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RESEARCH ARTICLE

ACUTE EFFECT OF CYPERMETHRIN ON NUTRIENT UTILIZATION AND HISTOPATHOLOGY OF CLARIAS GARIEPINUS (BURCHELL, 1822)

Whenu Olusegun Olufemi and Mekuleyi Gabriel Olarinde
Department of Fisheries, Lagos State University, Ojo, Lagos State, Nigeria.

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Abstract

The toxicity of cypermethrin (a pesticide) on fingerlings of African catfish, Clarias gariepinus was investigate with emphasis on histopathological effects. The juvenile were exposed to 0, 1.0, 2.0, 3.0, 4.0 and 5.0ppb of cypermethrin under experimental condition. The growth, nutrient utilization and feed utilization parameters (Weight gain, Average Daily Weight Gain, Percentage Weight Gain , Specific Growth Rate , Feed Intake ,Feed Conversion Ratio and Protein Intake were not significantly different($P>0.05$) at the different concentration. However, histopathological changes were observed on the gills and liver of the experimental fish. In the gills, filament cell proliferation, cellular infiltration, haemorrhage and gill damage were observed. In the liver, there was vacuolation of hepatocytes and necrosis. However, there was no observable histological change in the intestine at the various concentrations of 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 showed that mortality increased with increase in concentration. This study concluded that Cypermethrin is highly toxic to fish and therefore should be avoided in aquatic ecosystem.

Introduction:-

Cypermethrin is a common synthetic pyrethroid used as insecticide in agriculture. It enter into aquatic environment as a result of anthropogenic processes, via agricultural run offs, industrial effluent and other sources (Svoboda, 2001). Cypermethrin is highly toxic to fish and a 96 – low lethal concentration (96hr LC50) of 8µg/l and 10µg/l has been reported for rainbow trout and common carp respectively (Tabassum et al., 2003). Many studies have been carried out in the laboratory on the effects of toxicants on the haematology of Clarias gariepinus and these include exposure to Malachite green (Musa and Omoregie, 1999); Actellic25EC (Omoregie and Ufodike, 1991), Glyphostate (Gabriel and George,2005) and Monocrotophous (Yaji and Auta 2007).These studies showed that various degrees of haematological changes could be produced by exposure of C. gariepinus to different levels of toxicants under laboratory conditions.

Despite a number of studies on the effects of toxicants on haematology of C. gariepinus, little is known on the histological changes that C. gariepinus may suffer under exposure to cypermethrin, a pesticide commonly applied in agriculture operations. The present study therefore aimed to investigate the acute effect of cypermethrin on the growth and histological parameters of C. gariepinus raised under laboratory condition.

Corresponding Author:- Whenu Olusegun Olufemi
Address:- Department of Fisheries, Lagos State University, Ojo, Lagos State, Nigeria.

Materials and Methods:-

Preparation of Tank

Twenty rectangular plastic aquaria tanks (30cm x 60cm) of 30 litre volumes were disinfected with saline solution before they were filled with well aerated tap water (pH 7.8 – 8.0). The tanks were transparent and evenly spaced for proper observation of specimen. Water aerators and net were also affixed to the tanks.

Collection of Test Organisms

The test organisms were 300 fingerlings of African Catfish, Clarias gariepinus that were randomly selected from the population of fingerlings that were bred and raised at Lagos State University hatchery. The fish were from the same parents stock and bred at the same time. Also, they were visibly free of any deformities, lesions or disease. This species was chosen for this work because of its availability as the most cultured fish in Nigeria and Sub Saharan Africa and also due to its ability to adapt and yield easily to laboratory and scientific experiments. On the other hand, cypermethrin was selected because it is common in most insecticides used by Lagos farmers.

Acclimatization of Fish

The fish were acclimatized in tap de-chlorinated water for four weeks prior to the experiment. Fingerlings (15 fish in each tank) were distributed into the 20 plastic aquaria tanks. Unconsumed feed and faecal wastes were siphoned daily with a rubber hose and the water replenished regularly as recommended by Oyelese and Faturoti (1995). Dissolved oxygen in each tank was maintained, close to saturation by aeration. However, feeding were stopped 24 hours before the commencement of the experimental set-up.

Feeding of fish prior to addition of pesticides to the water

The fingerlings were fed with commercial feed (Coppens feed; 45% protein). The composition and proximate analysis of the feed used for this experiment is presented in table 1 while a fixed feeding regime of 3% of the body weight per day was employed throughout the experimental period.

Experiment Phases:

Definitive test

A 24 hours static bioassay was done daily for a period of 84days to determine the acute toxicity of Cypermethrin on fingerlings of Clarias gariepinus. The pesticide was obtained from the stocks available at Lagos State Agricultural Inputs Supply Authority, Ojo, Lagos.

The range finding or limit test was carried out to allow for comparison of chemicals and provide data with which a definitive test can be based. It was carried out according to the method described by Ogundiran et al.,(2010). Percentage mortality in each concentration was recorded every 3 hours for 24 hour for 84 days and was estimated.

Experimental set up-Recovery test

The experimental set-up consists of 12 transparent rectangular plastic tanks filled with 10 litres of chlorine-free tap water. Ten fingerlings of Clarias gariepinus of average weight of 5g were randomly selected into each tank. The tanks were arranged to accommodate 6 treatments (T_1, T_2, T_3, T_4, T_5,) and a control. The concentration of 1ppb, 2ppb, 3ppb, 4ppb, 5ppb of cypermethrin and control free of toxicant, were replicated twice.

Fish mortality and survival was monitored during toxicity phase for 24hours each day for 12 weeks at interval of ½, 1, 2, 4, 8, 16, 24hours. The toxicant treated water in all the 12 aquaria tanks were daily discarded after 24hours and replaced with fresh water mixed with the concentration of 1ppb, 2ppb, 3ppb, 4ppb and 5ppb of cypermethrin.

Feeding of the fish when pesticides are added to the water

Feeding was done twice a day at 3% body weight at 09:00hr and 16:00hr for a period of 12 weeks. Feed was dispensed evenly on the water surface of each tank to allow equal feeding opportunity. Feeding in all tanks was generally completed in about 20-25minutes. **Determination of Physico-Chemical Parameters of dilution water and test solutions**

Water quality parameters of the test tanks were monitored daily for the replicates using standard methods (APHA, 1985). The dilution water and the test solutions were tested for temperature, pH and dissolved oxygen (DO).Temperature and pH were measured daily at 9.00hrs and 16.00hrs by using a mercury –in glass thermometer

and pH meter(Extec407227) respectively, while dissolved oxygen was determined(in mg/l) by method described by APHA(1985).

Determination of Growth Parameters

The mean size of the fish {weight (g) and total length (cm)} for each treatment and its replicates were measured every 2 weeks.

Weight Gain (WTG) = W_1-W_O

WhereW_1= final mean body weight (g)

W_o= initial mean body weight (g)

Percentage Weight Gain (%) = $\frac{100 (W_1-W_0)}{W_0}$

Where W_1= final mean body weight (g)

W_0= initial mean body weight (g)

Specific Growth Rate (SGR) = $\frac{\text{Log } W_1 - \text{Log} W_0 \text{ x } 100}{T-t}$

Where, W_1 is the final weight, W_0 is the initial weight, T is final time, t is initial time and log is natural logarithm.

Average Daily Weight (ADG) = $\frac{W_1- W_O}{T}$

Where, W_1 is mean final weight, W_0 is mean initial weight and T is rearing period.

Length-weight relationship of the fish

The relationship between total length and weight of the fish in each experimental unit were estimated using the equation by Ricker (1973): $W= aL^b$

Where, W is weight of fish (g); L is length of fish (cm), a is y – intercept or the initial growth coefficient and b is slope or the growth coefficient.

The equation above was linearized by logarithmic transformation to enable the estimation of the values of constants a and b using least square linear regression (Zar, 1996). After linearization the equation become, LogW= Log a + b LogL.

Statistical Analysis

Analysis of variance (**ANOVA**) was used to test for significant difference in the growth performance and feed utilization. The ANOVA was done using computer package for social science (SPSS) for windows (version 17.0). Differences between the mean values were partitioned by Fisher's Least significant Difference (LSD) at $p < 0.05$.

Histopathological Analysis

The tissues (gills, livers and intestine) were removed and prepared for histopathological observation using standard method (Schalm et al.,1995). The tissues 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 later embedded in paraffin, sectioned at 4-5µm thick using a rotary microtome and the resulting tissues were then mounted on a glass slide and dried on a slide warmer. The sectioned tissues are stained with haematoxylin and eosin and the stained were examined using light microscope and photomicrograph with scale bar of 50µm (Keneko, 1989).

Results:-

Growth and Feed Utilization of the Specimen

The growth parameters -Weight Gain (WG), Percentage Weight Gain (PWG), Average Daily Weight Growth (ADWG) and Specific Growth Rate (SGR) of the specimen are as shown in Table 2.The highest value for WG (12.10±0.56g) was recorded in the control experiment while the lowest value (7.20±0.96g) was obtained at a concentration of 5ppb (Part Per Billion). There was no significant different ($p>0.05$) in the weight gain among the treatments except between the control and the treatments ($p<0.05$). The values of PWG (79.20%), SGR (4.47±1.25g) was highest at concentration of 2.0 ppb while the lowest values of PWG (78.20%), SGR (3.54±1.34g) occurred at a treatment of 4.0ppb. Highest ADWG (0.68±0.24g) occurred in the concentration with 2.0ppb while the lowest value of (0.60±0.30g) occurred in the concentration with 5.0ppb. No significant different ($p>0.05$) occur among the treatments for PWG, SGR and ADWG.

The feed utilization parameters such as Feed Intake (FI) and Feed Conversion Ratio (FCR) presented in Table 2 did not vary significantly ($p>0.05$) among the treatments. However, Protein Intake (PI) exhibited significant differences ($p<0.05$) between the treatments and control experiments.

Histopathological Studies

Gills:

No recognisable changes were observed in the gills of the fish in treatment 1.0, 2.0, 3.0 and 4.0 ppb of cypermethrin respectively. Normal gill cartilage and overlying epithelium free of pathology was observed in the gills as shown on Figures 1a-1d. However, damage to gill cartilage was observed on the gill of the fish exposed to 5.0 ppb cypermethrin concentration (Figure 1e).

Liver:

The histology of the liver of fish subjected to 1.0, 3.0 and 4.0 ppb cypermethrin concentrations 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 (Figures 2a, 2c and 2d). There were glycogen vacuolation, fatty infiltration, hemosiderosis and congested central vein and severe infiltration of leukocytes on liver of fish exposed to 2.0ppb cypermethrin concentration (Figure 2b).However, only few inflammatory cells were seen on the liver of fish in the 5.0 ppb cypermethrin concentration (Figure 2e)

Intestine:

There were no observable histological changes or differences in the intestine of the fish at all levels of cypermethrin concentration (Figure 3a-3e).

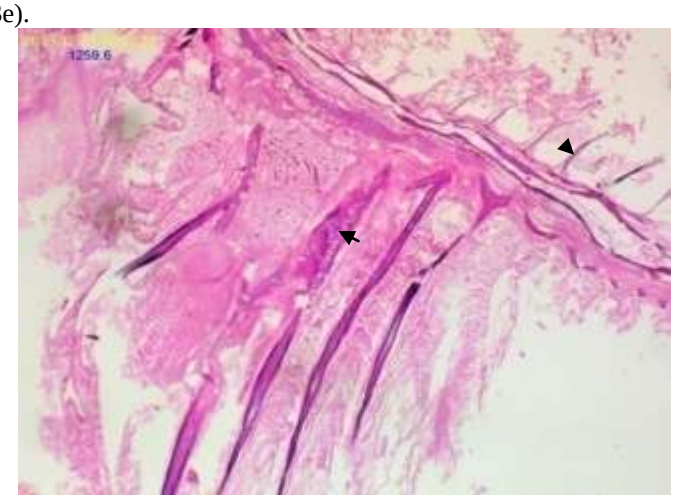


Fig.(1a)

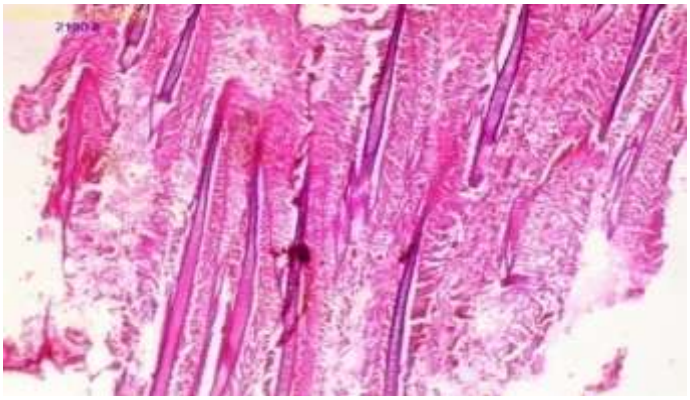


Fig. (1b):-

Figures (1a):- Gill of C. gariepinus in 1.0ppb cypermethrin showing normal gill cartilage and overlying epithelium (arrow) with no pathology.(1b):Gill of C. gariepinus in 2.0ppb cypermethrin with normal gill cartilage and overlying epithelium (arrow)and absence of pathology.

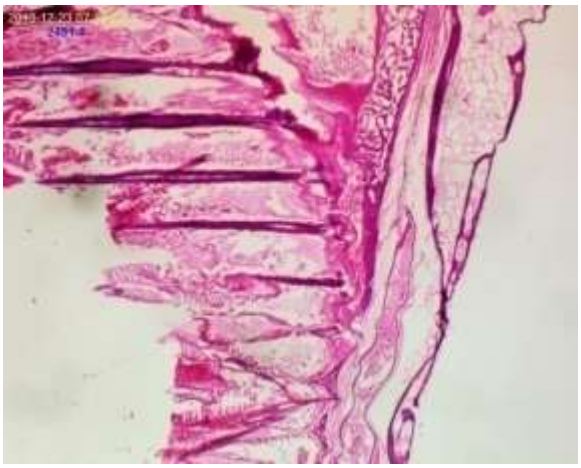


Fig.(1c)

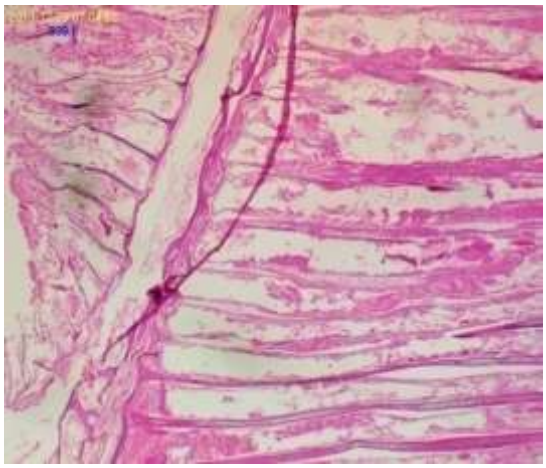
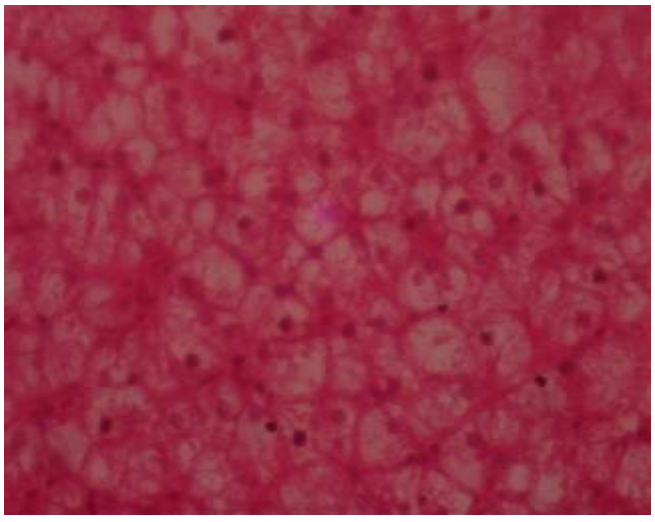


Fig.(1d)

Figures (1c):- Gill of C. gariepinus exposed at 3.0ppb concentration of cypermethrin showing normal gills. (1d): Gill of C. gariepinus exposed at 4.0ppb concentration of cypermethrin with no pathology.

Figure (1e): Gill of C. gariepinus in 5.0ppb cypermethrin showing great damage to gill cartilage.



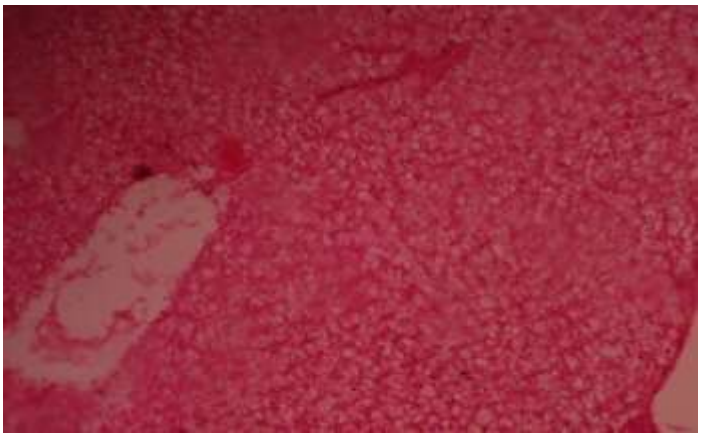


Fig.(2a) Fig.(2b)

Figures**(2a): Liver of C. gariepinus exposed at 1.0ppb concentration of cypermethrin showing normal liver with no pathology.(2b): Liver of C. gariepinus exposed at 2.0ppb concentration of cypermethrin showing infiltration of the leucocytes**

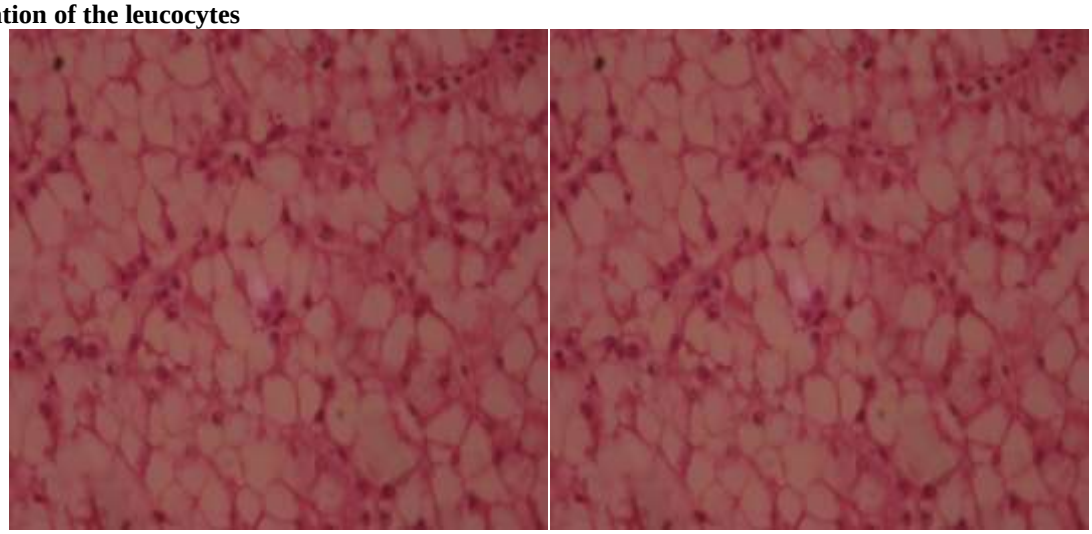


Fig.(2c) Fig.(2d)

(2b): Liver of C. gariepinus exposed at 2.0ppb concentration of cypermethrin showing infiltration of the leucocytes(2c): Liver of C. gariepinusexposed at 3.0ppb concentration of cypermethrin showing normal liver.

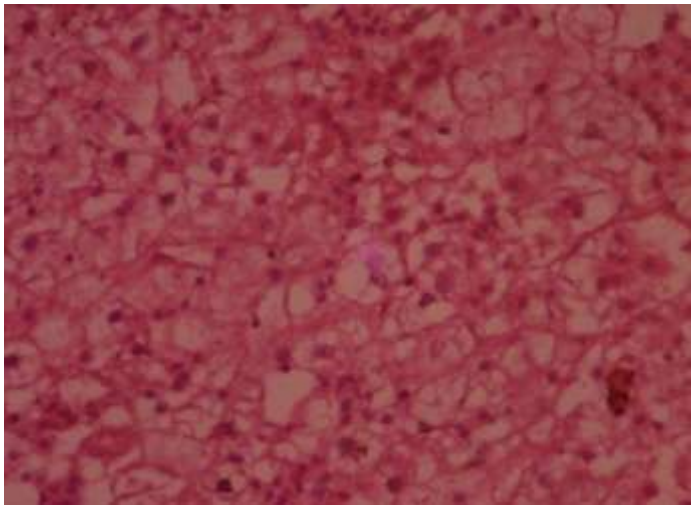


Fig.(2e):-

Figure (2e):- Liver of C. gariepinusexposed at 5.0ppb concentration ofcypermethrin showing few inflammatory cells.

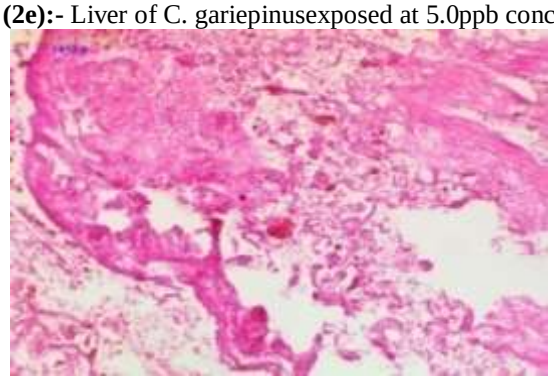


Fig.(3a)

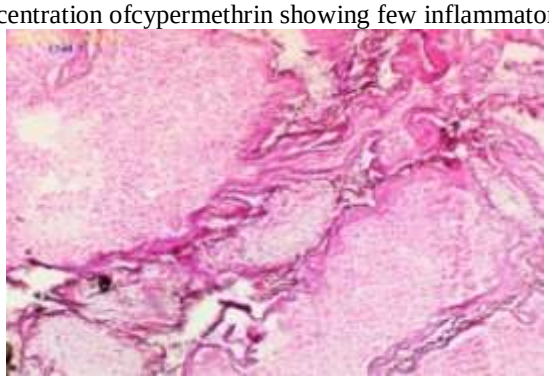


Fig (3b)

Figures (3a):- Intestine section of C. gariepinusexposed at 1.0 ppb concentration of cypermethrin showing no damage or alteration. (3b): Intestine section of C. gariepinus exposed at 2.0ppb concentration of cypermethrin with no observable changes.

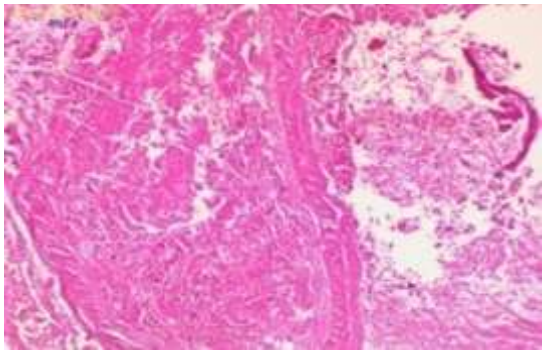


Fig.(3c)

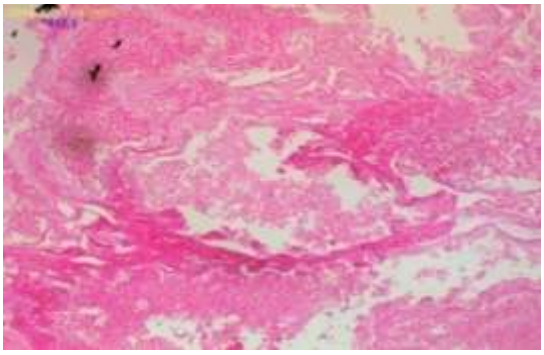


Fig. (3d)

(3c): Intestine section of C. gariepinus exposed to 3.0 ppb concentration of cypermethrin with no visible change.

(3d): Intestine section of C. gariepinus exposed at 4.0ppb concentration of cypermethrin with no pathology.

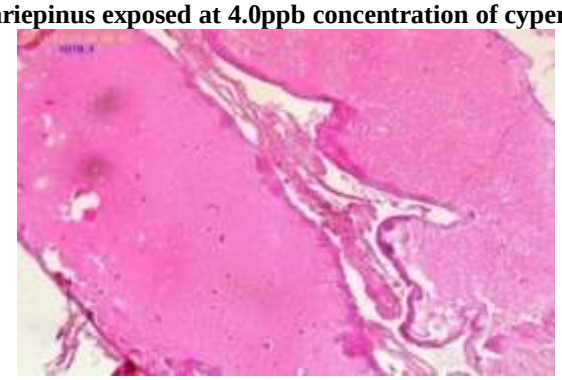


Fig.(3e):-

Figure (3e):- Intestine section of C. gariepinus exposed at 5.0 ppb concentration of cypermethrin with no visible changes.

Discussion:-

The observed behavioural changes are directly proportional to the concentration of the toxicant as the fishes became inactive at higher concentrations with increased time of exposure to the toxicant. Erratic swimming and excessive jumping were observed in the fish on immediate exposure to toxicant especially in tanks with 1.0 and 5.0ppb concentration of cypermethrin which could be due to skin irritation, respiratory rate impairment or a response to altered locomotors activity which is an indication of the effect of toxicant on the nervous system as reported by Jenyo-oni et al.(2011). Erratic swimming and settling at the bottom of the tank and subsequent immobilization

before death observed in the study agree with the reports of Adesina (2008), Omitoyin et al.(2006) and Fafioye (2002). The stressful behaviour exhibited by the fish may be attributed to the effect of toxicants on the gill and several authors such as Wade et al. (2002), Rahman et al.(2002), Aguigwo (2002) and Ajani (2006) have reported similar patterns of abnormal behavioural responses in fish exposed to toxicants.

The histopathological examination of the gill, liver and intestine of the exposed fish indicated that the liver and gills were the tissues most affected and this is similar to the observation of Rahman et al., (2002), Aguigwo (2002), and Omitoyinet 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.

Damage of the gill cartilage recorded in some treatment of the fish 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).

This present study shows that although there was no significant difference in the growth pattern of the fish at all level of exposure however, cypermethrin is affirmed to be toxic to fish as it has potential of causing histopathological changes in fish organs. Therefore, indiscriminate use of cypermethrin by people especially farmers should be discouraged particularly in area close to aquatic environment.

Conclusion:-

The growth, nutrient utilization and the feed utilization of Clarias gariepinus could be disturbed by Cypermethrin acute exposure. Also, the exposure of Clarias gariepinus to different concentrations of Cypermehrin as shown in this study indicated that cypermethrin is toxic to fish and causes histopathological changes in fish organs (gills and liver). Therefore, indiscriminate use of cypermethrin by farmers should be discouraged particularly in areas close to aquatic environment

Table 1:- Composition of experimental diets.

Ingredients	**Values**
Crude Protein	45
Crude Fat	15
Crude Fibre	0.4
Ash	10.9
Vitamin A	22.5 IU/kg
Vitamin D3	2.5 IU/kg
Vitamin E	200 mg/kg
Vitamin C	300 mg/kg
Phosphorus	1.8
Calcium	2.6
Sodium	0.7

Source: Information from Coppens

Table 2:- Growth and nutrient utilization parameters of Clarias gariepinus fingerlings exposed to different concentrations of Cypermethrin.

Conc (ppb)	**WG (g)**	**ADWG(g)**	**PWG (%)**	**SGR(g)**	**FI (g)**	**PI**	**FCR**
1.0	7.22±0.41[a]	0.61±0.23[a]	78.60[a]	3.62±1.54[a]	0.08±0.02[a]	0.37±0.26[a]	0.03±0.02[a]
2.0	8.13±0.33[a]	0.68±0.24[a]	79.20[a]	4.47±1.25[a]	0.08±0.01[a]	0.38±0.17[a]	0.09±0.03[a]
3.0	8.09±0.95[a]	0.67±0.20[a]	78.70[a]	3.61±1.49[a]	0.20±0.15[a]	0.49±0.41[a]	0.03±0.02

							[a]
4.0	7.48±0.48 [a]	0.62±0.30 [a]	78.20[a]	3.54±1.34 [a]	0.01±0.01 [a]	0.48±0.29 [a]	0.07±0.03 [a]
5.0	7.20±0.96 [a]	0.60±0.30 [a]	78.40[a]	3.61±1.87 [a]	0.09±0.02 [a]	0.43±0.24 [a]	0.07±0.03 [a]
Control	12.10±0.56 [b]	1.02±0.20 [b]	82.6 [b]	3.98±0.62 [a]	0.24±0.10 [a]	1.03±0.94[b]	0.05±0.02 [a]

Same column values= non-significant. Conc = concentration

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