

# The Toxicity and Effects of Chlorpyrifos 40 EC on the Fingerlings of African Catfish (*Clarias gariepinus*)

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## Abstract

The study examined the acute toxicity of Chlorpyrifos 40EC, a soluble pesticide, on *Clarias gariepinus* fingerlings under laboratory conditions using static non-renewable bioassays for 96hrs. The fish (weight 0.6- 0.8 kg) were exposed to four different concentrations of 0.4 ml/l, 0.8 ml/l, 1.2 ml/l, 1.6 ml/l, and 0.0 ml/l as control. The physicochemical parameters of the test media were relatively stable except for the total dissolved solids (TDS) and conductivity, which increased with increased concentration and exposure time. The LC50 of Chlorpyrifos 40 EC was observed at 0.76 ml/l while the LT50 was found to be 0.4mg/l, 0.8mg/l, 1.2mg/l and 1.6mg/l for 120.22hrs, 95.50hrs, 66.07hrs, and 36.08hrs respectively. The ANOVA revealed significant variation between treatments and control for fish mortality ( $P < 0.05$ ). The physiological changes analyzed revealed that Tail Beat Frequency (TBF) decreased while the Opercula Beat Frequency (OBF) increased with an increase in concentration and exposure time. The fish exposed to the extract displayed behavioral changes like prolonged vertical movement, rapid movement, jumping, and changes in skin colour with the heavy secretion of mucus. This study shows that Chlorpyrifos is toxic to fish, which implies that stringent measures should be taken to ensure the restraint of its usage by the local fishers to reduce the potential risk of poisonous fish consumption and pollution of the aquatic ecosystem.

**Keywords:** Health implication, Toxicology, Water Quality, Agrochemical

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## Introduction

Agriculture affects the environment locally, regionally, and globally with both on-site and off-site effects known as primary and secondary effects. These effects are commonly attributed to unregulated and injudicious discharges of agrochemicals like pesticides into the aquatic environment, resulting in ecological problems for all aquatic organisms (Adewumi *et al.*, 2018). Pesticides are substances used to prevent, repel and control pests to improve crop yield per hectare (Amaeze *et al.*, 2020). The use of pesticides has been noted to be of significant importance in agricultural development and the protection of public health in many developing countries where pest breeding is increasing (Sunanda *et al.*, 2016). Unfortunately, several researchers have reported that only 0.1% of pesticides applied on agricultural fields and households

reach the target pests. At the same time, a more significant percentage of it is delivered via runoff/discharges from agricultural, commercial, and domestic sources into the aquatic ecosystem (Kanu *et al.*, 2019). Prominent among these pesticides is Chlorpyrifos.

Chlorpyrifos, sold under many brand names, is an organophosphate pesticide used to control pests such as insects and worms on crops, animals, and buildings (Rathod and Garg, 2017). This pesticide is known with the IUPAC nomenclature: 0, 0-diethyl 0-3, 5, 6-trichloropyridin-2-yl phosphorothioate, soluble at a temperature range of 19.5°-25° and density of 0.39-1.4mg/l (Kanu *et al.*, 2021). Chlorpyrifos was introduced in 1965 by Dow chemical company. Today, it is extensively used with an increased toxicity load on the aquatic ecosystem, causing adverse effects on non-target organisms such as fish (Sunanda *et al.*,

2016). It acts on the nervous system of insects by inhibiting the enzyme acetylcholinesterase, and the mode of entry acts on pests primarily as contact poison with its actions as a stomach poison (Woke and Aleleye-Wokoma, 2009). This action results from the lipophilic characteristics of Chlorpyrifos since it is more persistent than other organo-phosphate in use (Chambers *et al.*, 1993).

In Sub-Sahara African countries like Nigeria, assessing the impacts of pesticides on aquatic ecosystems and fish species will only be complete by evaluating the effect of sub-lethal and lethal exposure of Chlorpyrifos on *Clarias gariepinus*, one of the most commonly farmed fish. *Clarias gariepinus* is one of the most standout cultured fish in developing countries like Nigeria and Tanzania. Its preference could be attributed to fast growth, disease resistance, and good market value (Wokeh *et al.*, 2020). However, fishes like *Clarias gariepinus* are relatively sensitive to changes in their environment, particularly at the early stages, since they are known to be susceptible to toxicants and pollution (Sikoki and Zabbey, 2006; Amaeze *et al.*, 2020). It implies that fishes like African catfish, under extreme environmental conditions, will have their physiological and biochemical make-up altered, causing adverse effects on the health of the fish and as well serve as an indicator of the health status of a particular water body (Ayanda *et al.*, 2017). According to Sunanda *et al.* (2016), sub-lethal toxicities of Chlorpyrifos in an aquatic ecosystem can cause histopathological, haematological, oxidative, biochemical, morphological, and neurological interferences. In contrast, lethal toxicities can result in mass mortalities of non-target organisms like fish.

In order to bring to public knowledge the risk associated with the use of pesticides, particularly Chlorpyrifos on fish and aquatic ecosystems, the study on the toxicity and effects of Chlorpyrifos 40EC on fingerlings of African catfish was conducted to determine the effects of Chlorpyrifos on *Clarias gariepinus*, being a significant source of protein to the common person and by implication, the impacts of this widely used pesticide on water which is primarily consumed by man, animals and used

for irrigation purposes.

## Materials and Methods

### Collection and Handling of Experimental Organisms

A total of four hundred (400) fingerlings of the African catfish (*Clarias gariepinus*) were obtained from the African Regional Aquaculture Centre (ARAC) in Rivers State. The fingerlings were of the mean weight range of 0.6- 0.8 kg and length of 7-9 cm. The fingerlings were carefully transported to the laboratory in the evening in an open plastic bucket covered with a nylon net. They were immediately transferred into holding tanks containing dechlorinated tap water in the laboratory. They were acclimatized for 14 days (2 weeks) prior to the commencement of the experiment. The holding tanks were aerated and cleared, and the water was replaced (with clean water) once in three days. The test fish were fed during acclimatization, using feed obtained from Kinfaavour Ideal Concept, Ozuoba, in Rivers State, Nigeria. The fish was fed based on 5% of their weight twice (9 am and 4 pm) daily. Feeding was stopped 24 hours before the commencement of the acute toxicity experiment. Dead fish were removed immediately to reduce pollution of the water.

### Test Chemical

The pesticide used was Chlorpyrifos 40EC. Chlorpyrifos 40EC was bought from the open market (Mile 3 market) in Port Harcourt, Nigeria. It was stored in an airtight bottle and kept in a cool, dry place to avoid possible loss of the volatile component of the chemical.

### Preparation of the Toxicant

Chlorpyrifos 40EC is a soluble pesticide and was diluted into four different concentrations of 0.4 ml/l, 0.8 ml/l, 1.2 ml/l, 1.6 ml/l, and 0.0 ml/ as control. The test media were allowed to stabilize for 30 minutes for proper mixing before introducing the fish (Odioko *et al.*, 2016).

### Exposure to Test Organisms

A static non-renewal bioassay method was used for the experiment to determine the LC50 at 96 hours acute toxicity test. A range finding test was carried out to ascertain the

concentrations of the test solution (Chlorpyrifos 40EC) needed to cause mortality of fingerlings within 96 hours. Fish mortalities were observed and recorded at 24, 48, 60, and 96 hours. Four (4) different concentrations were used for the experiment: 0.0 ml/l (control), 0.4 ml/l, 0.8 ml/l, 1.2 ml/l, and 1.6 ml/l (the experiment consists of two replicates).

The control did not have the test solution (toxicant) in it. Therefore, the fingerlings were placed in the containers randomly from the acclimatization tanks. The fish were placed in 15 plastic containers at a rate of ten (10) fingerlings per container, which were covered with nylon nets to prevent fish from jumping out of the plastic containers.

### **Acute Toxicity Test**

The test for the lethal concentration was a short interval exposure of 96 hours. The fish during this period of exposure was not fed. Fish mortalities were monitored every 24 hours and recorded once any fish was confirmed dead. Death was confirmed when the fish no longer responded to touch with an object (gentle prodding with an object). The dead fish was removed and was not allowed to decay inside the exposure container.

### **Determination of physicochemical parameters of Water**

The water was changed once in 3 days, and the containers were cleaned. Fish were fed twice daily, and the test medium's water quality parameters (Temperature, pH, Dissolved oxygen, and conductivity) were monitored throughout the experiment using multi-parameter kit previously used by (Fubara *et al.*, 2022; Okey-Wokeh *et al.*, 2023).

### **Physiological Response of Test Fish**

Opercula Beat Frequency (OBF): The opercula Beat Frequency is the rate at which the operculum of a fish beats per unit of time. The operculum beats of the fish were measured after every 24 hours, and the experiment lasted for 96 hours. With the aid of a stopwatch, the numbers were recorded as the opercula beat frequency (OBF).

Tail Beat Frequency (TBF): The tail

beat frequency is the rate at which the tail of a fish beats per unit of time. The tail beat of the fish was measured after 24 hours, and the experiment lasted 96 hours. Therefore, using a stopwatch, the number of tail beats per minute were recorded as the Tail Beat Frequency (TBF).

### **Growth**

This is the rate at which the fish develops and increases in size. Its length and weight measure this. The length is measured using a meter rule, and the weight is measured using a sensitive weighing balance. Performance on growth was calculated according to the following formulae (Rashid *et al.*, 2010; Orose *et al.*, 2018).

### **Standard length and Total length**

The standard length of a fish is measured from the top of the snout to the posterior end of the last vertebra. This measurement excludes the length of the caudal fin. The total length of a fish is measured from the top of the snout to the top of the longer lobe of the caudal fin.

From the acute toxicity test result, sub-lethal concentrations (0.00ml/l "control," 0.004 ml/l, 0.008ml/l, 0.012ml/l and 0.016ml/l) were prepared.

Fifteen plastic containers were used with two replicates per treatment and ten groups of fish each. These were exposed to different concentrations of Chlorpyrifos 40Ec for eight weeks. During this period, freshly prepared test solution was regularly added to maintain the concentration level after removing the wastewater. In addition, length and weight were measured every two weeks to determine the growth performance.

### **Statistical Analysis**

Microsoft Excel 2010 and statistical package for social sciences (SPSS) version 20 were used to enter and analyze data at 5% probability.

### **Results**

#### **Dissolved Oxygen (DO)**

The mean  $\pm$  standard error of dissolved oxygen content of various concentrations is presented in Table (1). The dissolved oxygen content of the control (0.0) was found to be  $6.6 \pm 0.1$ , which is within the range of Nigeria

Standardized Aquaculture Practice (NSAP) and Federal Ministry of Environment (FMEnv) standard of water quality for aquatic life. DO content of 0.4 ml/L concentration was  $5.9 \pm 0.2$ , while for 0.8 ml/L concentration was  $5.6 \pm 0.1$ , and that of 1.2 ml/L concentration had dissolved oxygen of  $3.2 \pm 0.1$ . Dissolved oxygen was absent in the 1.6 ml/L concentration of Chlorpyrifos 40EC. The dissolved oxygen result clearly shows that DO decreases with increased Chlorpyrifos 40EC. In 1.6 ml/L concentration, dissolved oxygen was absent, and in such an environment, aquatic organisms, especially fish, cannot survive.

not much variation in temperature during the temperature measurement concerning the increase in concentration. It varied between 26°C and 27°C

#### Conductivity

Table 1 shows the mean value  $\pm$  standard error (SE) of conductivity of various concentrations. The conductivity of the water samples from various test concentrations showed some fluctuations.

#### Acute Toxicity Test

The acute toxicity test results of mortality

**Table 1: Physicochemical parameters of the different concentrations for the Acute toxicity test**

| Concentration of Chlorpyrifos 40EC (ml/L) | DO (mg/L) $\pm$ SE | pH             | T (°C)           | Conductivity (Scm-1) |
|---|--------------------|----------------|------------------|----------------------|
| 0.0 (control)                             | $6.60 \pm 0.1$     | $6.50 \pm 0.1$ | $26.90 \pm 0.01$ | $25.00 \pm 0.2$      |
| 0.4                                       | $5.90 \pm 0.2$     | $6.50 \pm 0.3$ | $27.40 \pm 0.2$  | $18.70 \pm 0.1$      |
| 0.8                                       | $5.60 \pm 0.1$     | $6.50 \pm 0.2$ | $27.00 \pm 0.1$  | $19.60 \pm 0.3$      |
| 1.2                                       | $3.20 \pm 0.1$     | $6.50 \pm 0.1$ | $27.10 \pm 0.2$  | $17.00 \pm 0.8$      |
| 1.6                                       | -                  | $6.40 \pm 0.1$ | $26.90 \pm 0.1$  | $19.00 \pm 0.3$      |
| FME                                       | 6.00-8.00          | 6.00-9.00      | 27.00 - 38.00    | -                    |
| NSAP                                      | 5.00-9.00          | 6.50-9.00      | 25.00 – 32.00    | -                    |

*Mean values  $\pm$  standard error (SE) of physicochemical parameters*

*FME-, NSAP=*

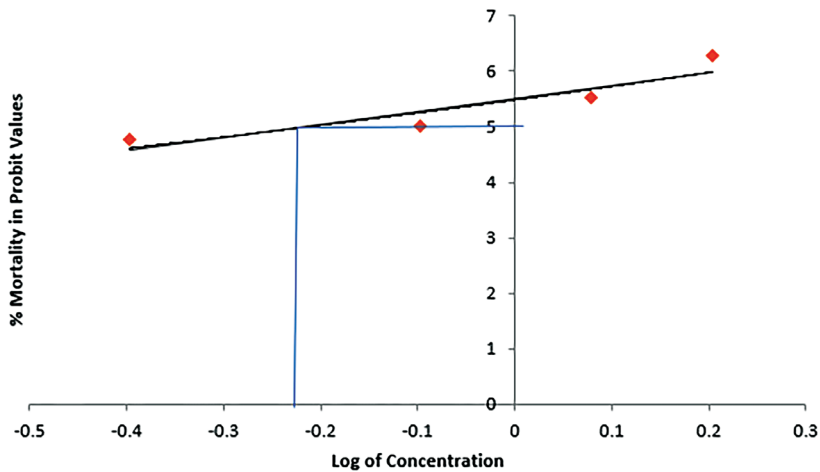
#### Hydrogen Ion Concentration (pH)

The mean value of pH shown in Table 1 revealed that the pH of the control and bioassay were found to be within the acceptable range (6.00–9.00) of the Federal Ministry of Environment (FMEnv) and (6.50–9.00) of Nigeria Standardize Aquaculture Practice (NSAP). However, the pH of the water samples from the various test concentrations showed some fluctuations.

#### Temperature

The mean values  $\pm$  standard error (SE) of temperature at various concentrations of Chlorpyrifos, as shown in Table 1, revealed that the temperature of the control and treatment measured every 24 hours were found to be within the acceptable range for the sustenance of aquatic life. It was noted that there was

for *Clarias gariepinus* exposed to water-soluble fractions (WSF) of Chlorpyrifos 40EC for 24 to 96 hours are shown in Tables 2, 3, 4, and 5. During the experiment, the lowest mortality was observed in the lowest concentration. No mortality was recorded in control; mortality tends to increase with concentration and time. It was observed that mortality increased with an increase in exposure time (from 24 to 96 hours), but there were fluctuations in mortality within the different concentrations and exposure times. Therefore, the acute toxicity test results suggest that the increase influences the increase in mortality in concentration and time. The 96 hours LC50, the lethal concentration that kills 50% of test organisms in 96 hours, was observed at 0.76 ml/L (0.76 ppt) concentration (Fig. 1). The ANOVA revealed significant variation between treatments for fish mortality ( $p < 0.05$ ).



**Figure 1: Graph of Percent Mortality against Log of Concentrations of Chlorpyrifos 40 EC (LC50 Graph)**

LC50 = Antilog of =0.24 = 0.76 ml/L

**Table 2: Percentage Mortality and Mortality in Probit Values**

| Time in Hr./ Log of Time | Concentrations of Chlorpyrifos 40 EC in ml/L |                  |                 |                 |
|--------------------------|--|------------------|-----------------|-----------------|
|                          | 0.4 ml/L (-0.40)                             | 0.8 ml/L (-0.10) | 1.2 ml/L (0.08) | 1.6 ml/L (0.20) |
| 24 (1.38)                | 0.0 (0.00)                                   | 0.0 (0.00)       | 20.0 (4.16)     | 30.0 (4.48)     |
| 48 (1.68)                | 10.0 (3.72)                                  | 20.0 (4.16)      | 30.0 (4.48)     | 50.0 (5.00)     |
| 72 (1.86)                | 20.0 (4.16)                                  | 40.0 (4.76)      | 50.0 (5.00)     | 80.0 (5.84)     |
| 96 (1.98)                | 40.0 (4.76)                                  | 50.0 (5.00)      | 70.0 (5.52)     | 90.0 (6.28)     |

*Note:* the log of time is in the bracket while mortality in probit is also in the bracket

**Table 3: Opercula Beat Frequency (OBF) at Different Concentrations of Chlorpyrifos 40EC**

| The concentration of Chlorpyrifos 40EC in ml/L | 24 hours    | 48 hours    | 72 hours    | 96 hours    |
|--|-------------|-------------|-------------|-------------|
| 0.0 (control)                                  | 21.50 ± 0.1 | 41.50 ± 0.8 | 55.00 ± 0.9 | 50.10 ± 0.8 |
| 0.4  | 24.00 ± 0.2 | 45.00 ± 0.6 | 62.00 ± 0.7 | 58.20 ± 1.4 |
| 0.8  | 26.50 ± 0.2 | 49.00 ± 0.9 | 63.00 ± 1.4 | 61.02 ± 2.4 |
| 1.2  | 30.00 ± 0.8 | 52.50 ± 0.3 | 67.50 ± 1.2 | 64.50 ± 3.5 |
| 1.6  | 34.00 ± 1.2 | 56.20 ± 2.1 | 70.20 ± 0.7 | 69.40 ± 2.0 |

**Physiological Responses**

Opercula Beat Frequency (OBR): Table 3 shows the mean values ± standard error obtained for opercula movement of test fish from the 24<sup>th</sup> to 96<sup>th</sup> hour. The results showed that the opercula beat frequency increased with an increase in concentration and time.

Tail Beat Frequency (TBF): Table 4 shows the mean values obtained for a tail beat or movement off test fish or various concentrations and exposure time. The result showed that tail beat frequency decreased with increased concentration and time after 48 hours of exposure.

**Table 4: Tail Beat Frequency (TBF) of Fish at Different Concentrations of Chlorpyrifos 40 EC**

| The concentration of Chlorpyrifos 40 EC in ml/L | 24 hours    | 48 hours    | 72 hours    | 96 hours    |
|---|-------------|-------------|-------------|-------------|
| 0.0 (control)                                   | 10.50 ± 0.5 | 10.20 ± 0.7 | 10.80 ± 1.4 | 10.50 ± 0.9 |
| 0.4   | 15.00 ± 0.6 | 13.50 ± 0.5 | 11.00 ± 1.6 | 8.20 ± 1.6  |
| 0.8   | 17.50 ± 0.3 | 10.00 ± 0.8 | 9.50 ± 1.0  | 5.70 ± 0.7  |
| 1.2   | 18.50 ± 0.5 | 7.50 ± 0.6  | 5.50 ± 0.0  | 6.10 ± 1.3  |
| 1.6   | 20.00 ± 0.3 | 4.50 ± 0.4  | 3.0 ± 1.8   | 3.80 ± 0.5  |

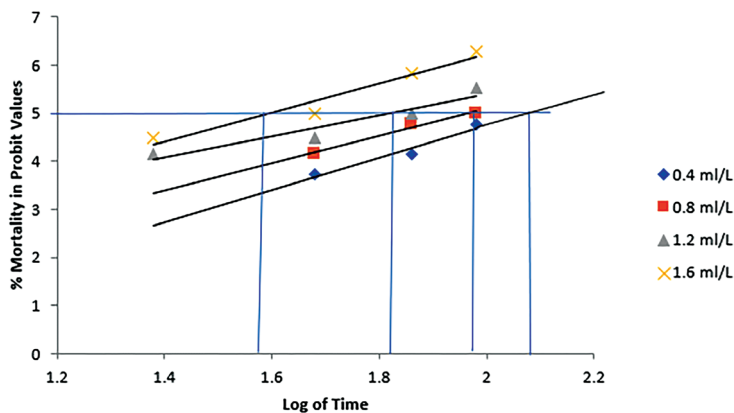
**Table 5: Growth Performance for the weight (g) at the end of 8 weeks**

| Parameters | Concentrations |             |             |              |              |
|------------|----------------|-------------|-------------|--------------|--------------|
|            | Control        | 0.004       | 0.008       | 0.012        | 0.016        |
| IL         | 6.6±0.08 b     | 6.5±0.16b   | 7.46±0.21a  | 6.92±0.17 ab | 6.88±0.32 ab |
| FL         | 11.82±0.04a    | 10.65±0.18b | 10.37±0.26b | 8.47±0.11c   | 8.66±0.27 c  |
| IW         | 5.99±0.14ab    | 6.23±0.5a   | 5.84±0.45ab | 6.16±0.24a   | 5.02±0.21b   |
| FW         | 11.19±0.24a    | 9.18±0.25b  | 7.66±0.22c  | 7.72±0.02c   | 7.09±0.37c   |
| WG         | 5.20±0.37a     | 2.94±0.55b  | 1.81±0.34bc | 1.57±0.26c   | 2.06±0.30bc  |
| FI         | 40.93±0.29a    | 36.83±1.20b | 32.57±1.48c | 31.82±0.35c  | 28.40±1.48d  |
| FCR        | 0.13±0.01a     | 0.08±0.02b  | 0.07±0.01b  | 0.58±0.01    | 0.05b±0.01b  |
| SGR        | 9.29±0.66a     | 5.26±0.98b  | 3.24±0.61bc | 2.80±0.47c   | 3.68±0.53bc  |

**Behavioral Responses**

Behavioral responses were observed at the end of 24 hours, 48 hours, 72 hours, and 96 hours. At the end of 24 hours, there were no observable or significant changes in behavior

compared with those in control. However, from the 48<sup>th</sup> hour to the end of the 96<sup>th</sup> hour, fishes at different concentrations were seen coming to the water's surface and gasping for air. This increased with increased concentration,



**Figure 2: Graph of Percentage Mortality in Probit Value against Log of Time (LT50 Graph)**

LT50 for 0.4 ml/L = Antilog of 2.08 = 120.22 hours  
 LT50 for 0.8 ml/L = Antilog of 1.85 = 95.50 hours  
 LT50 for 1.2 ml/L = Antilog of 1.82 = 66.07 hours  
 LT50 for 1.6 ml/L = Antilog of 1.56 = 36.08 hours.

and other responses observed during the test include erratic movement, increased irritability, and loss of skin pigmentation. All these behavioural responses could be attributed to the contaminating effect of the Chlorpyrifos 40EC present in water.

**Growth**

The result of growth performance presented in Table 5 shows the mean value obtained for fish growth at different concentrations. The results revealed that growth was stunted with increased concentration and time, respectively. The control increased in size (length and

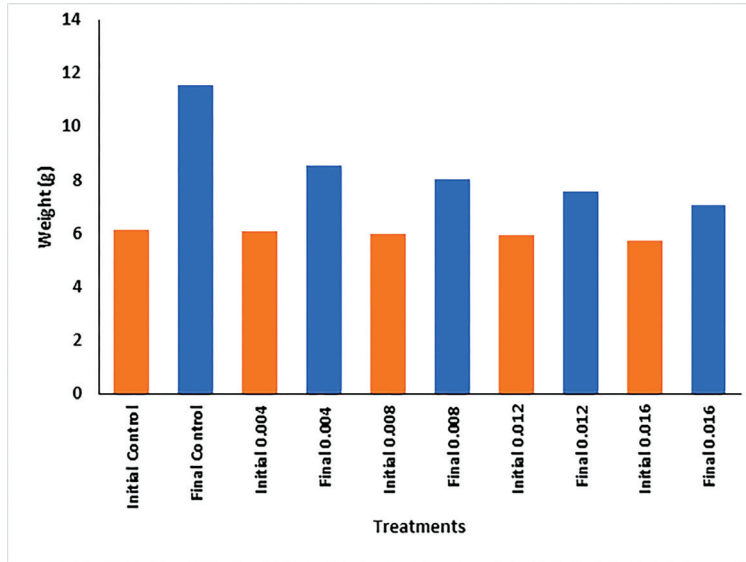


Figure 3: Barchart for Growth Performance for the weight(g)

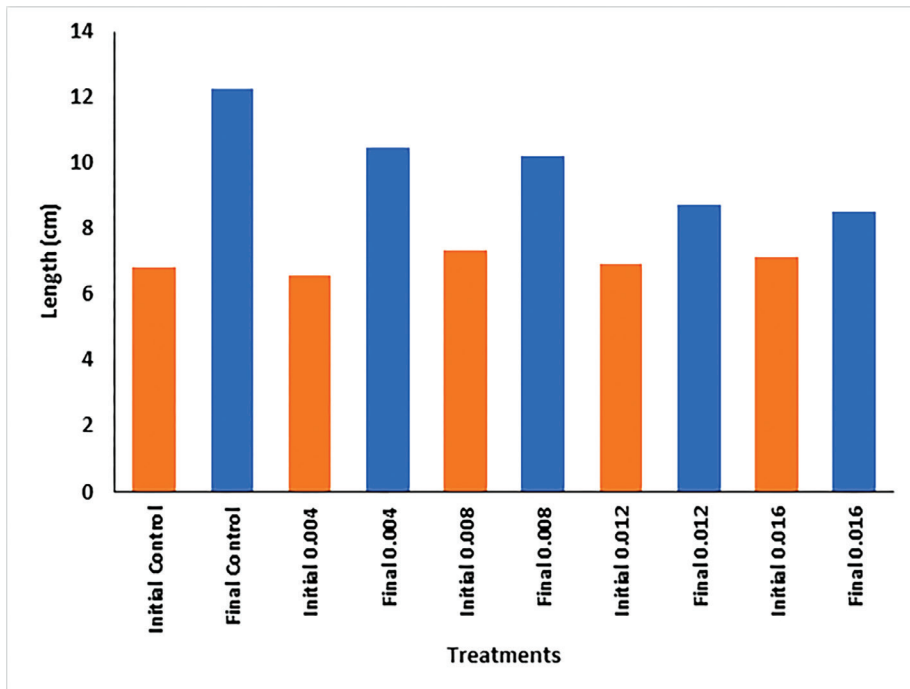


Figure 4: Barchart for Growth Performance for Length (cm)



weight). The concentrations 1.2 ml/L and 1.6 ml/L seemed to have less appetite and could not feed as much as those in concentrations 0.0 ml/L, 0.4 ml/L, and 0.8 ml/L (Fig. 3&4)

### Discussion

The exposure of *Clarias gariepinus* fingerlings to Chlorpyrifos 40EC showed mortality at a 1.2 ml/L concentration. This finding is in accordance with previous reports on the effects of toxicants, especially hydrocarbons, pesticides, herbicides, and insecticides, on aquatic life (Adewumi *et al.*, 2018; Amaeze *et al.*, 2020). Furthermore, the increase in mortality rate with increased concentration of Chlorpyrifos 40EC and increased exposure time suggest dose-dependent survival and concentration-graded lethality, which is indicative of the possible toxic effects and danger of the toxicant on fish species and highlights its negative impact on the aquatic environment (Tjeerderma, 2005). Notably, fishes are highly susceptible to aquatic ecosystems contaminated with pesticides or insecticides, as any form of exposure for a long time often results in behavioral, physiological, and morphological responses (Hussain *et al.*, 2015; Kanu *et al.*, 2019).

The environmental and ecological implications of indiscriminate use of Chlorpyrifos 40EC could lead to bioaccumulation, fish kill, and biodiversity loss in the natural environment (Amaeze *et al.*, 2020). It is also of great importance to note that LC50 derived are specific to this experiment due to the concentration of Chlorpyrifos 40EC used, the choice of the toxicant, the age of the fish, feeding habits, time of exposure and laboratory condition at the time of the experiment affected toxicant toxicity.

The dissolved oxygen at the various test media in which mortality was observed during the experiment was found to be below the Federal Ministry of Environment (FMEnv) and Nigerian Standardized Aquaculture Practice (NSAP) accepted limits (6.0 – 8.0) and (5.0 – 9.0 mg) of water quality for the sustenance of aquatic organisms. In addition, the concentration of 1.6 ml/L of the toxicant shows the total depletion of dissolved oxygen; the dissolved oxygen stress aquatic organisms found in water contaminated

with pesticides is due to the insufficient supply of oxygen for respiration in the medium. The low dissolved oxygen observed in higher concentrations of Chlorpyrifos 40EC, apart from death, can lead to impaired fish development and maturation (Okey-Wokeh *et al.*, 2020). The inability of young fishes to withstand oxygen deficiency in the medium is a result of oxidative stress caused by the pressure of the toxicant. The result obtained from this experiment agrees with the work by Kanu *et al.*, (2019). The pH and temperature of the water were within the Federal Ministry of Environment (FMEnv), and Nigeria Standardized Aquaculture Practice (NSAP) accepted limits for the sustenance of aquatic life. Water pH measures the acidic and alkaline condition of an aquatic ecosystem, which influences most of the chemicals and biochemical reactions as well as the aquatic productively (Okey-Wokeh *et al.*, 2021). The moderate pH and temperature values in this experiment reveal that both parameters contribute little to the toxicity of Chlorpyrifos 40EC.

The impacts of the toxicant on the physiological functions observed are related to the fact that the fish maintained direct and constant contact with the water (Chindah *et al.*, 2001). The impaired respiratory ability observed in the increased opercula movement (stressed breathing, gasping for air) is due to depleted oxygen content with increased concentration. The fish tends to compensate for reduced dissolved oxygen in the media by increasing the opercula movement as they gasp for breath at the water's surface. The increase in Opercula Beat Frequency (OBF) with exposure to the toxicant indicates disturbance and stress. The reduction in the rate of Tail Beat Frequency (TBF) with an increase in concentration and exposure time indicates retarded physiological response in the fish body function as a result of the weakness of the fish caudal muscles. This implies that behavioural responses such as Opercula Beat Frequency (OBF), Tail Beat Frequency (TBF), Erratic Movement, gasping at the surface of the water for air, suspending at an odd angle, and change in skin pigmentation are sensitive and appropriate indices for toxicological studies (Grilltsch *et al.*, 1999; Kanu *et al.*, 2019; Ayanda



*et al.*, 2017).

Similarly, growth indices are extremely sensitive to stressors. They show a very sharp difference between control and fish exposed to different concentrations of toxicants, which inhibits growth (Odioko *et al.*, 2016). For example, Chlorpyrifos causes inhibition of AchE in fish (Assis *et al.*, 2012; Oruc, 2012). According to a review report by the European Commission, Chlorpyrifos is considered very ecotoxic to fish (EPA, 2012) and LC50 (96 hours) = 0.00054-520 mg/L (EC, 2005), while chronic = 0.00014 mg/L (EC, 2005). From the report of Huynh and Nugegoda (2012) on exposure of Australian Catfish (*Tandanus tandanus*) to a short pulse of Chlorpyrifos at 2µg/l resulted in reduced growth. However, there was no difference in food intake. Reduction in swimming activity has also been reported in Coho Salmon (*Oncorhynchus kisutch*), and a reduction in feeding occurred at 1.2µg/l (Sandahl *et al.*, 2005). This also supports the result of reduced Tail Beat Frequency (TBF) as concentration increased. The report of Richard and Kendel (2003) also supports that an increase in the concentration of Chlorpyrifos brings about a reduction in body length and stunted growth.

### Conclusion

It is important to note that despite the usefulness of the static non-renewable bioassay to determine the toxic effect of Chlorpyrifos 40 EC and the relative sensitivity of *Clarias gariepinus*, care should be taken extrapolating data on pesticide pollution situations. Also, it should be noted that an experiment in the laboratory is different from the natural environment, which is made up of multi-variable systems. The LC50 values observed in this study showed the toxicity of Chlorpyrifos 40 EC to fish. It has both acute and chronic effects on *Clarias gariepinus* and indicates that its effect on lower biota could be far more devastating. Therefore, the government should discourage the use of synthetic pesticides in the control of insect pests by farmers and encourage the application of other pest management strategies like biological, mechanical, and cultural strategies or the application of these strategies in combination with one another.

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### Declaration of Conflict of Interest

No notable conflict of interest was observed among the authors.

### Ethical Approval

This work was done based on the institutional guideline provided by the University of Port Harcourt, Nigeria.

### References

- Adewunmi, B., Ogunwole, G.A., Akingunsola, E., Falope, O.C. and Eniade, A. (2018). Effects of sub-lethal toxicity of Chllorpyrifos and DD force pesticides on haematological parameters of *Clarias gariepinus*. *International Research Journal of Public and Environmental Health*, 5(5), 62-71.
- Amaze, N.H., Komolafe, B.O., Salako, A.F., Akagha, K.K., Briggs, T.D., Olatinwo, O.O. and Femi, M.A. (2020). Comparative assessment of the acute toxicity, haematological and genotoxic effect of ten commonly used pesticides on the African Catfish, *Clarias gariepinus*. Burchell 1822. Heliyon, 6 e04768.
- Assis, C.R.D., Linhares, A.G., Oliveira, V. and Carvalho, L.B. (2012). Comparative effect of pesticides on brain acetylcholinesterase in tropical fish. *Science of Total Environment*, 441, 141-150.
- Ayanda, O.I., Oniye, S.J. and Auta, J.A. (2017). Behavioural and some physiological assessment of glyphosate and paraquat toxicity to juvenile of African catfish, *Clarias gariepinus*. *Pakistan Journal of Zoology*, 49(1), 183-190.
- Chambers, J.E. and Carr, R.L. (1993). Inhibition patterns of brain acetylcholinesterase hepatic and plasma aliesterases following exposure to three phosphorothionate insecticides and their oxions in rat. *Applied Toxicology*, 21, 111-119.

- Chindah, A.C. Braide, S.A. and Nduaguibe, U.M. (2001). Tolerance of periwinkle (*Tympanotonus fuscatus* Linne) and shrimp (*Palaemonetes africanus*) to waste water from Bonny light crude oil tank farm. *Polish Journal of Environmental Protection and Natural Resources*, 21(22), 61-72.
- EC 2005. Review report for the active substance Chlorpyrifos. SANCO/3059/99-1.5. Health and Consumer Protection Directorate General, European Commission.
- Fubara, R.I., Wokeh, O.K. and Okey-Wokeh, C.G. (2022). Physicochemical Characterisation and Water Quality Status of the Lower Orashi River, Niger Delta, Nigeria. *West African Journal of Applied Ecology*, 30(2), 24-31.
- Grillitsch, B., Vogl, C. and Wyttek, R. (1999). Qualification of spontaneous undirected locomotor behaviour of fish for sublethal toxicity testing. Part II variability of measurement parameter under toxicant induced stress. *Environmental Toxicology and Chemistry*, 18(12), 2743-2750.
- Hussain, M.I., Kumar, B. and Ahmad, M. (2015). Acute Toxicity, Behavioral response and Biochemical Composition of Blood of common carp, *Catla catla* (Hamilton) to an Organophosphate Insecticide, Dimethoate. *International Journal of Current Microbiology and Applied Sciences*, 4(5):1189-1199
- Huynh, H.P.V. and Nugegoda, D. (2012). Effects of Chlorpyrifos exposure on growth and food utilization in Australian catfish *Tandanus tandanus*. *Bulletin of Environmental Contamination and Toxicology*, 88(1), 25-29.
- Kanu, K.C., Ogbonna, O.A. and Mpamah, I. C. (2019). Acute toxicity and biological responses of *Clarias gariepinus* to environmentally realistic Chlorpyrifos concentration. *Pollution*, 5(4), 839-846.
- Kanu, K.C., Otitolaju, A.A. and Amaeze, N.H. (2021). Assessment of the risk of death of *Clarias gariepinus* and *Oreochromis niloticus* pulse-exposed to selected agricultural pesticides. *Scientific Reports*, 11, 14652.
- Odioko, E., Sikoki, F.D., Vincent-Akpu, I.F. and Utibe, D.I. (2016). Acute Toxicity of Sponge Plant (*Luffa cylindrica*) Fruit extract on the Africa Catfish (*Clarias gariepinus*, Burchell 1822) Juveniles. *International Journal of Life Sciences Research*, 4, 148-159.
- Okey-Wokeh, C.G., Obunwo, C.C. and Boisa, N. (2020). Assessment of physicochemical characteristics of Mini-Ezi Stream in Elele-Alimini, Emohua Local Government Area of Rivers State, Nigeria. *International Journal of Advances in Scientific Research and Engineering (IJASRE)*, 6(1), 196-202.
- Okey-Wokeh, C.G., Obunwo, C.C. and Wokeh, O.K. (2021). Evaluation of water quality index using physicochemical characteristics of Ogbor River in Aba, Abia State, Nigeria. *Journal of Applied Science and Environmental Management*, 25(1), 47-51.
- Okey-Wokeh, C.G., Wokeh, O.K., Orose, E., Lananan, F. and Azra, M.N. (2023). Anthropogenic Impacts on Physicochemical and Heavy Metal Concentrations of Ogbor Hill River Water, Southern Nigeria. *Water*, 15(7), 1359.
- Orose, E; Woke; G.N and Bekibele, D.O (2018). Growth Response and Survival of Nile Tilapia (*Oreochromis niloticus*) using Steroid Hormone, Animal Testes and Pawpaw Seed based diet. *Nigerian Journal of Fisheries*, 15(1): 1336-1341.
- Oruc, E.O. (2010). Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to Chlorpyrifos. *Pesticides Biochemistry and Physiology*, 96, 160-166.
- Rashid, M.H., Hossain, M.T., Mortuza, M.G., and Chowdhury, A.S (2010). Utilization of sunnhemp (*Crotalaria juncea*. L) seed as a protein supplement in fish feed. *Journal Agro for Environment*. 4(2): 21-24
- Rathad, A. and Garg, R.K. (2017). Chlorpyrifos poisoning and its implications in human fatal cases: Aforensic perspective with reference to Indian scenario. *Journal of Forensic and Legal Medicine*, 47, 29-34.
- Richard, S.M. and Kendall, R.J. (2002). Biochemical effects of Chlorpyrifos on two

- developmental stages of *Xenopus laevis*. *Environmental Toxicology and Chemistry*, 21, 1826-1835.
- Sandahl, J.F., Baldwin, D.H., Jenkins, J.J. and Scholz, N.L. (2005). Comparative thresholds for Acetylcholinesterase inhibition and behavioural impairment in Coho Salmon exposed to Chlorpyrifos. *Environmental Toxicology and Chemistry*, 24(1), 136-145.
- Sikoki, F.D .and Zabbey, N. (2006). Aspects of fisheries of the middle reaches of Imo river, Niger Delta, Nigeria. *Environment and Ecology*, 24:309-312
- Sunanda, M., Chandra, J., Neelima, P., Govinda, K.R. and Sinhachalam (2016). Effects of Chlorpyrifos (An organophosphate pesticide) in Fish. *International Journal of Pharmaceutical Sciences, Review and Research*, 39(1), 299-305.
- Tjeerderma, R.S. (2005). Acute and chronic effects of crude dispersed oil on Chinook Salmon Smolts (*Onchoryndis tshawtscha*). A report submitted to the Coastal Response Research Centre. Nation Ocean and Atmosphere Administration United States, 1-15.
- Woke, G.N. and Aleleye-Wokoma, I.P. (2009). Effects of pesticides (Chlorpyrifos Ethyl) on the fingerlings of *Clarias gariepinus*. *Global Journal of Pure and Applied Sciences*, 15(3), 265-266.
- Wokeh, O.K., Woke, G.N., Orose, E. and Odioko, E. (2020). Comparison of growth performance of African catfish (*Clarias gariepinus*) fed with different standard feed. *International Journal of Fisheries and Aquatic Studies*, 8(5), 394-397.