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International Journal of Toxicological and Pharmacological Research 2016; 8(5); 360-367

ISSN: 0975-5160

Research Article

# Toxicological Evaluation and Histopathological Changes of Synthetic Pyrethroid Pesticide (Cypermethrin) Exposed to African Clariid Mud Catfish (*Clarias gariepinus*) Fingerlings

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*Available Online: 25th October, 2016*

**ABSTRACT**
The study of the toxicological and histopathological changes of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin is of great importance due to the fact that it helps reveal the extent of toxicological damages of cypermethrin on fishes. Cypermethrin is globally used for control of pest, but it is washed along with run-offs into the aquatic ecosystem, and this has an effect on the non-target species, thereby killing various fauna within the aquatic eco-system. Ten (10) fingerlings of *Clarias gariepinus* were introduced into each aquarium containing twenty (20) litres of water with 25ppm, 50ppm, 75ppm, 100ppm, 125ppm and 0.0ppm (Control) concentrations of cypermethrin and its effects were observed for 96hrs. The *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin exhibited signs of stress and erratic movement. All the time-effect relationship was found to be concentration dependent. The fingerlings of *Clarias gariepinus* showed normal gill lamella, erosion of secondary gill lamella and haemorrhage of secondary gill lamella in the gill tissues when exposed to different concentrations of cypermethrin. The fingerlings exposed to the cypermethrin showed normal distribution hepatocytes, hyperplastic hepatic cells necrosis of hepatic cells in the liver tissues. A log concentration probit regression analysis was significant ($p<0.05$) yielding a co-efficient of determination, $r^2$ of 0.973 and having a 96 hours $LC_{50}$ with 95% confidence value of 1.80±0.28ppm. The study revealed that cypermethrin has toxicological and histopathological effects on different organs of *Clarias gariepinus* fingerlings.

**Keywords:** Toxicological, Histopathological changes, Cypermethrin, Catfish, Fingerlings

## INTRODUCTION

Water covers about 70% of the earth, and happens to be the most essential natural resources[1]. Despite this awareness of the essentiality of water, humans have ignored its importance by polluting it[2]. The advancement in industrialization has coincided with the problem of aquatic pollution. The use of mechanical and biological means of pest control has been abandoned for an easier and faster use of agricultural pesticides for control of pest, in order to generate massive crop yield, so as to meet-up with the ever growing human population[3-5]. The careless and indiscriminate use of these synthetic pesticides has led to the global pollution of water bodies[6,7], leading to mortality of aquatic organisms and a general deterioration of the aquatic ecosystem[8,9]. Cypermethrin is a globally used for the control of pest, in order to improve food productivity[10], but their use could create a risk of food contamination as well as affects the non-target aquatic species; like invertebrates and vertebrates[11]. It is a synthetic pyrethroid, with a very high activity and stability[12]. Of all the pesticides available in the market, pyrethroids make about 25% of global pesticides sale[13]. Over 200 types of synthetic pesticides exist[14] and they all contain several heavy metals. These metals enter the water bodies, thereby affecting growth, physiology, reproduction and survival of fish[15]. The toxicity of pyrethroids varies between biological species, due to the difference in elimination and metabolic degradation from the body[16]. Globally, Cypermethrin is used for the control of cotton, fruits and vegetables pest[12], copepod parasite infestation[17], aquatic and terrestrial ectoparasites[18] and for illegal fishing[12]. Agricultural run-off happens to be the main route of entry of cypermethrin into the aquatic eco-system, and this affects the non-target species[19]. The histological study of the deformation and disruption of gills, liver, gonads and other organs of fish has become a very important indicator of the toxic effects of pollutants[14,20,21] reported the presence of lesions, due to the effect of pesticides. Pesticides affect the physiology, functions and morphology of various organs of biological organisms[22,23]. The main function of liver is to breakdown toxic and harmful substances during metabolism, but this ability of detoxification depreciates when the toxicity of substances gets to a certain limit,

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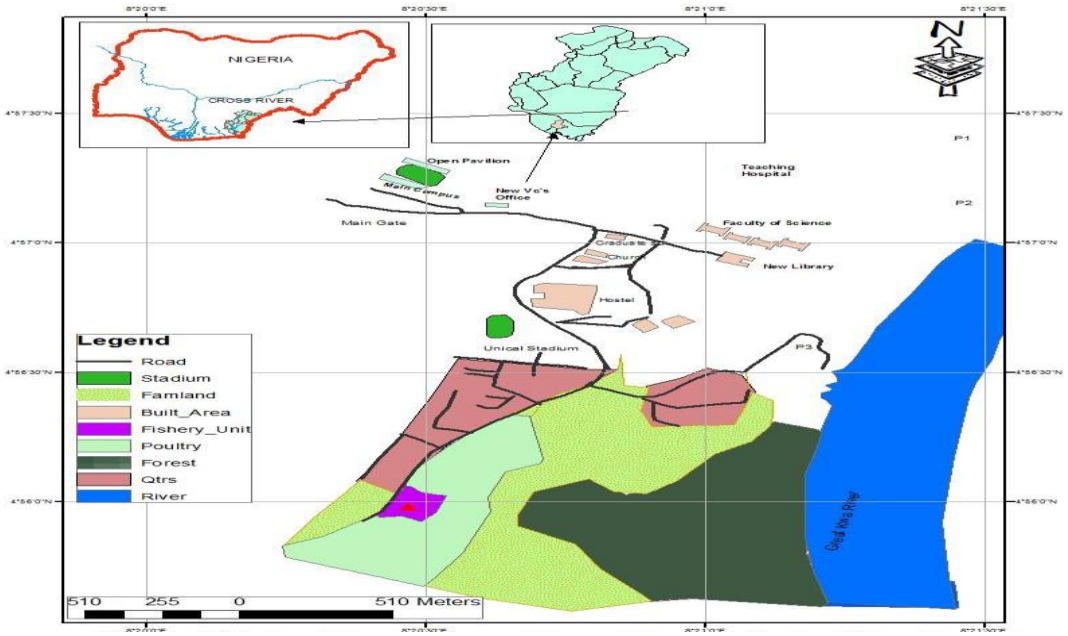


Figure 1: Map of University of Calabar Showing Sampling Area (Fishery Unit).

Table 1: Manifestation, Overturning and Survival Time of *Clarias gariepinus* Exposed to concentrations of cypermethrin.

| Concentration (ppm) | Manifestation Time (Hrs) | Over-turning Time (Hrs) | Survival Time (Hrs) |
|---|---|---|---|
| 0.00 | 0.00 | 0.00 | 0.00 |
| 25.0 | 4.10 | 4.55 | 5.30 |
| 50.0 | 3.45 | 4.46 | 5.14 |
| 75.0 | 3.56 | 4.37 | 4.35 |
| 100.0 | 3.35 | 4.30 | 4.24 |
| 125.0 | 3.31 | 4.25 | 3.45 |

thereby causing morphological alteration[24,25]reported that Cypermethrin causes necrosis, hyperplasia of primary epithelial cells, oedema, epithelial hypertrophy, epithelial lifting, fusion of secondary lamellae and desquamation in the gills of *Clarias gariepinus*. The study was aimed at determining the acute toxic effects and Histopathological changes of cypermethrin in the gills and liver tissues of African mud catfish (*Clarias gariepinus*) fingerlings.

## MATERIALS AND METHODS

*Test chemical*

Cypermethrin used for this research was purchased from Federal Ministry of Agriculture, Barrack Road, Calabar, Cross River State.

*Collection and Transportation of Test Fish*

*Clarias gariepinus* fingerlings were collected from fishery hatchery complex of University of Calabar fish farm, Calabar, between latitude $04^0$ 56''021 N and Longitude $08^0$ 20''45 E (Fig. 1), with the aid of a scoop net in the early hours of the morning to avoid heat, high intensity, stress and were transported to the laboratory in an open plastic bucket containing aerated habitat water[26]. The container containing the fingerling was handled with care to avoid agitation which may cause damage to the fingerling and possibly death.

*Acclimation and Maintenance of Test Fish*

The *Clarias gariepinus* fingerlings were kept in glass holding tanks for 21 days (three weeks) to allow them acclimatized to the prevailing laboratory conditions in one tank (30x30x50) cm 2/3 filled with water which were continuously aerated with electrical air pumps. The fingerlings were fed with coppens feed containing 40% crude protein at 2.5% of body weight twice daily. *C. gariepinus* was selected because it is an ecologically and economically important group of the tropical inland waters[26]. The fingerlings holding water were changed daily to avoid accumulation of toxic waste products and metabolite and food particles. Tap water was dechlorinated by vigorously aerating and allowing the water to settle for at least 48 hour.

*Range Finding*

A preliminary range finding test was carried out based on the concentration of the active ingredient in the test chemical. The range finding was done using the following concentrations 1ppm, 10ppm and 100ppm of cypermethrin, for 24 hours in triplicates. The result when obtained from the range finding test would provide a guide for the definitive test. Following this, the definitive test was carried out using 25ppm, 50ppm, 75ppm, 100ppm, 125ppm and 0.0ppm of cypermethrin.

*Preparation of Cypermethrin*

A stock solution of pesticide (cypermethrin) was prepared by dissolving 5mg of technical grade CYP (cypermethrin) containing cyano 3-(phenoxyphenyl, methyl 3-/2-2-dich loroethenyl)-2,2-dimethyl cyclopropane-carboxylate) in 5ml of 80% acetone. Then 1ml solution was taken in 10ml volumetric flask with the help of micropipette and 80% acetone was added up to mark to make concentration. 1mg CYP per 10ml, the flask was thoroughly shaken or stirred to ensure a homogenous

Table 2: Probit Transformation/analysis of mortality data of *Clarias gariepinus* exposed to concentration of cypermethrin.

| Conc (ppm) | Log Conc (x) | N | R | P | $M_R$ | Y | $R_P$ | *P* |
|---|---|---|---|---|---|---|---|---|
| 0.00 | 0.00 | 30 | 0 | 0 | 0 | 5.32 | 0.00 | 0.00 |
| 25.0 | 1.398 | 30 | 15 | 0.50 | 50 | 9.70 | 5.30 | 0.32 |
| 50.0 | 1.698 | 30 | 17 | 0.56 | 56.7 | 15.1 | 1.89 | 050 |
| 75.0 | 1.875 | 30 | 22 | 0.73 | 73.3 | 20.5 | 1.51 | 0.68 |
| 100.0 | 2.000 | 30 | 24 | 0.80 | 80.0 | 24.8 | -0.81 | 0.83 |
| 125.0 | 2.090 | 30 | 26 | 0.87 | 86.7 | 27.6 | -1.62 | 0.92 |

n = Number of fish fingerling tested at each concentration, r = Number of fish fingerling responding, p = Response rate, r/n, $M_R$ = Mortality rate, Y = Expected probit from visual regression line, $R_P$ = Residual probit, *P* = Probability

Table 3: Results of regression analysis of Log Concentration–probit relationship of *Clarias gariepinus* fingerlings exposed to concentration of cypermethrin.

| Conc. (Log Unit) | Response rate, p | Equation | Co-efficient of determination, $r^2$ | Significant level, α |
|---|---|---|---|---|
| 0.00 | 0.00 | | | |
| 1.398 | 0.50 | | | |
| 1.698 | 0.56 | Y = 2.49 + 39.91x | 0.973 | 0.05 (S) |
| 1.875 | 0.73 | | | |
| 2.00 | 0.80 | | | |
| 2.09 | 0.87 | | | |

Table 4: Chi-square Tests of *Clarias gariepinus* fingerlings exposed to concentration of cypermethrin.

| | Chi square | df[a] | Sig. |
|---|---|---|---|
| PROBIT Pearson Goodness-of-FitTest | 12.94 | 3 | 0.005[b] |

Table 5: Covariance's and correlation of *Clarias gariepinus* fingerlings exposed to concentration of cypermethrin.

| PROBIT | Concentration | Natural Response |
|---|---|---|
| Concentration | 0.00 | 0.492 |
| Natural Response | 0.00 | 0.038 |

Table 6: $LC_{50}$ with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of cypermethrin.

| $LC_{50}$ with ± 95%CL | Confidence limits | |
|---|---|---|
| | Lower | Upper |
| 1.80 ± 0.28 | 0.013 | 0.025 |

solution. The further dilutions were made from the stock solution.

*Experimental Procedures*

A total of eighteen (18) glass aquaria were used for the definitive toxicity test. Ten (10) fingerlings of *Clarias gariepinus* were introduced into each aquarium containing 20 litres of water with 25ppm, 50ppm, 75ppm, 100ppm, 125ppm and 0.0ppm (Control) concentrations of cypermethrin at the same time. Each of the toxicant (poison) concentration was replicated three (3) times. The experiment was carried out using a static non-renewal bioassay for 96hrs; mortality and general behavior of fish was noted 24 hourly.

*Histopathological Study*

Gills and liver of *Clarias gariepinus* were carried out after 96hrs of exposure period to the various concentration of the toxicant (CYP).

*Tissue Processing*

Organs of gills and liver were collected and fixed in 10% formal saline for one week. Then they were processed for routine paraffin histological sectioning. The tissues were dehydrated through graded concentration of ethanol (30%, 50% and 90%), absolute ethanol and cleared in xylene. The tissues were pre-impregnated in xylene paraffin wax in the oven and embedded in pure paraffin wax. The organs were sectioned at 7µm thickness and tissues were stained with haematoxylin and eosin (H & E) for light microscopic examination. Photography was done by using digital camera

*Data Analysis*

The mortality-concentrations data was subjected to probit transformation, regression analysis and $LC_{50}$ values were computed using Predictive Analytical Software (PASW) version 20. The significant of the slope was tested using Chi-square. Graphs was plotted using Microsoft excel (MSE) version 2013[26].

## RESULTS

*Manifestation time*

Time – concentration effect relationships such manifestation, overturning and survival times were carefully monitored and recorded. Manifestation time (the time interval between the initial exposure of fish to toxicant and the first appearance of symptoms of toxicity in fish) was observed to decrease with increase in

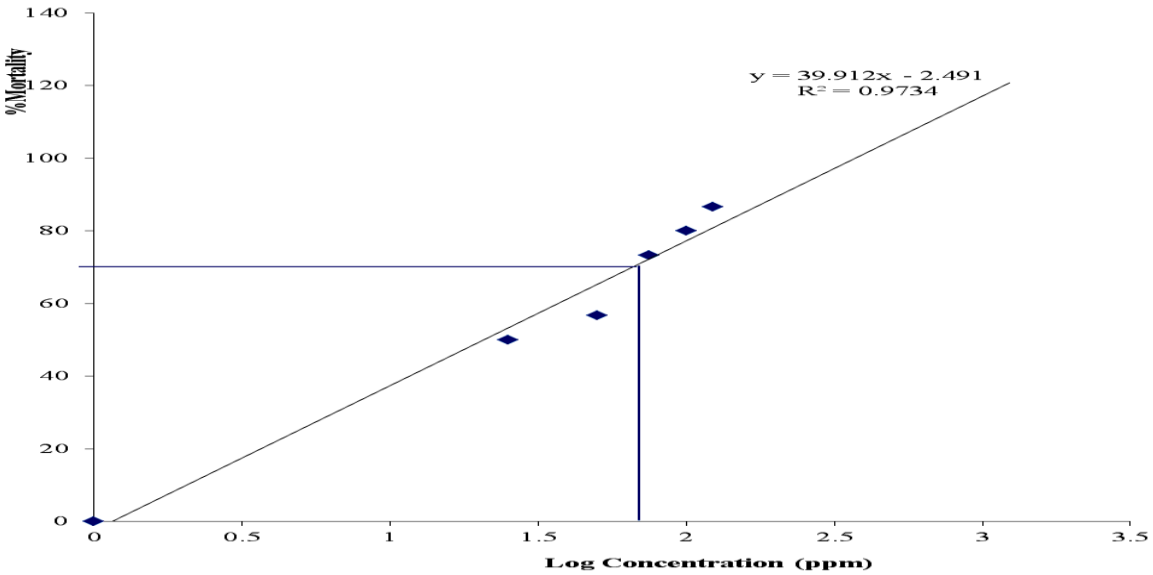


Figure 2: Linear relationship between percentage mortality of probit response and log concentration of cypermethrin on fingerlings of *Clarias gariepinus*.

concentration of cypermethrin. *Clarias gariepinus* exposed to 0.00, 25.0, 50.0, 75.0, 100.0 and 125ppm of cypermethrin indicated manifestation times of 0.00, 4.10, 3.45, 3.56, 3.35 and 3.31hours respectively, decreasing steeply from 4.10 to 3.31 hours as the concentration of plant extract increased (Table 1).

*Overturning time*

Overturning time, (the time between the introduction of the toxicant and the loss of equilibrium or righting balance) showed a similar trend with manifestation time, decreasing with increase in toxicant concentration. In all concentrations, overturning was preceded by uncoordinated, fast swimming bursts, swimming head up and tail down and swimming upside down. Overturning times of 0.00, 4.44, 4.46, 4.37, 4.30 and 4.25 hours were recorded for to 0.00, 25.0, 50.0, 75.0, 100 and 125ppm of cypermethrin respectively (Table 1)

*Survival time*

Survival time, the time interval between the initial exposure of *Clarias gariepinus* to toxicant and the time the first mortality occurs. Survival time of *Clarias gariepinus* exposed to concentrations of cypermethrin decreased as exposure concentration increased (Table 1). *Clarias gariepinus* exposed to 0.00, 25.0, 50.0, 75.0, 100.0 and 125.0ppm of cypermethrin yield survival time of 0.00, 5.30, 5.14, 4.35, 4.24 and 4.45 hours respectively. Drops in survival times were steeper from 25.0 to 75.0ppm but ended more gradually towards 125.0ppm.

*Probit Analysis Results*

Shows the results of probit analysis performed on mortality data. The trends in mortality data of fingerlings indicate that fingerling's mortality rate increased with increasing concentration of cypermethrin (Figure 2). Probit transformation of mortality rate-log concentration relationship determined to be linear by regression analysis (Figure 2). A log concentration probit regression analysis was significant ($p<0.05$) yield a co-efficient of determination, $r^2$ of 0.973 (Figure 2), and the $LC_{50}$ with 95% confidence limit was also determined to be 1.80 (Figure 2).

*Histopathological study of cypermethrin on Gill and Liver*

Histopathological study of cypermethrin on the gill and liver of *Clarias gariepinus* fingerlings carried out after 96hrs of exposure period to the various concentrations of the toxicant (cypermethrin). Plate 1a microphotograph of the gill tissues of *Clarias gariepinus* (x40) showing normal structure of the gill lamella, no significant lesion seen and the primary lamellae and secondary lamellae (respiratory lamellae) are not damaged. Plate 1b to 1e microphotograph of the gill tissues of *Clarias gariepinus* (x40), the 96hrs exposed group at 25.0 to 100.0ppm concentrations of cypermethrin showing erosion of secondary gill lamellae (ESGL) across the tissues. Plate 1f microphotograph of the gill tissues of *Clarias gariepinus* (x40) the 96hrs exposed group at 125ppm of cypermethrin, showing haemorrhage of secondary gill lamellae (HSGL). Plate 2a shows a normal distribution of hepatocytes (NDH) of the liver tissues at the 96hrs exposed group of 0.00 and 25.0ppm concentration of cypermethrin. Plate 2b, 2c and 2d shows hyperplastic hepatic cells (HHC) of the liver tissues at the 96hrs exposed group of 50.0 and 75.0ppm concentrations of cypermethrin and Plate 2e and 2f shows necrosis of hepatic cells (NHC) of the liver tissues at the 96hrs exposed group of 100.0 and 125.0ppm concentrations of cypermethrin, all at (x40) magnification.

## DISCUSSION

Cypermethrin is a globally used for the control of pest, in order to improve food productivity[10], but their use could create a risk of food contamination as well as affects the non-target aquatic species; like invertebrates and

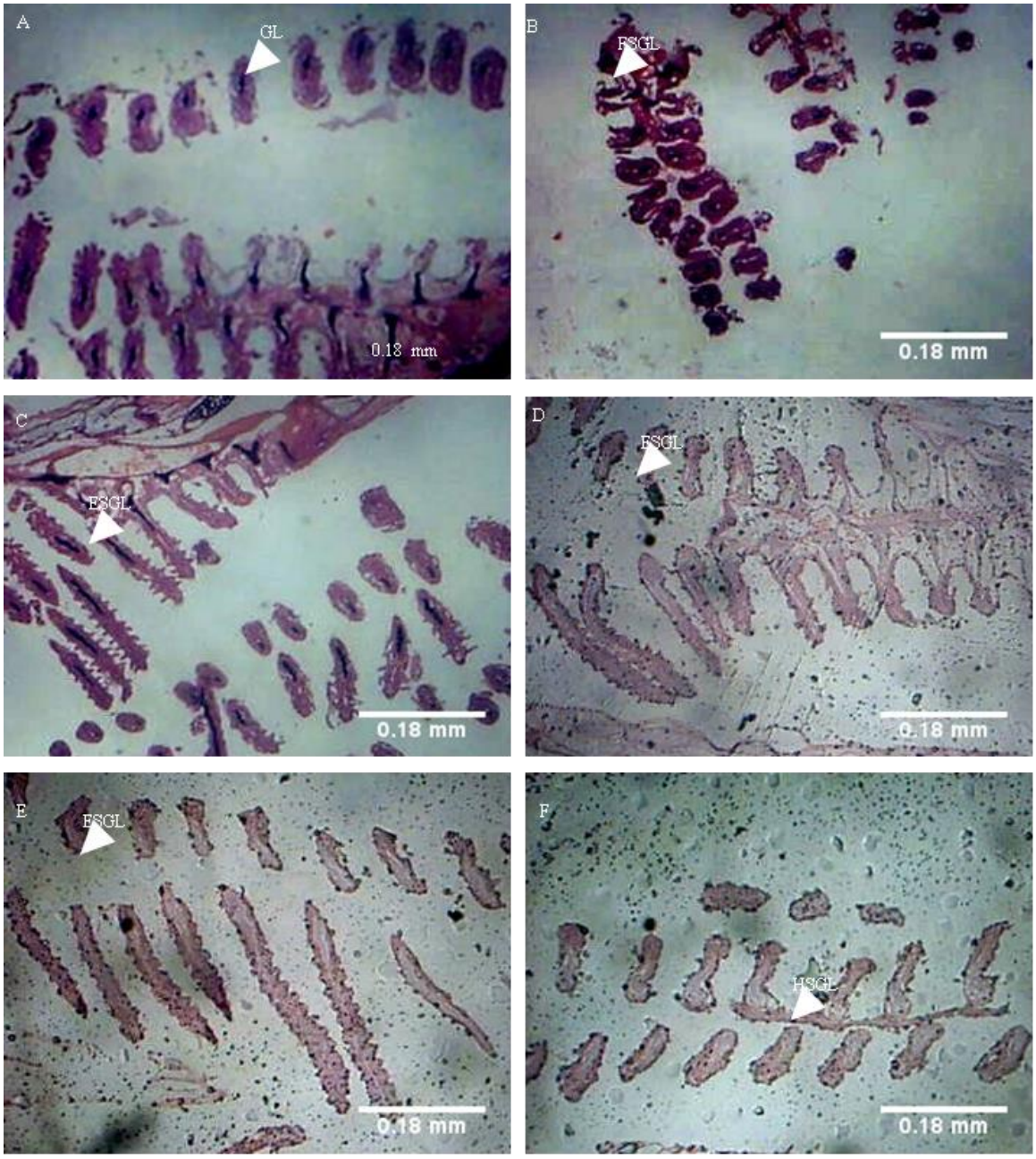


Plate 1a-1f: Microphotograph of the gill tissues showing histopathological changes at different concentrations (0.00-125ppm) of cypermethrin of the test organism (*Clarias gariepinus*).

vertebrates[11]. In this study, the fingerlings of *Clarias gariepinus* showed signs of stress and erratic behaviour when exposed to different concentrations of cypermethrin pesticides, due to respiratory impairment and similar observations were made and reported by[27]. The toxicity of cypermethrin in this study was concentration dependent, and this corroborated with the findings of[28], who reported that the toxicity effects of pesticides is concentration and exposure dependent. Mortality of *Clarias gariepinus* fingerlings was observed and this confirms the fact that synthetic pesticides causes mortality of aquatic organisms and deterioration of the aquatic ecosystem[8,9]. The histological study of the deformation and disruption of gills, liver, gonads and other organs of fish has become a very important indicator of the toxic effects of pollutants[14]. Pesticides affect the physiology, functions and morphology of various organs of biological organisms[22,23,20,21] reported the presence of lesions, due to the effect of pesticides. The fingerlings of *Clarias gariepinus* exposed to various concentrations of cypermethrin showed various histopathological deformations of gills and liver. The fingerlings of *Clarias gariepinus* in gill showed erosion of secondary gill lamella and haemorrhage of secondary gill lamella when exposed to different concentrations of cypermethrin, but the control group had a normal gill lamella. Some of

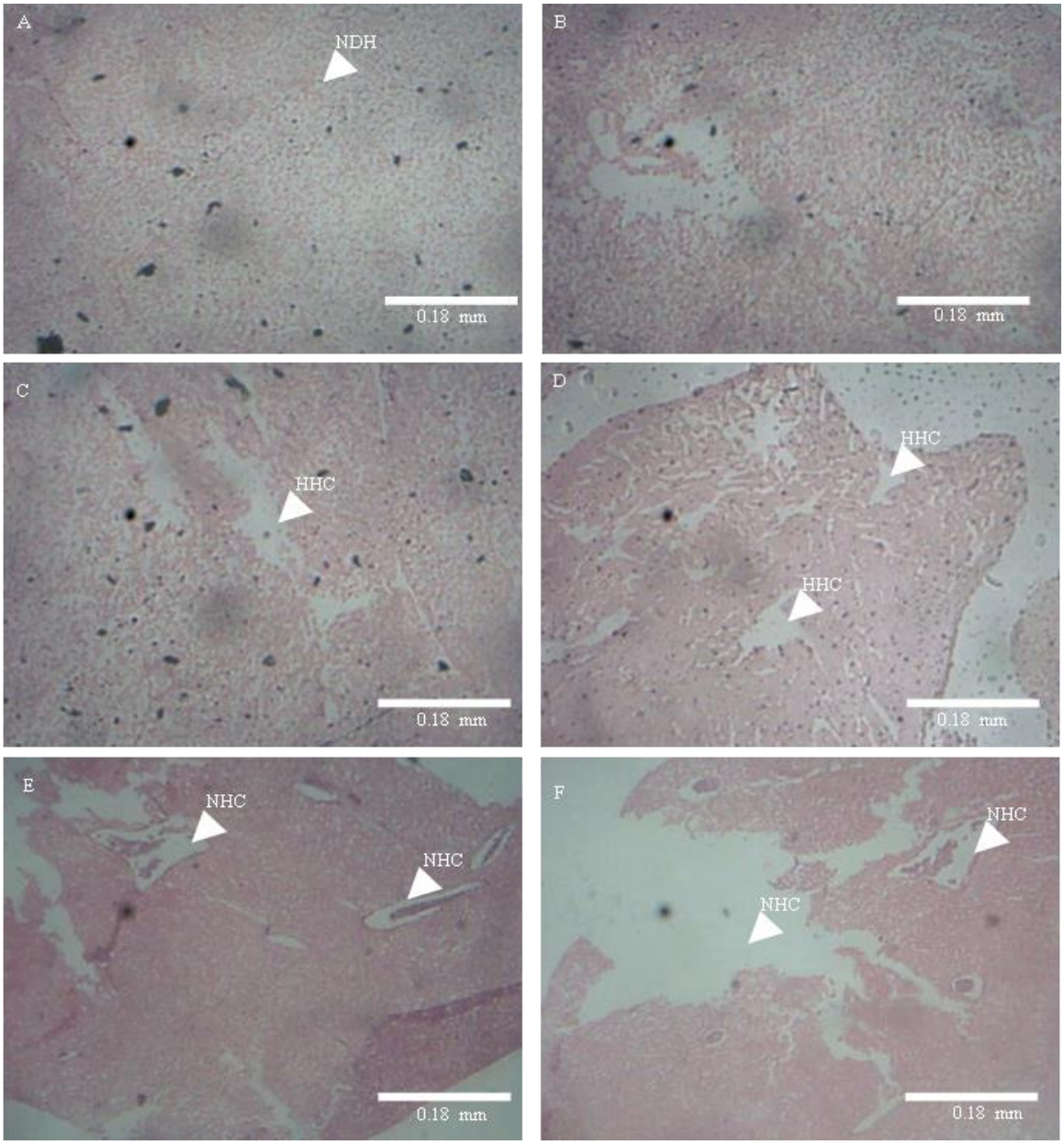


Plate 2a-2f: Microphotograph of liver tissues showing histopathological changes at different concentrations (0.00-125ppm) of cypermethrin of the test organism (*Clarias gariepinus*).

these histopathological deformations were reported by[25,29], who also reported raised filament, cellular infiltration, conjestion and swollen tip of gill filament. Although some deformations like; rising of lamellae, oedema of lamellae, oedema of lamellae epithelia reported by[25,29] were not observed in the gills of *Clarias gariepinus* exposed to cypermethrin. These differences in histopathological deformations may be due to the fact that the degree of lesions and histopathological deformations depends on concentration and duration of pesticide exposure[28]. The liver of *Clarias gariepinus* in the present study showed normal distribution of hepatocytes in the control group, but the other group exposed to the cypermethrin showed hyperplastic hepatic and necrosis of hepatic cells. Similar observations were made in[28] findings. The Necrosis in the liver could be due to the extra work load on hepatocyte during detoxification of the cypermethrin[28]. The 96 hours $LC_{50}$ of *Clarias gariepinus* fingerlings exposed to cypermethrin at 95% confidence limit was 1.80ppm for this study, which did not tally with the findings report of[30] who reported a lower 96 hours $LC_{50}$ value of 0.04ppm, when he tested the toxicity of cypermethrin on *Oreochromis mossambicus*. This discrepancy in the $LC_{50}$ value of fish exposed to cypermethrin could be due to the difference in fish species, duration of exposure, due to the difference in elimination and metabolic degradation from the body, as well as the fact that *Clarias gariepinus* adapts to more environmental stress, because of its toughness[16]. The low $LC_{50}$ value of *Clarias gariepinus* fingerlings exposed to

cypermethrin indicates that cypermethrin is toxic to biological organisms even at low concentration. Cypermethrin had toxicity effects on *Clarias gariepinus* fingerlings, thereby causing mortality to the fingerlings. The low $LC_{50}$ of cypermethrin proves its high level of toxicity. The toxicity and mortality of *Clarias gariepinus* fingerlings exposed to cypermethrin was concentration dependent. Several histopathological deformations of the gills and liver of *Clarias gariepinus* exposed to various cypermethrin concentrations were observed, further revealing the damaging effects of cypermethrin on biological organism's organs.

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