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Acute Effect Of Atrazine Exposure On Juveniles Of *Clarias Gariepinus*

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Abstract

Atrazine is an herbicide of the triazine class which is used in agriculture to kill plants weed and they can be washed into waterbodies where they cause toxic pollution. The common Atrazine (2 - chloro - 4 - ethylamino - 6 - Isopropylamino - - s - trazine) is one of the most commonly used herbicides among rural farmers in Nigeria. These studies aimed at determining the effects of atrazine on haematology of juveniles of *Clarias gariepinus.* Healthy juveniles were randomly selected and weighed then exposed to five different concentrations of atrazine including control. Haematological, behavioural and mortality parameters were investigated in the exposed fish. Result obtains shows that behavioural and mortality parameters were dose dependent as the concentration of the atrazine increased, the tail beats and operculum ventilation increased. There was significant increase in mortality rate and change in behaviour ($P<0.05$) in 12 and 24 hours of the exposed fish compared to control. Behavioural effects observed include increased mucous secretion, restlessness, swirling irregular movement and fish progressively became sluggish and lethargic. Fish mortality was dose dependent and the 96 hours LC_{50} for the fish determined using probit analysis was 0.55mg/l. Significant changes ($p<0.05$) in haematological analysis were observed as the toxicant concentration increased. Mean Red Blood Cells (RBC), Haemoglobin content (Hb), Packed Cell Volume (PCV) reduced as the concentration of toxicant increased while other parameters increased proportionally with the toxicant concentration. It is thereby recommended that proper measures should be taken as regards to the discharge of chemicals or waste water arising from chemical industries into aquatic bodies to prevent the dead and possibly the extinction of many other fish species.

Keywords: Atrazine, Behaviour, *Clarias gariepinus*, Haematology, Mortality.

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INTRODUCTION

Crop production is one of the sectors that the technology of herbicide utilization is well adopted without required knowledge of its impacts. Agriculture is in the chemical age of development in which the intensive use of chemicals is practiced. The increasing population of developing countries and the attendant need to increase food production is a pointer that herbicides must be used in an increasing proportion in order to increase the much-needed food, and to provide it sufficiently [26]. Pesticides in rivers, lakes and groundwater in Europe. Indicators and signals – European Zero Pollution Dashboards. Retrieved from the EEA website. This report states that atrazine and its metabolites are among pesticides still exceeding environmental quality standards in groundwater, despite being banned in many places [13]. There is almost no weed problem that cannot be solved by herbicides. Today, high-yield agriculture heavily depends on herbicides, as they constitute a vital and integral component of weed management practices. Although herbicides lead to increased food production, there is a very reason to use them properly to safeguard the people and environment.

Atrazine interferes with the liver functions markers and disrupts the normal architectural and structural components of the liver resulting in non-infections of liver injury which may result in repeated cycles, cell deaths and inflammation which could result in the eventual death of the exposed fish if exposure duration is prolonged [33].

[20] reported that atrazine could be toxic to *Clarias gariepinus* and its use in agricultural systems close to aquatic bodies should be strictly monitored. The use of herbicides in weed control has been recognized as a part of Agricultural practices worldwide (Meng *et al*., 2022; NPIC. 2024). Unregulated use of herbicides to boost agriculture productivity may negatively affect non target organisms especially in the aquatic environments (El Nahhal and El Nahhal 2021). The application of herbicides generally occurs in the dry season or early raining season which often coincides with the breeding season of many fish species (Dinh *et al*., 2022).

The constant flow of agricultural effluents into fresh water often leads to a variety of pollutant accumulation, which becomes apparent when considering toxic pollution [32]. Herbicides may produce toxicant - induced behavioral impaired impairment that interferes with ecologically relevant behaviors of fish such as predator avoidance, reproduction and social interactions which are essential to the wellbeing and survival of fishes in natural ecosystems [32]. FAO (1997) asserted that the use of chemical in agriculture, if carried out properly, can be regarded as wholly beneficial with no attendant adverse environmental effects or increased risk to the health of the farmers. African fresh water catfish, *Clarias gariepinus* is commonly found in Nigeria rivers, ponds and streams. Fish is used for toxicity test because they are sensitive indicators of chemical pollution and an integral part of aquatic communities and can be used as surrogate species for other species in comparative toxicology [27]. Therefore, the main objective of this study was to examine the deleterious effect of atrazine on the juveniles of *Clarias gariepinus.*

METHODS AND MATERIALS

Experimental fish

Healthy juveniles of *Clarias gariepinus* of mixed sexes and fairly uniform size were obtained from Aqua Consult Barkallahu Kaduna-Zaria Express Way, Kaduna and transported in plastic container to the Zoological laboratory in the Department of Biological Sciences, Kaduna State University, (Lat.10°31´N; Long.7°26´E) and 6.14m above sea level, Nigeria. Atrazine (herbicide) was purchased from Central Market of Kaduna State. The juvenile fishes were kept in the fish pond, separately containing tap water of about 150L, for a period of two weeks (14 days) to acclimatize to the environment before they were used in the bioassays.

The fish were fed twice daily at 3% body weigh with coppens (1.5mm) with 35% crude protein and the water was changed every two (2) days to enhance oxygen content in the water. Feeding was stopped 24 hours prior to the bioassay to avoid contamination of the water due to their excretory product.

Acute Bio-assay

Pilot studies were carried out to determine the definitive concentration range for testing Atrazine following the methods of Solbe (1995). Ten fish per concentration of toxicant were used with 2 replicates each. Mortality of fish was determined at 12, 24, 48, 72 and 96 hours. This was carried out until about 80% to 90% of the fish died in the highest concentration and 20% to 30% at the lowest concentration. The methods of acute toxicity tests as described by [43] and [5] was employed. Ten juvenile fishes were exposed to five different concentrations of the toxicant in each test plastic tank in duplicate and the control.

Experimental Design

Juveniles of fairly equal weight (mean ± standard deviation) and length (mean ± standard deviation) were selected randomly, weighed and distributed into 12 plastic aquaria containing definitive concentration of Atrazine and 2 controls with only tap water without Atrazine. The bioassay test was carried out in a 44 × 29.5 × 24cm dimension of 12 plastic aquariums with a water holding capacity of 30 litres in a close water into which approximate quantity of Atrazine were taken and gave a final volume of 20 litres of water per aquarium. The solutions were stirred for homogenous mixing before each aquarium was randomly stocked in duplicates with 10 juveniles of fish while the test solution and control were renewed daily.

Behavioural Studies

After exposure of the fish to various concentrations of the toxicant, observations were made on the behavioura land morphological responses of the fish at 12, 24, 48, 72 and 96 hours [12]. Behavioural and morphological characteristics monitored are erratic swimming, loss of equilibrium, lethargy, increased excitability, mortality, vertical suspension, mucous secretion, sluggishness, startle response, deformities and haemorrhage. The investigation of opercular ventilation count and tail fin movement rate at 12, 24, 48, 72 and 96 hours in every two minutes. Three fish were observed in each test tank and the control and was recorded respectively.

Haematological Studies

Blood was sampled as described by [10] for the assessment of the various blood parameters and were collected by severance of caudal peduncle from the caudal artery at 2cm away from the caudal peduncle.

Total Erythrocyte Count

Hendricks solution was used for the erythrocyte count. Blood was drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool and adjusted the volume to exactly 0.5 mark. The pipette was filled to 101mark with the diluting fluid and shaken for 30 minutes to ensure thorough mixing. The diluted suspension of cells was thereafter drawn in to the Neubauer’s chamber haemocytometer. Haemocytometer was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer (Hesser, 1960) were counted with 4mm objectives and ×40 eyepiece of the microscope. The number of cells was multiplied by ×10 and this gave the total number of cells per cubic millimeter (mm^3) of blood (Hesser, 1960).

Total Leucocyte Count

Leucocytes were counted by using Shaw's solution A (neutral red (25mg), sodium chloride (0.9g), distilled water (100mls) and B (crystal violet (12mg), sodium citrate (3.8g), distilled water (100mls)). Blood was drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn to shake the bulb of the pipette half way and then filled to 101 mark with solution B. A few drops were dispensed in to the haemocytometer. The cells in the four large squares of the chamber (Hesser, 1960) were counted with 4mm objective and ×40 eyepiece microscope. The number of cells was multiplied by 500 to obtain the total number of leucocytes per cubic millimeter (mm^3) of blood (Hesser, 1960).

Leucocytes Differential Count

Two drops of blood were placed on a slide, made into a thin smear with another slide and left to dry. The smear was fixed with absolute methanol, then stained with giemsa's stain and buffered distilled water. It was allowed to stand for about 20-30 minutes after which the slide was rinsed again with buffered distilled water and allowed to air dried. Counting was made by the use of microscope and the parameters counted include neutrophils, lymphocytes, basophils, eosinophils, monocytes. This was applicable for both acute and sub-lethal bioassay.

Haematocrit (Packed Cell Volume)

Determination of packed cells volume (PCV) was carried out by micro-westegren method as described by [10]. The well-mixed sampled blood from the severed caudal penducle was drawn in to micro-haematocrit tube (75mm long and 1.1-1.2mm internal diameter). The tubes were then centrifuged for five minutes. The reading was taken with the aid of a micro-haematocrit reader and expressed as the volume of the erythrocytes per $100cm^3$.

Haemoglobin.

It was determined by adding 0.02mls of well mixed blood to 0.4mls of Drabkins reagent. The solution was mixed gently by the method of inversion and allowed to stand for conversion of haemoglobin to cynanomethaemoglobin. Small wooden steel was used to remove any coagulating formed. A well-mixed blood was placed in the curved of the colorimeter and reading recorded using optical density from a standard haemoglobin table. The method used was the cyanomethaemoglobin method [25].

Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)

The values for Mean Cell Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration were calculated based on the equations given below (Adewuyi, 2007),

$$\text{MCV } (m^3) = \frac{Haematocrit\ (\%) \times 100}{Red\ Blood\ Cell\ Count\ (\frac{Cell}{mm3})}$$

$$\text{MCH (g/cell)} = \frac{Haemaglobin\ (g/100ml) \times 100}{Red\ Blood\ Cell\ Count\ (\frac{Cell}{mm3})}$$

$$\text{MCHC (g/100ml)} = \frac{Haemaglobin\ (g/100ml) \times 100}{Haematocrit\ (\%)}$$

Mortality Studies

Observations to determine the mortality rate of *Clarias gariepinus* were made at 12, 24, 48, 72 and 96 hours. Juveniles of *Clarias gariepinus* were considered dead when there was no sign of opercular movement or no response to gentle prodding. The number of dead fishes in each group was recorded against the time of their death [43]. The dead fish were removed immediately to avoid fouling.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS software to test for the significant differences between means and where significant differences were found, the Duncan's Multiple Range Test (DMRT) was used to separate the means.

RESULTS AND DISCUSSION

The result of this study as presented in Table 1 and 2 revealed that *Clarias gariepinus* exposed to various concentrations of Atrazine recorded decrease packed cell volume (mm^3), total red blood cell (RBC) and haemoglobin (Hb) but an increase in total white blood cells (WBC) could be due to an inhibition in erythrocyte pproduction and haemodilution. Erythropenia (deficiency in the number of red blood recorded in this study as seen by the reduced haemoglobin content and haematocrit value as well as erythrocyte sedimentation rate (ESR). Decreased erythrocyte count and haemoglobin content in freshwater fish *Channapunctatus* after acute exposure to diazonin was also reported by [4]. Changes in the erythrocyte profile induced by acute effect of dichclorvos in *Clariasbatrachus*, malathion in *Cyprinuswabsoni* (Khattak and Hafeez, 1996), trichlorphon in *Patractus mesopotamicus* (Tavares *et al*., 1999) and formothion in *Heteropneustesfossilis* [41]. The significant decrease in the packed cell volume (PCV) in this study could be attributed to gill damage and or impaired osmoregulation causing anaemia and haemodilution. These findings are similar with anaemia associated with erythropenia that was reported by [44] in *Colisafasciatus* after acute exposure to lead.[47] reported similar results when exposing the fish *Sarotherodon mossambicus* to dimecron. While [41] showed another organophosphate insecticide, formothion, gave a significant increase in the total erythrocyte count and haemoglobin content in fish. Similar results have been reported for several freshwater fishes (Khalaf Allah, 1999; Balathakur and Bais, 2000; Rehulka, 2000; Gbem *et al*., 2003; Aderolu *et al*., 2010). *Oreochromis niloticus* exposed to cadmium also showed significant reduction in RBC, Hb and PCV [22].

The increase in white blood cell in acute bioassay studies could be associated with an increase in antibody production which help in survival and recovery in the fish exposed to acute concentration of Atrazine. Similar trend was also reported by [21] and [15]. The present findings suggest hypersensitivity of leucocytes to Atrazine. Similar results were obtained by [40] [29] who showed increase in total leucocyte count of *Channapunctatus* and *Clarias batrachus* after exposure to mercuric chloride. The fluctuation in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the present study, clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, depicting aneamic condition.[9] observed similar fluctuations. The Mean Corpuscular Haemoglobin Concentration is a good indicator of red blood cell swelling [49], the Mean Corpuscular Haemoglobin Concentration, which is the ratio of haemoglobin concentration as opposed to the haemotocrit, is not influenced by the blood volume for the number of cells in the blood, but can be interpreted incorrectly only when the new cells, with a different haemoglobin concentration, are released into blood circulation [38].

The fluctuation in neutrophils and lymphocytes could possibly be as result of antibodies released in the circulatory system of the fish as a form of defense. [6] stated that lymphocytes consist the majority of white blood cell present in peripheral blood of *O. Niloticus*. This study also showed neutrophils and lymphocytes increased as the concentration of the toxicant increased compared with basophils, eosinophils and monocytes.[18] reported lymphopenia on both neutrophils and eosinophils and also granulocytosis in *Heteropneustes fossils* after exposure to 96h LC_{50}. These changes in differential leucocytes count also give evidence for decreased level non-specific immunity in fish after the exposure to toxic substances (Svoboda *et al*., 2003).

Table 1: Haematological parameters of *Clarias gariepinus* exposed to acute toxicity of Atrazine

Conc. (mg/l)	Hb	WBC	PCV	RBC	MCV	MCH	MCHC
0.00	8.33±0.88[a]	23.6±0.35[f]	22.67±0.88[a]	246.5±0.058[a]	0.96±0.008[c]	0.33±0.015[d]	33.5±0.09[d]
0.45	6.5±0.15[b]	24.39±0.19[e]	17.67±0.88[b]	242.6±0.63[b]	0.773±0.17[d]	0.26±0.0115[e]	33.2±0.04[d]
0.50	6.3±0.115[c]	25.42±0.16[d]	16±0.577[c]	178.3±0.79[c]	0.92±0.012[c]	0.363±0.01[d]	35.9±0.024[c]
0.55	5.85±0.65[d]	26.57±0.09[c]	12.33±0.88[d]	126.6±0.55[d]	1.24±0.015[b]	0.46±0.0115[c]	36.8±0.046[c]
0.60	5.53±0.04[d]	27.64±0.02[b]	11.20±0.88[e]	113.9±1.76[e]	1.27±0.006[a]	0.51±0.011[b]	39.4±0.049
0.65	5.32±0.07[d]	28.85±0.028[a]	10.33±0.88[f]	97.2±0.83[f]	1.22±0.008[b]	0.55±0.017[a]	43.43±0.05[a]

Means with the same superscript along the columns are not significantly different (P>0.05)

Table 2. The Effect of Acute Dose of Atrazine on Some Leucocytes Differential Count of *Clarias gariepinus*

Conc.(mg/L)	Neutrophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)
0.00	125.33±0.882[a]	85.33±0.882[f]	ND	ND	ND
0.45	122.00±1.155[b]	91.30±0.882[d]	ND	ND	ND
0.50	116.67±0.882[c]	92.33±0.882[c]	ND	ND	ND
0.55	114.67±0.882[d]	94.00±0.577[b]	ND	ND	ND
0.60	112.33±0.882[e]	94.00±1.155[b]	ND	ND	ND
0.65	110.00±1.155[f]	98.33±0.882[a]	ND	ND	ND

Means with the same superscript along the columns are not significantly different (P>0.05); ND = Not Detected

The increase in opercular ventilation count and tail fin movement rate as shown in Fig 2, is attributed to the increase in the concentration of the toxicant. The result showed that the opercular count of the exposed fish at 12 and 24 hours were higher than that of the control fish. As the concentration of atrazine increased the beats of the operculum increases except at the 48th hour when the beats started to decrease. Further duration of exposure led to more decrease in the opercular ventilation count of the fish. By the 96th hour, the opercular ventilation count of the exposed fish were significantly (P<0.05) lower than those of the control group. Similar findings were reported by [31] on 96hr of exposure of Diazinon to *Clarias gariepinus*. [31] also observed that there were marked increase in the opercular and tail fin beats of the fish within the first 24 hours compared to that of the control, while further exposure resulted in the decrease of the opercular and tail fin beat.

The increase in tail fin movement rate is due to the increase in the concentration of the toxicant. The tail fin movement rate of the exposed fish to the toxicant at 12 and 24 hours were higher than the one in the control fish (Fig. 3). Even though the concentration of Atrazine increased the movement of tail also increases except at the 48th hours when the beat started to decrease. Similar results were obtained by [8] [11] [3].

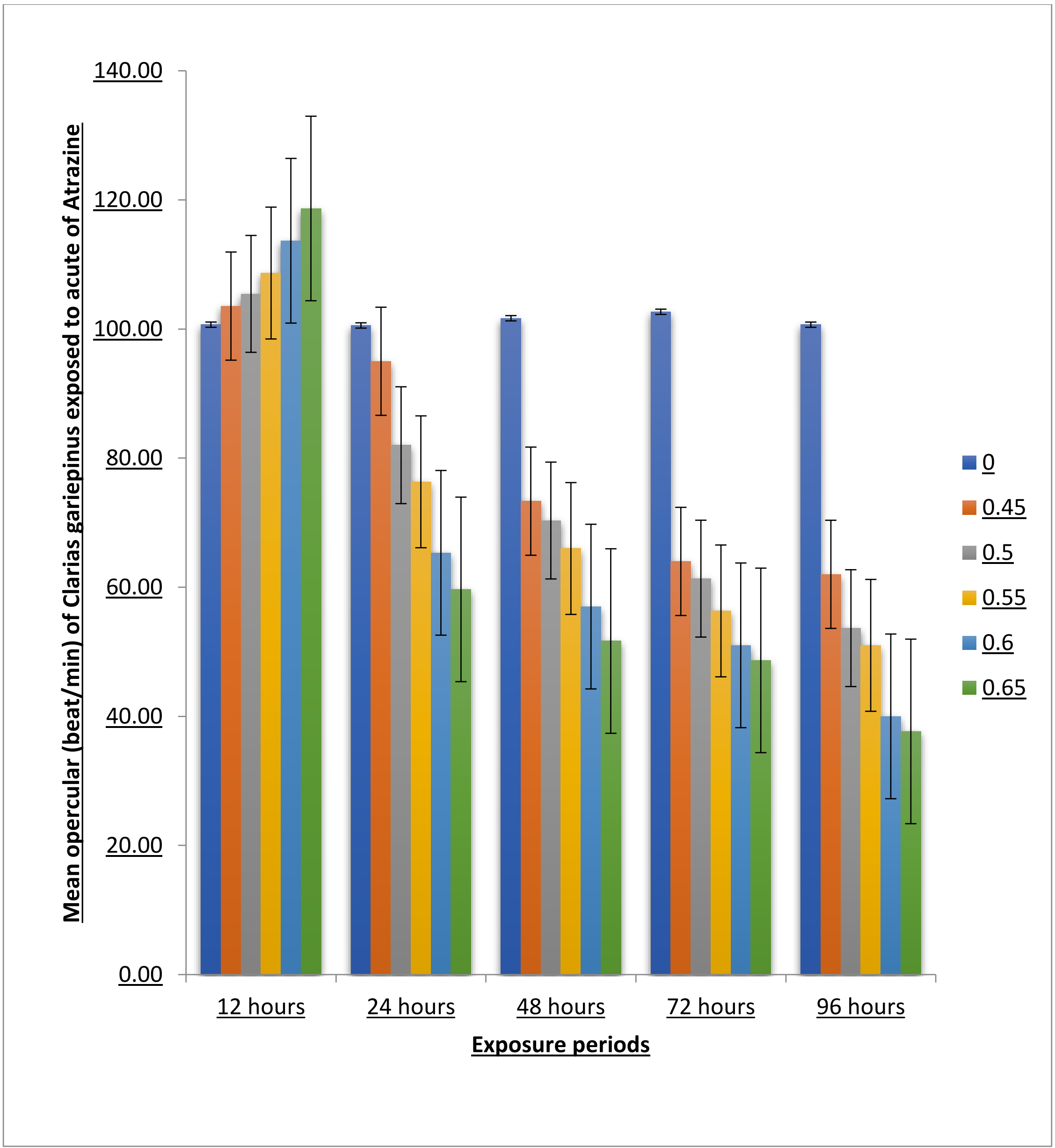

Fig. 2. Opercular ventilation rate of *Clarias gariepinus* exposed to acute concentrations of Atrazine.

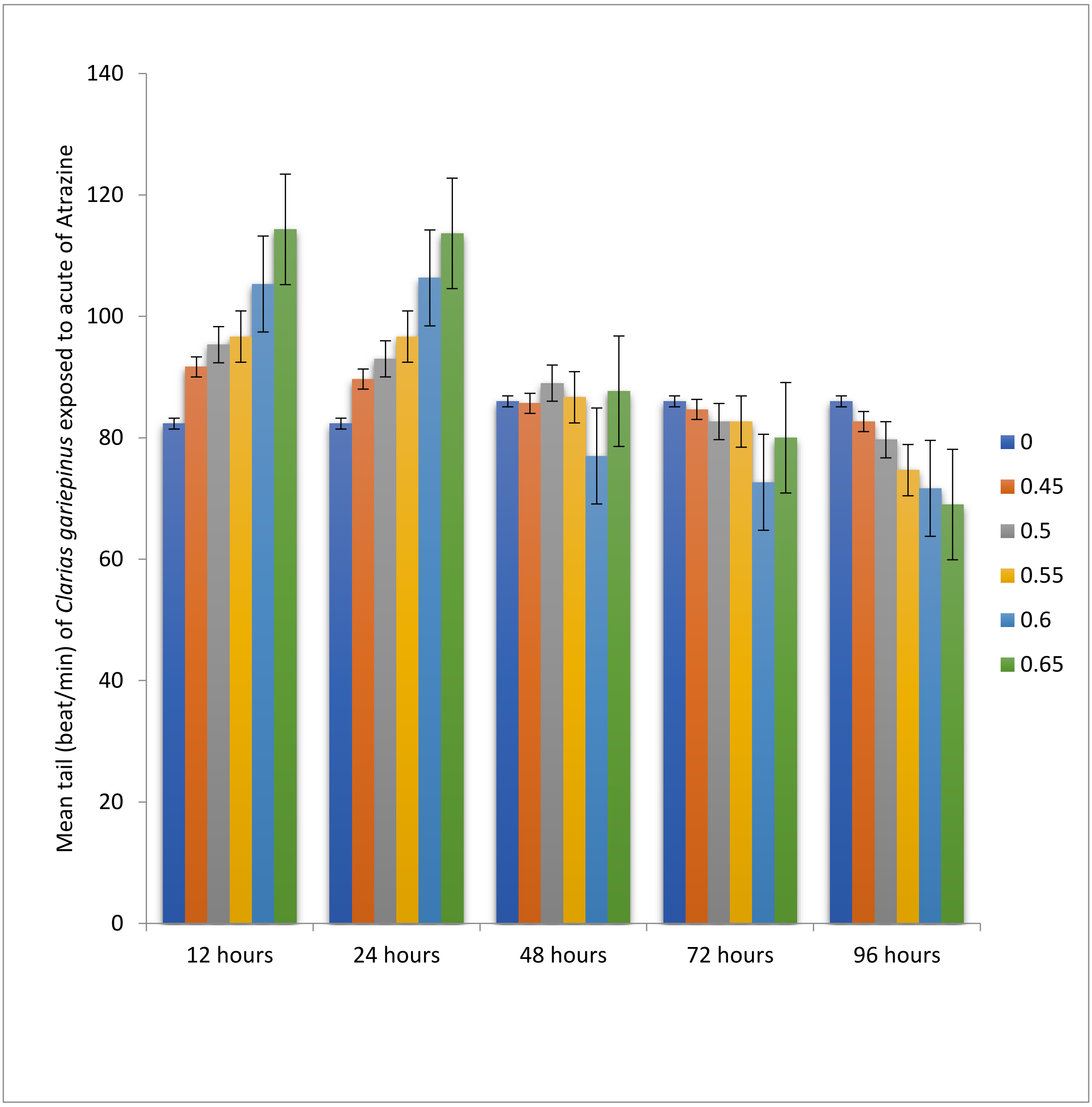

Fig. 3. Tail fin movement rate of *Clarias gariepinus* exposed to acute concentrations of Atrazine.

Fish mortality was observed in all the tanks except in the control tanks as presented in Table 3. The first mortality was observed at 24 hours at 0.45mg/L, 0.55mg/L, 0.60mg/L and 0.65mg/L of atrazine. Mortality rates and Log of concentration in *Clarias gariepinus* exposed to acute concentration of atrazine indicates that the concentration 0.65mg/L as having the highest number of mortality rate (15) and Log of concentration value (-0.187087) while concentration 0.45mg/L as having the lowest number of mortality rate (5) and Log of concentration value (-0.346787) with the control having the value 0.0000 respectively. The Lc_{50} 0f 96hrs of atrazine on *Clarias gariepinus* is 0.55mg/L as shown in Fig. 4.

Table 3: Mortality rates and Log of concentration of *Clarias gariepinus* exposed to acute concentration of Atrazine.

Concentration (mg/l)	Log. of Conc.	No. exposed	Mortality rates	% mortality	Probit kill
0.00	0.000000	20	0	0	2.4
0.45	-0.346787	20	5	25	4.3
0.50	-0.301030	20	8	40	4.7
0.55	-0.259637	20	13	65	5.3
0.60	-0.221849	20	13	65	5.3
0.65	-0.187087	20	15	75	5.6

Values representing mortality rates and values of log conc. at different concentration.

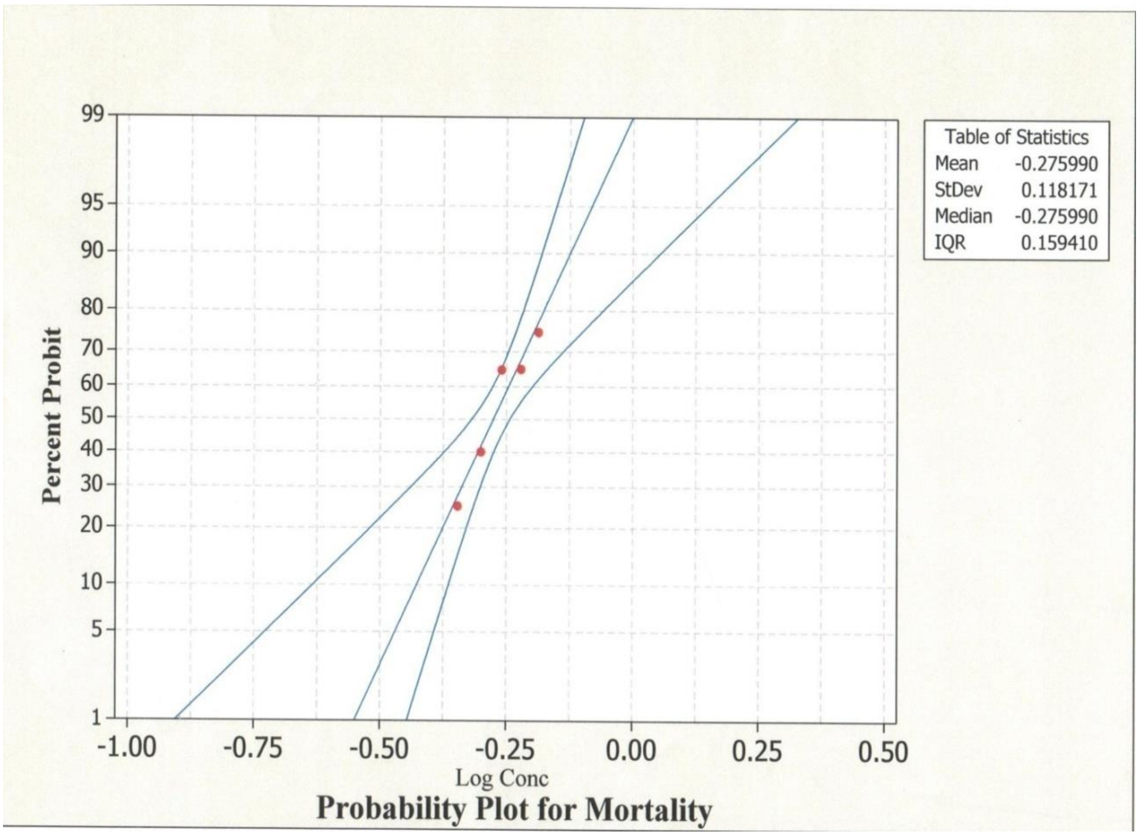

Fig. 4. Probability plot for mortality of Atrazine on juveniles of *Clarias gariepinus*

CONCLUSIONS

Clarias gariepinus exposed to atrazine elicited behavioural responses such as opecular ventilation movement and tail movement rate. Hence, atrazine is harmful to juveniles of *Clarias gariepinus* and resulted to altering of behaviour. Atrazine also resulted to marked reduction in the values of haemoglobin, erythrocyte count and packed cell volume and increased in white blood and lymphocytes in juveniles of *Clarias gariepinus*. It is thereby recommended that proper measures should be taken as regards to the discharge of chemicals or waste water arising from chemical industries into aquatic bodies to prevent the extinction of fish species.

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