiltration. (Plates 1b-1f).

Liver

The histology of control fish liver revealed normal typical paranchymatous appearance. The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus (plate 2a). There were glycogen vacuolation, fatty infiltration, hemosiderosis and congested central vein at the concentration of 1.9 to 9mg/l, severe infiltration of leukocytes, pyknotic and hepatic necrosis were also observed at 21 and 45mg/l concentration (plates 2b-2f) severe necrotic, haemrrhage and vacuolation were observed accordingly as described in Table 3.

Kidney

No recognisable changes were observed in the kidney of the control fish (plate 3a). At the light microscopic level, the renal corpuscle was composed of the glomerulus and Bowman's capsule. The kidney tissue from *C. gariepinus* exposed to different concentrations of cypermethrin showed necrosis, degenerated kidney tubules pyknosis, exfoliated and swollen with pyknotic neclei (Plates 3b-3f).

Brain

No significant lesion was seen in the brain of the control fish, and the morphological structure is normal (plate 4a). The histopathological observation on the brain showed that there was discolouration on the brain of fish exposed to different concentration of cypermethrin. Fish exposed to concentration of 1.9 to 45mg/L showed mononuclear infiltration neuronal degeneration, infiltration and severe spongiosis. (plates 4b to 4f).

Table 2: Rate of mortality of *Clarias gariepinus* juvenile on exposed to Cypermethrin per treatment

Treatment/hr	1	3	6	9	12	15	18	21	24	27	30	33	36	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	Total Mortality	% mortality
T_{OA}																																	00	00
T_{OB}																																	00	00
T_{OC}																																	00	00
T_{1A}																																	00	00
T_{1B}																																	00	00
T_{1C}																																	00	00
T_{2A}														01	01									01	02			00				01	06	60
T_{2B}														00	01									01	03			01				01	07	70
T_{2C}														00	01									00	01			03				02	07	70
T_{3A}								01	02							02					01				01			00		01			09	90
T_{3B}								02	02							01					01				01			00		00			08	80
T_{3C}								01	01							01					01				00			02		00			09	90
T_{4A}		01			01				01	02						01	01							01	02								10	100
T_{4B}		01			01				01	02						00	01							01	02								10	100
T_{4C}		02			01				01	02						01	01							01	02								10	100
T_{5A}	01	01		01		01			01		02			01		01	01																10	100
T_{5B}	00	02		01		02			01		02			01		01	01																10	100
T_{5C}	01	02		01		01			01		02			01		01	01																10	100

T_0= Control treatment without toxicant, T_1=1.9mg/l of toxicant T_2=4.1mg/l of toxicant, T_3=9mg/l of toxicant, T_4=21mg/l of toxicant, T_5=45mg/l of toxicant,

Table 1: Mean physico-chemical parameters of the test concentrations (cypermethrin) on *Clarias gariepinus*

Concentration	Physico-chemical parameters		
(mg/l)	Do (mg/l)	pH	Temp ^{0}C
0.0	6.3 ± 0.1^f	7.0 ± 0.1^a	26.0 ± 0.8^a
1.9	5.4 ± 0.1^e	6.9 ± 0.3^a	27.0 ± 0.1^b
4.1	5.3 ± 0.6^d	6.7 ± 0.1^b	27.2 ± 0.1^b
9	5.2 ± 0.1^c	6.6 ± 0.3^c	27.4 ± 0.3^c
21	4.9 ± 0.3^b	6.3 ± 0.3^d	27.5 ± 0.1^c
45	4.7 ± 0.1^a	6.3 ± 0.1^e	28.0 ± 0.3^d

*Mean values followed by the same superscript in each column are not significant different (p<0.05)

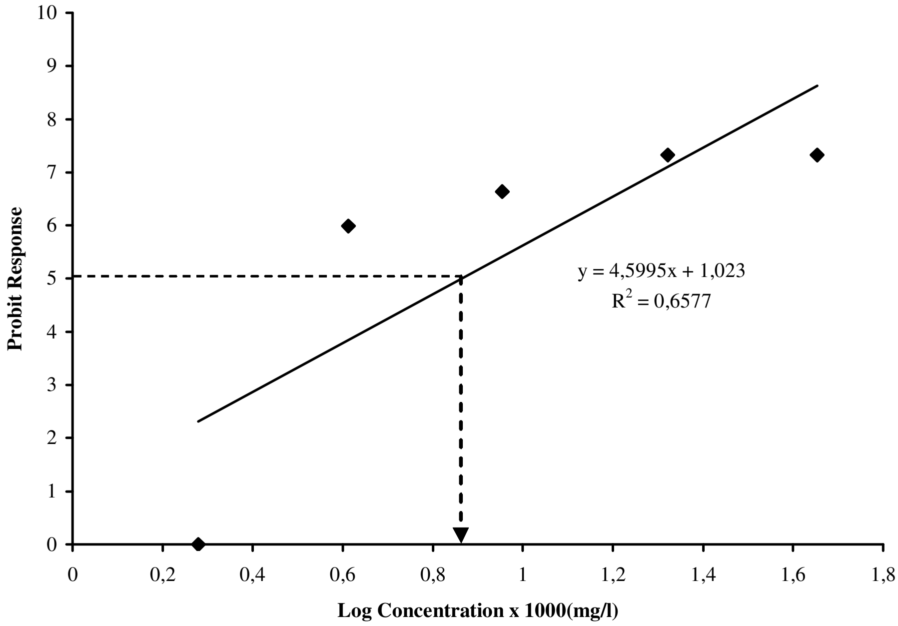

Fig.1: Linear relationship between probit response and log concentration of Cypermethrin on juveniles of *Clarias gariepinus* .

Table 3: Summary of histopathological changes observed in the brain, gill, liver and kidney of *Clarias gariepinus* juveniles subjected to different concentrations of cypermethrin (cyperforce) for 96h

Treatment Concentration	Hour of Merit	Organs	Congestion	Necrosis	Cellular Infiltration	Spongiosis	Pyknosis	Haemorrhage
O (Control) mg/l	96	B	-	-	-	-	-	-
		G	-	-	-	-	-	-
		L	-	-	-	-	-	-
		K	-	-	-	-	-	-
1.9 mg/l	96	B	½	-	-	-	-	½
		G	-	-	½	-	-	+
		L	-	½	-	-	-	-
		K	-	-	-	-	-	-
4.1 mg/l	96	B	½	-	-	-	-	½
		G	-	-	½	-	-	+
		L	-	½	½	-	-	-
		K	-	-	½	-	-	-
9 mg/l	96	B	+	-	-	+	-	½
		G	+	-	+	-	-	+
		L	-	+	½	-	-	½
		K	-	-	½	-	+	½
21 mg/l	96	B	+	+	-	++	-	++
		G	++	+	+	-	-	++
		L	+	++	+	-	+	+
		K	+	+	+	-	++	+
45mg/l	96	B	++	++	++	++	+	++
		G	++	+	++	++	+	++
		L	+	++	+	-	+	++
		K	+	++	+	-	++	++

Key: B = Brain, G = Gill, L = Liver, K = Kidney, - = Completely absence, + = Present, ½ = Mild, ++ = Severe.
Note: Treatment with negative signs indicated no histopathological changes were observed.

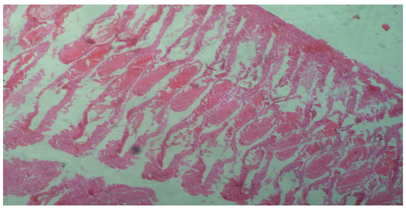

Plate 1a: **Gill of *C. gariepinus* X25. The 96-h exposed in the control group no significant lesion seen**

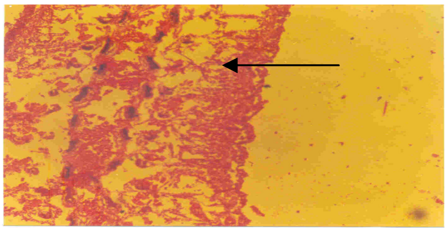

Plate 1b: **Gill of *C.gariepinus* X25. The 96-h exposed at 1.9mg/l (cypermethrin) showing mild cellular infiltration**

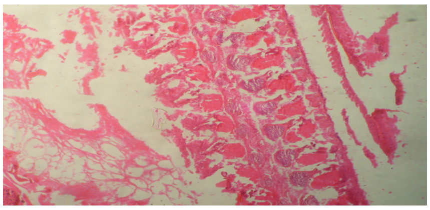

Plate 1c: **Gill of *C. gariepinus* X25. The 96-h exposed at 4.1mg/l (cypermethrin) showing swollen tip filament.**

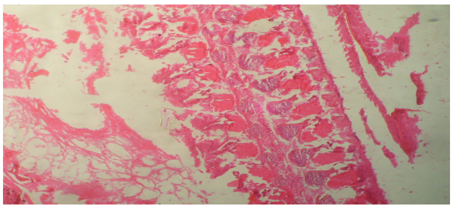

Plate 1d: **Gill of *C.gariepinus* X25. The 96-h exposed at 9mg/l (cypermethrin) showing infiltration and haemorrhage.**

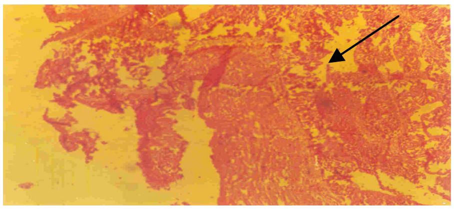

Plate 1e: **Gill of *C.gariepinus* X25. The 96-h exposed at 21mg/l (cypermethrin) showing infiltration, congestion and gill damaged.**

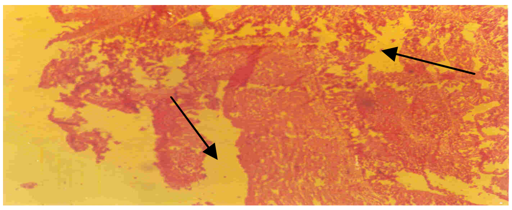

Plate 1f: **Gill of *C.gariepinus* X25. The 96-h exposed at 45mg/l (cypermethrin) showing infiltration, haemorrhage and hererophilic infltration.**

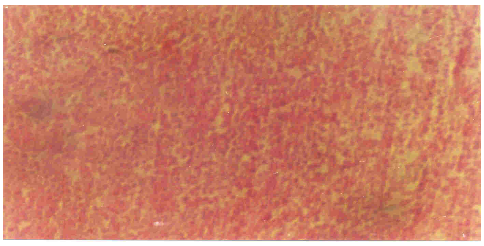

Plate 2a: Liver of *C. gariepinus* X25. The 96-h exposed in the control group no significant lesion seen

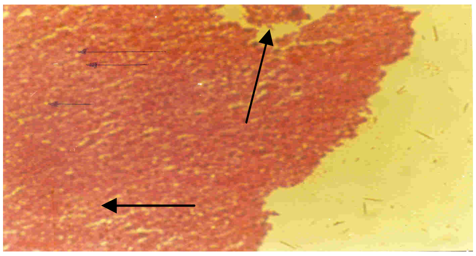

Plate 2b: Liver of *C. gariepinus* X25. The 96-h exposed at 1.9mg/l (cypermethrin) showing glycogen vacuolation

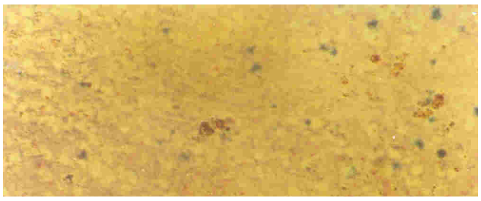

Plate 2c: Liver of *C. gariepinus* X25. The 96-h exposed at 4.1mg/l (cypermethrin) showing (1)fatty infiltration (2) hemosiderosis

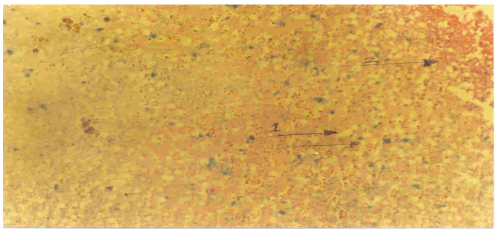

Plate 2d: Liver of *C. gariepinus* X25. The 96-h exposed at 9mg/l (cypermethrin) showing darkly stained specks of necrotic, glycogen vacuolation and congested central vein.

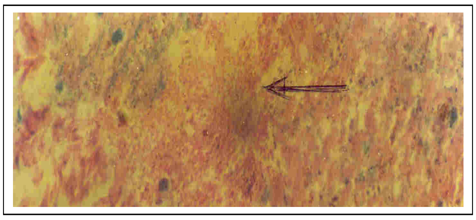

Plate 2e: Liver *C. gariepinus* of X25. The 96-h exposed at 21mg/l (cypermethrin) showing severe infltration of leukocytes (arrow) pyknotic (N) and lipid vacuoles (L)

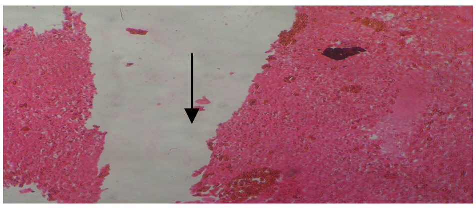

Plate 2f: Liver of *C. gariepinus* X25. The 96-h exposed at 45mg/l (cypermethrin) showing diffuse hepatic necrosis

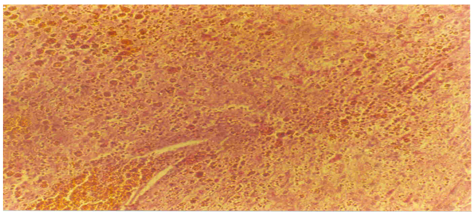

Plate 3a: Kidney section of *O. C. gariepinus* X25. The 96-h exposed in the control group no significant lesion was seen.

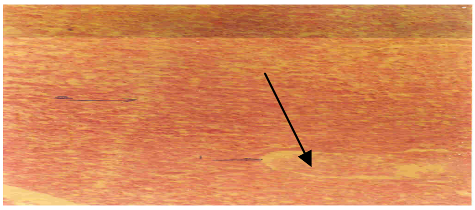

Plate 3b: Kidney section of *C. gariepinus* X25. The 96-h exposed at 1.9mg/l (cypermethrin) showing mild pyknotic nuclei

Plate 3c: Kidney section of *C. gariepinus* X25. The 96-h exposed at 4.1mg/l (cypermethrin) mild pyknosis

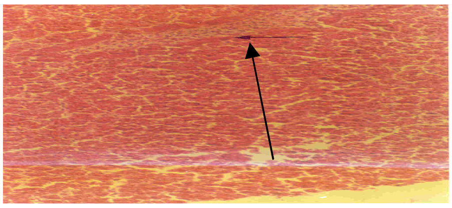

Plate 3d: Kidney section of *C. gariepinus* X25. The 96-h exposed at 9mg/l (cypermethrin) showing homosiderosis and pyknosis

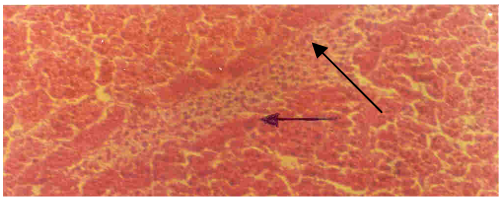

Plate 3e: Kidney section of *C. gariepinus* X25. The 96-h exposed at 21mg/l (cypermethrin) showing fatty infiltration and pyknosis

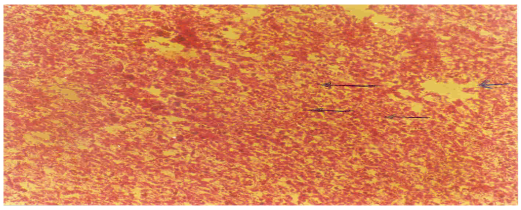

Plate 3f: Kidney section of C. *gariepinus* **X25. The 96-h exposed at 45mg/l (cypermethrin) exfoliated and swollen with pyknotic nuclei.**

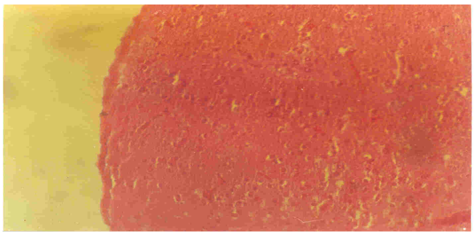

Plate 4a: Brain of *C. gariepinus* X25. The 96-h exposed in the control group no significant lesion was seen.

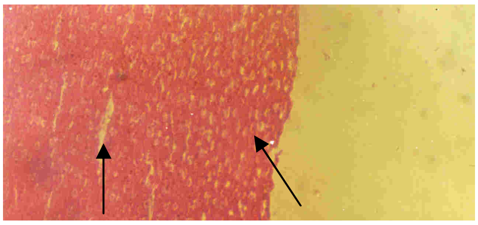

Plate 4b: Brain section of *C. gariepinus*. The 96-h exposed at 1.9mg/l (cypermethrin) showing mononuclear infiltration

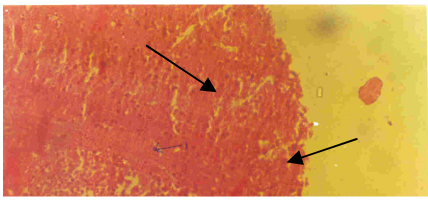

Plate 4c: Brain section of *C. gariepinus*. The 96-h exposed at 4.1mg/l (cypermethrin) showing congestion and mononuclear infiltration

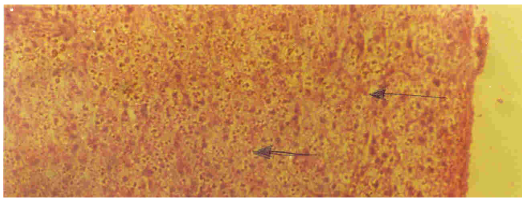

Plate 4d: Brain section of *C. gariepinus*. The 96-h exposed at 9mg/l (cypermethrin) showing neuronal degeneration and spongiosis

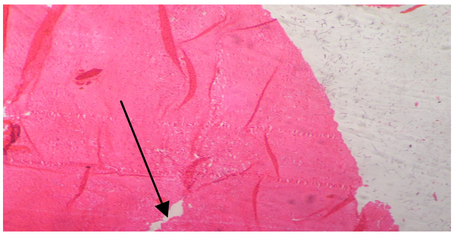

Plate 4e: Brain section of *C. gariepinus*. The 96-h exposed at 21mg/l (cypermethrin) showing infiltration and spongiosis

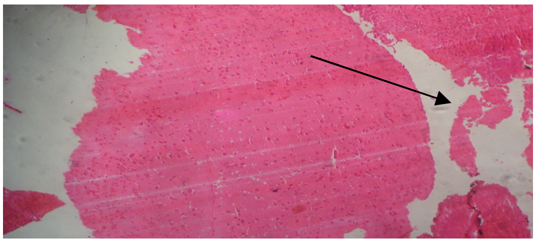

Plate 4f: Brain section of *C. gariepinus*. The 96-h exposed at 45 mg/l (cypermethrin) showing severe spongiosis

Discussion

The present research showed that the 96h LC_{50} value of cypermethrin insecticide was 0.063mg/l. the LC_{50} value in the present study for *C. gariepinus* juvenile is lower then the findings of Aguigwo (2002) that used formulation of cymbush pesticide on *Clarias gariepinus*. The fact may be related to toxicity level of cypermethrin, which has been found to be toxic than other pesticides like Mirex (Tagatz *et al* (1975), Keptone (Walsh *et al* 1977) and gammalin 20 (Ojike, 1980).

Cypermethrin toxicity is shown to increase with increased concentration. The observation is in consonance with earlier reports of Omoregie and Ufodike (1991), Gesamp, 1991). Neibor and Richardson (1980) reported that the level of toxicity of any pesticide depends on its bioaccumulation, the different chemistries of the compound forming the pesticide and the reaction of the organism receiving the toxicant. The three physico-chemical parameters of the test were fluctuated slightly during the toxicity test. The values were normal for toxicity test (FAO, 1977). The water quality parameters may have probably contributed to the variation in behavioural pattern and the mortality of the test fish during the study period. There was a significant negative correlation between pH and dissolved oxygen values. In case of dissolved oxygen, the treatments did not only show a dose dependent decline in concentration, but also rapid depletion of dissolved oxygen with time. Warren (1977) had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolve oxygen concentration, which will impair respiration leading to asphyxiation.

C. gariepinus juveniles were stressed progressively with time before death. The stressful behaviour of respiratory impairment due to the toxic effect of cypemethrin on the gills was similar with the report of Omitoyin et al (2006) and Aguigwo (2002) that pesticide impairs respiratory organs.

Death could therefore have occurred either by direct poisoning or indirectly by making the medium unconducive or even by both, which ever is the case, the source of death was cypermethrin. Several abnormal behaviour such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercula activities, surface to bottom movement, sudden quick movement, resting at the bottom were similar to the observations of Omoniyi *et al* (2002), Rahman *et al* (2002) and Aguigwo (2002). The stressful and erratic behaviour of *O.niloticus* juvenile in the experiment indicates respiratory impairment,probably due to the effect of the toxicant cypermethrin on the gills.The fishes became inactive at higher concentrations with increased time of exposure to toxicant. According to Kulakkatolickal (1997), this is a normal observation in acute and chronic toxicity test.

It was also observed that the higher the concentration of the toxicant, the higher the mortality rate. This demonstrates the observation of Fryer (1977), that in all toxicant, a threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance. The time of toxicity disappearance and mortality were observed from the record of the relative mortality time in different concentrations of cypermethrin for 96hours. The histopathological examination of the brain, gill, and kidney of the exposed fish indicated that the liver and kidney were the organ most affected.

Damages of the gills indicated that the lethal concentrations of insecticide caused impairment in gaseous exchange efficiency of the gills.

The histopathological examination of the gill, liver, kidney and brain of the exposed fish indicated that the liver and kidney were the organs most affected and this is similar to the observation of Rahman *et al* (2002), Aguigwo (2002), and Omitoyin *et al* 2006.

The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification and similar to the observation of Rahman *et al* (2002). The inability of the fish to regenerate new liver cells may also have led to necrosis. The liver of the exposed fish had slightly vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed 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.

The kidney cells were observed to have been massively destroyed. The renal corpuscles of the kidney were scattered resulting in their disorganisation and consequently obstruction to their physiological functions. The renal corpuscles of the kidney were scattered resulting in their disorganization and consequently obstruction to their physiological functions.Some of the kidney cell were found clogging together while they were disintegrated in some tissues of the organ. This also agreed with the findings of Omoniyi *et al* (2002), and Rahman *et al* (2002).

Damages of the gills indicated that impairment in gaseous exchange efficiency of the gills Oedematous of the lamella and hyperplasia were observed and this is similar to the observation of Omoniyi *et al* (2002).

The brain also indicated severe congestion and generalised spongiosis that indicate severe brain damage. This agreed with the findings of Omitoyin *et al* (2001). This study shows that cypermethrin is toxic to fish and causes histopathological changes in fish organs. Therefore, indiscriminate use of cypermethrin by farmers should be discouraged particular in area close to aquatic environment.

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