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Research Article

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Lethal effect of Sniper (Organophosphate Insecticide) on *Clarias gariepinus* Fingerlings and Post fingerlings under laboratory condition.

Uedeme-Naa, B[1]; John-Amadi, S.V[1]; Opara, J.Y[2] and Godwin R. R[3]

[1]Department of Animal and Environmental Biology. Rivers State University, Oroworukwo-Nkpolu. Rivers State, Nigeria.
[2]African Regional Aquaculture Centre (ARAC), Aluu. Port Harcourt, Rivers State.
[3]Department of Fisheries, Faculty of Agriculture, University of Port Harcourt, P.M.B 5323, Rivers State, Nigeria.
*Corresponding author: barikpoa.uedeme-naa@ust.edu.ng

Abstract The toxicity of sniper - containing 2, 3-dichlorovinyl dimethyl phosphate (DDVP) on fingerlings and post fingerlings of *Clarias gariepinus* (African catfish) was investigated in a static bioassay for 96 hours. 100 pieces of fingerlings and 100 post fingerlings fishes weighing between 1.2g – 1.4g were obtained from a fish farm and transported to the Laboratory where they were kept for seven days to acclimate. A range finding test was conducted prior to the main test which had the following concentrations (0.0 – control, 0.10, 0.05, 0.025. and 0.0125mg/l) of Snipper in each static tank respectively. 10 *Clarias* fish were introduced into each static tank containing 20 litres of water about 30 minutes after treatment with the toxicant and observed for 96 hours. Data on fish mortality as well as the physico-chemical parameter (temperature, pH, dissolved Oxygen, nitrate) of water were recorded. The water quality parameters in the treatment tanks showed no significant difference with that of the control. Behavioral responses in the fishes include erratic and uncoordinated swimming which were observed to be more pronounced at higher concentration and time. Bleached body was the only external change observed and this was more pronounced in the fingerlings in higher concentration. The 96-hr LC_{50} for fingerling determined by probit analysis was 0.041mg/L. The result indicated that DDVP is highly toxic to fingerlings and post fingerlings of *Clarias gariepinus*. As such, caution should be exercised in the use and disposal of unused cans of sniper close to aquatic- systems.

Keywords 2,3-dichlorovinyl dimethyl phosphate (DDVP), Clarias gariepinus, probit analysis

1. Introduction

Organophosphate compounds are a diverse group of chemicals used in both domestic and industrial settings, examples of which include insecticide, nerves gases and anti helminthes. Over a hundred organophosphate compounds representing a variety of chemical, physical and biological properties are in commercial use [1]. Organophosphate insecticides are common household product in Nigeria, where they are used mainly to control mosquitoes and other household pest such as cockroaches and bedbugs [2]. The most popular source of organophosphate insecticide/pesticide in Nigeria is locally made variety called 'otapiapia'. The active ingredient in 'Otapiapia' is dichlorvos or 2 ,2 – dichloro vinyl dimethy phosphate (DDVP). Sniper', which is another common variety of DDVP has a more refined packaging and is available in stores nationwide at a higher cost. Dichlorvos (Sniper) is used as agricultural insecticide on crops, stored products, and animals. It is estimated worldwide that 60% DDVP is used in plant protection, 30% for public health and vector control and 10% for protection of stored

product. In Nigeria, agrochemicals that contains pesticides especially chlorinated hydrocarbons and the organophosphates, are routinely employed as part of the family practice to protect crops and animals from insects, weeds and diseases thus, toxicity test become imperative to estimate potential hazards as part of risk assessment protocols in agriculture, especially in fish farming [3].

Pesticide contaminated water will have undesirable effects on fish and other aquatic life biota, runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream [4]. Herbicides can accumulate in water bodies at levels that kill zooplankton, the main source of food for young fish [5]. The toxicity of some chemicals can also be enhanced or mitigated in the presence of other chemicals [6]. Toxicity test are performed for the specific purpose of predicting what biological functions would be perturbed by the toxicant exposure or explicitly to quantify the effect of a toxicant on the health of an organism [2]. This work aims at investigating the effects of organophosphate insecticide (snipers) on the wellbeing of aquatic organisms particularly on two life stages of African catfish *C. gariepinus*. Acute levels of organophosphate (sniper) products and their mixtures have shown to be toxic to early life stages of fishes, producing mortality and various abnormal behavioural changes which may be deleterious to the survival [7]. Results from investigations involving the use of fish species support the concept that toxicant induced stress on organisms can be quantified by methods other than mortality [8],[9],[10] Hence, changes in fish behaviours can be used as a sensitive indicator of acute and sublethal toxicant exposure. The studies involving the clarids seem to suggest that changes in operculum beat frequency (OBF) and tail beat frequency (TBF) of fish exposed to various toxicants is directly related to toxicant concentration but inversely related to times of exposure [11] and could be used to monitor the negative effects of herbicide application before mass mortality that may result from indiscriminate use of the herbicide.

The use of pesticides to control pests (insects) has been recognized as a part of agricultural practice throughout the world. Unfortunately, the indiscriminate use of these pesticides to improve agricultural production and yield have negative impacts on non-target organisms, especially aquatic life forms and their environment. Pesticides-surface runoff into rivers and streams can be highly lethal to aquatic life [11]. Pesticides can accumulate in water bodies at levels that kill zooplankton, the main source of food for young fish. Accidental spills and dumpsites also account for a part of the environmental Pesticides input [5]. In addition to killing the organisms, some pesticides can have negative but non-lethal effects on individual organisms and populations, such as reduced reproduction, reduced mobility to escape predation, or alternations in behavior [6]. Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety [12]. The application of environmental toxicology studies on non-mammalian vertebrates particularly fish is rapidly expanding, for the evaluation of the effects of environmental contamination by noxious compounds [3]. Genotoxic evaluation of pollutants in fish is of great concern because of their potential adverse effects on human health after consumption. There is also a great susceptibility of the liver of fish to be damaged as a result of its primary role of metabolism of these foreign substances [11].. Liver damage alters the lactate dehydrogenase and glutamic oxaloacetic transaminase activities which are two of the most important liver function test enzymes [13]. Thus toxicity tests or studies are essential for determining sensitivity of animals to toxicants and also useful for evaluating the degree of damage to target organs and the consequent physiological, biochemical and behavioural disorders. To supplement risk assessment studies of these pesticides, it is important to obtain information on their toxicity and effects on some local species. *Clarias gariepinus* belongs to the claridae family and it is geographically located in Africa (Nigeria), the Middle East, Brazil and Indonesia. They make fresh water, lakes, rivers and swamps and human made oxidative ponds and urban sewage system their habitats. The adult catfish can be 1 - 1.5m in length and weigh up to 60kg with flat body, head, broad terminal mouth with four pairs of barbells and large accessory breathing organs made up of modified gill arches. *Clarias gariepinus* is a popular species in fresh water aquaculture [14]. It is widely distributed and accepted by many farmers in Africa because of its fast growth, large size; low bone content, hardiness high yield, tolerance to poor water quality, omnivorous feeding habit, fine flavor, adaptability to overcrowding, high market value and has been successfully propagated artificially thereby making its fry and juveniles easily available [15].

2. Materials and Methods

Fish (*Clarias gariepinus)*, sniper-insecticide, plastic transparent containers, digital scales, normal fish feeds, borehole water. The fish were acclimatized to laboratory condition for 7 days and fed with top fish feed with crude protein of 35% on daily basis. The container was cleaned and the water changed every morning. One Hundred fingerlings and one hundred post fingerlings of *Clarias gariepinus* were obtained from a private fish farm known as Amadi fish farm from Igwuratali, Port Harcourt, Rivers State and transported in 25 litre of jerry can to the fisheries Demonstrations farm at Choba, University of Port Harcourt, where they were distributed to 10 fish per aquarium in fifteen rectangular aquarian tank filled with 20 litres borehole water. For the present study a commercial formulation of Dichlorvos (1000g/L), with the trade-name sniper 1000EC DDVP manufactured by Forward (Beihaj) Hepu Pesticide Co. Ltd. Guangxi China, was used as the toxicant or Stock Solution. The sniper-insecticide was purchased at Mile 3 Market, which is one of the most popular markets in Port Harcourt. Only one insecticide was used in the investigation. The control solution was made up of only borehole water. The experiment was conducted using standard static bioassay procedure [16]. This Involved controlled environmental conditions as to define the response of the test organism to Sniper (DDVP) insecticide. Sniper (DDVP) insecticide with trade name sniper was used for this experiment.

Range Finding Text (Trial Test)

The preliminary tests to determine the range of concentrations used in this experiment were performed by exposing 10 *Claris gariepinus* catfish to 20 liters of water containing 0.1, 0.05, 0.025, 0.0125mls of organophosphate insecticide (sniper) respectively for 96 hours until suitable concentration that produced motality was obtained. The fish were not fed for 24 hours before and during the exposure time.

Experimental Procedure

The two different stages of *Clarias gariepinus* were exposed to varied concentrations of 0.1m, 0.05m, 0.025m and 0.0125 of sniper in 20 liters of water. The mortality rate, behavioural characteristics of the catfish and the physico-chemical properties of water such as pH, temperature, nitrate, ammonia and dissolved oxygen were analyzed after 96hours period. The 96 hours lethal concentration of insecticide (sniper) was determined following the probity analysis method. Dead fishes were removed from the experimental tank every morning to avoid contamination at every 24hours interval for 96hours.

Determination of Temperature

Temperature was determined using the mercury-inglass thermometer, which was inserted in water and the temperature reading was taken after few minutes.

Determination of Hydrogen Ion

Concentration of pH was determined using a Jenway (R) type of pH meter (Model 3015). The probe was first inserted in the buffer for 5 minutes to standardize the meter to pH 7, thereafter, it was dipped into the water and the static pH was read after 60 seconds.

Determination of Dissolved Oxygen

Dissolved oxygen (DO) was measured with digital Dissolved oxygen meter which was done by inserting the tip of the digital dissolved oxygen meter into the aquaria containing the water and removed after 10 minutes.

Exposure of Fish to Acute Concentrations of Sniper to Determine Fish Behavioural alterations and 96hr LC_{50} Values

Test was conducted in the laboratory (static bioassay) following [17] guidelines to determine the toxicity of Sniper to *Clarias gariepinus*. From freshly prepared stock solutions, four concentrations of 0.1, 0.05, 0.025, 0.0125 mg/L for DDVP was dispensed with a 10ml measured Syringe repeatedly using serial dilution to get the desired concentration levels separately, and inserted inside the 60L tanks containing.

Borehole water. Ten (10) fishes were randomly distributed into each test tank. Survival and mortality rate were noted during this period. Fishes were considered dead when the opercula movement ceased and there was no response to gentle prodding. This was used as a measure of mortality. One fish species (*Clarias gariepinus*) was used for the study. The insecticide was administered at four levels of concentration except the control. Appropriate volumes of stock solution were dispersed using syringe to measure appropriately into the 60l tank containing

borehole water and measuring into each of the tanks except the control. The fingerlings and post fingerlings were exposed to nominal concentrations of toxicants for 96hrs.

3. Results and Discussion

Physico-chemical parameters of the experimental water

The mean physicochemical parameters of the experimental water from the study after 96 hours are presented in Table 1. The concentration of Ammonia varied between 0.15±0.00 in 0.1 ml/l to 0.60±0.00 in the control. The highest pH value of 6.70±0.00 was recorded in the control and the least value (6.00±0.00) was in the 0.1 and 0.05 concentration of the treatments. The Dissolve Oxygen (mg/l) varied between 2.00 ± 0.0 and 4.00 ± 0.0 with significant difference in the treatment. The highest value was recorded in control while the least was recorded in 0.1 ml/l concentration of the test chemical. The Nitrate values were not significantly different (P>0.05) between the treatments. The temperature values ranged between 27±0.00 to 28±0.00°C.

Table 1: Mean Physicochemical parameters of the experimental water

Conc. (ml/l)	Ammonia	pH	DO	Nitrate	Tempt
Control	0.60±0.00 [a]	6.70±0.00 [a]	4.00 ± 0.0 [a]	0.01±0.00 [a]	28±0.00 [a]
0.0125	0.40±0.00 [b]	6.40±0.00 [ab]	3.00 ± 0.0 [ab]	0.01±0.00 [a]	27±0.00 [b]
0.25	0.40±0.00 [b]	6.40±0.00 [ab]	3.00 ± 0.0 [ab]	0.01±0.00 [a]	27±0.00 [b]
0.05	0.15±0.00 [c]	6.00±0.00 [b]	3.00 ± 0.0 [ab]	0.01±0.00 [a]	27±0.00 [b]
0.1	0.15±0.00 [c]	6.00±0.00 [b]	2.00 ± 0.0 [b]	0.01±0.00 [a]	27±0.00 [b]

*Means with the same superscript down the column are not significantly different.
**Means with different superscripts down the column are significantly different.

The number of mortalities recorded for the fingerlings stage of the test fish sample exposed to different concentrations of the test chemical is presented in table 2. There was statistical significance (P<0.05) in the number of mortalities observed in the different concentrations from 24 hours to 96 hours and highest mortality was recorded in the 0.1ml/l concentrations of the test chemical. There was an increased cumulative mortality within the 96 hours exposure time of the test chemical with increase in concentrations of the test chemical (Figure 1 and 2). The Median lethal concentration of (LC_{50}) of 0.025 ml/l was recorded after 96 hours of exposure. The LC_{50} and the acute toxicity test after exposing the fish to the test chemical recorded the linear and regression equation with lower 95% and upper 95% values of 0.015 % and 0.041 % respectively (Table 3). The Plot of Log of Concentration Versus Probit at 96Hrs exposure to the toxicant are represented in figure 3.

Table 2: The Mortality Rate for fingerlings

Conc. (ml/l)	24hrs/day1	48hrs/day2	78hrs/day3	96hrs/day4
0.0125	1.00 ± 0.58 [a]	2.0 ± 0.71[c]	3.3 ± 0.71[a]	3.3 ± 0.0 [c]
0.025	0.00 ± 0.33 [b]	3.5 ± 0.71[b]	4.3 ± 0.0 [b]	6.8 ± 0.71 [b]
0.05	0.00 ± 0.00 [b]	3.8 ± 0.51[b]	5.9 ± 1.15 [ab]	7.9 ± 0.0 [b]
0.1	1.00 ± 0.00 [a]	4.5 ± 0.50 [a]	6.2 ± 0.08 [a]	9.7 ± 0.0 [a]

*Means with the same superscript down the column are not significantly different
**Means with different superscripts down the column are significantly different.

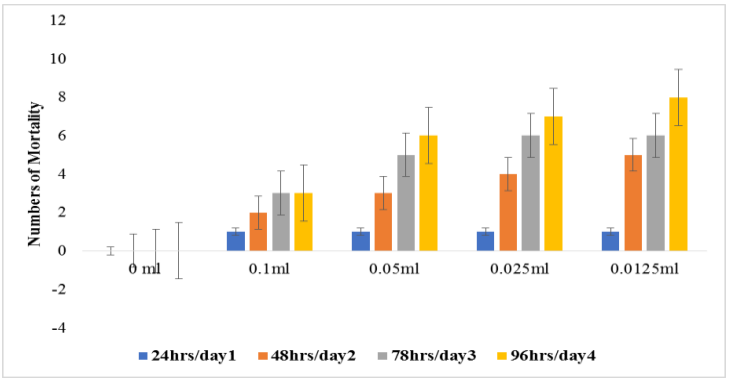

Figure 1: Variations in the numbers of Fingerlings Mortality From 24 hrs to 96 hrs

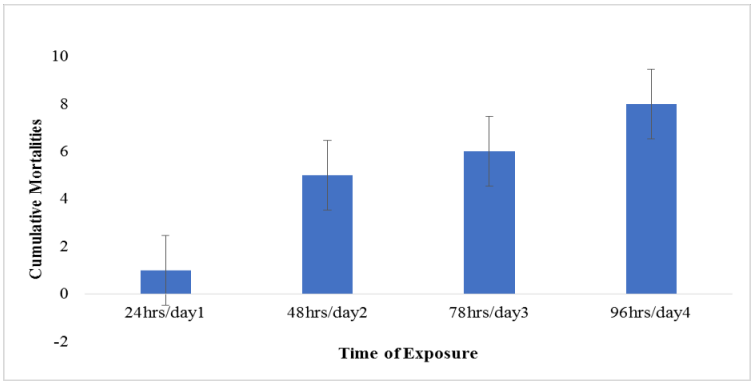

Figure 2: Cumulative Mortalities of the Fingerlings stage of C. gariepinus exposed to different concentrations of the test treatments.

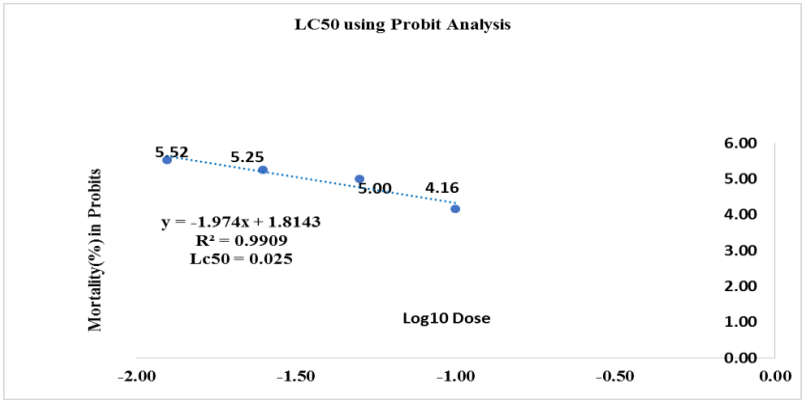

Figure 3: The Plot of Log of Concentration Versus Probit at 96Hrs exposure of the fingerlings stage of C. gariepinus exposed to different concentrations of the test treatments.

Table 3: The LC_{50} and the Acute Toxicity Test After exposing the fingerlings stage of *C. gariepinus* exposed to different concentrations of the test treatments.

Time (hrs.)	LC_{50}	Lower 95%	Upper 95%	Regression Equation
96	0.025	0.015	0.041	y = -1.974x + 1.8143 R^2 = 0.9909

The number of mortalities recorded for the post fingerlings stage of the test fish sample exposed to different concentrations of the test chemical is presented in Table 4. There was no significant variation (P>0.05) in the number of mortalities observed in the difference concentrations after 24 hours but some variation (P<0.05) was observed from 48 to 96 hours. The highest mortality was recorded in the 0.025ml/l concentrations of the test chemical. An increase in the cumulative mortality after 96 hours exposure time of the test chemical with increase in concentrations of the test chemical was also observed as seen in (Figure 4 and 5). The Median lethal concentration of (LC_{50}) of 0.035 ml/l was recorded after 96 hours of exposure. The LC_{50} and the acute toxicity test after exposing the fish to the test chemical recorded the linear and regression equation with lower 95% and upper 95% values of 0.023 % and 0.055 % respectively (Table 4). The Plot of Log of Concentration Versus Probit at 96Hrs exposure to the toxicant are presented in Figure 6.

Table 4: The Mortality Rate for post fingerlings.

Conc. (ml/l)	24hrs/day1	48hrs/day2	78hrs/day3	96hrs/day4
0.0125	0.00 ± 0.00 [a]	1.00 ± 0.67 [a]	0.3± 0.30 [c]	2.3 ± 1.00 [b]
0.025	0.00 ± 0.00 [a]	0.00 ± 0.00 [b]	1.7± 1.70 [b]	2.0 ± 1.30 [b]
0.05	0.00 ± 0.00 [a]	0.00 ± 0.00 [b]	1.3± 1.07 [b]	3.0 ± 0.60 [b]
0.1	0.00 ± 0.00 [a]	1.60 ± 0.70 [a]	3.3 ± 0.62 [a]	6.0 ±0.00 [a]

*Means with the same superscript down the column are not significantly different
**Means with different superscripts down the column are significantly different.

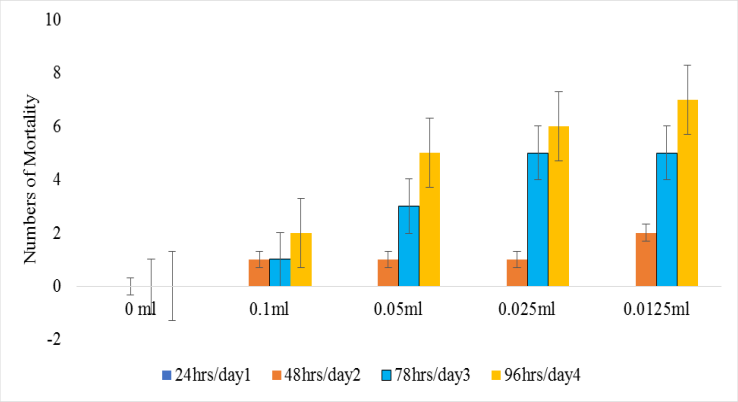

Figure 4: Variations in the numbers of Post Fingerlings Mortality from 24 hrs to 96 hrs.

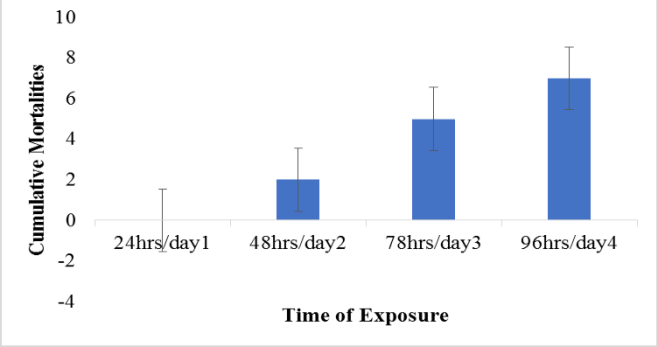

Figure 5: Cumulative Mortalities of the Post Fingerlings stage of C. gariepinus exposed to different concentrations of the test treatments.

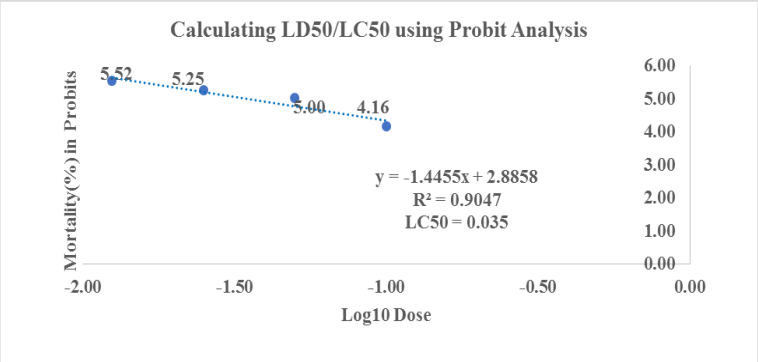

Figure 6: The Plot of Log of Concentration Versus Probit at 96Hrs exposure of the fingerlings stage of C. gariepinus exposed to different concentrations of the test treatments.

Table 5: The LC_{50} and the Acute Toxicity Test After exposing the fingerlings stage of *C. gariepinus* exposed to different concentrations of the test treatments.

Time (hrs.)	LC_{50}	Lower 95%	Upper 95%	Regression Equation
96	0.035	0.023	0.055	y = -1.4455x + 2.8858 R^2 = 0.9047

The lethal toxicity test showed that all the fish in the control medium survived while mortalities were observed in all treatments. The mortality increased with increasing concentration of the toxicant in water showing a dose – response relation which has been reported by many authors including [18]; [19] and [20] Mucus observed in the gills of the dead fish might be responsible for the mortality recorded in this study. [21] reported that accumulation of mucus on gills reduces respiratory activity because of the inability of the gill surface to actively carry out gaseous exchange, thus the recorded mortalities. The result of the water quality of media was within the optimal range recorded [22]; [19] as requirements for *Clarias gariepinus* culture implying that the parameters did not seem to influence the toxicity of the pesticide to the test fish. Mortality observed during the acclimation period must have been due to stress at handling and transportation from the fish farm to the experimental laboratory, similar observations were made by [21]. [1], reported that behviour provides a unique perspective, linking the physiology and ecology of an organism and its environment and allows the organism to adjust to external and internal stimuli to best meet the challenges of surviving in a changing environment. The results showed that sniper insecticide affected the normal behavioures of the two life stages of *C. gariepinus.* The control specimens were not hyperactive and showed normal swimming patterns, skin colour, equilibrium status and caudal movements throughout the exposure period. The exposed fish to DDVP insecticide (sniper) showed initial stress response such as gasping for air, abnormal swimming, restlessness, slow in swimming, bleached body with lesions. [23] reported erratic swimming, loss of equilibrium, resting, motionless at the bottom of the bowl and caudal bending. It was observed in this investigation that mortality of the exposed fish is not only due to impaired metabolism, but could in addition be due to nervous disorder. This is similar to the findings of

[1]. The LC_{50} of 0.025mg/L obtained in this investigation is in close affinity to the 0.58mg/L and 0.035mg/L reported by [24] when Grass carp and Zebra fish were respectively exposed to mercury. The observed LC_{50} in this study is however quite low when compared to that recorded by [25] . The LC_{50} value of 0.09mg/L reported by [26] is comparatively lower than the recorded LC_{50} value in this report. It is equally higher than the 24, 48, 72 and 96h LC_{50} values of 0.015, 0.041, 0.025 and 0.055ppm respectively as reported by [27]. Thus, revealing the insecticide sniper as a pesticide of high toxic potential. The variations in theLC_{50} values from different reports are attributable to the use of different toxicants concentrations as well as test organisms.

4. Conclusion

The abnormalities in behaviours and mortalities of *Clarias gariepinus* as a result of exposure to the sniper insecticide under laboratory conditions suggest that the use of Dichlorvos by farmers and fisher folk for domestic insecticide be curtailed or restricted so as to preserve the lives of non-target organisms, especially the fishes in the water bodies. Therefore, care should be taken during the use and discarding of unused cans of sniper especially close to aquatic environment.

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