

## TOXICITY ASSESSMENT OF THREE PESTICIDES ON AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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The toxic effects of dragon (A), sniper, (B) and kartodim 315ec, (C) on test specimens (*Clarias gariepinus*) of average weight  $56.4 \pm 31.1$  g and length  $18.5 \pm 3.50$  cm were examined. These pesticides have pervaded the markets, and have become common items in farms, homes, and food storage houses, hence are indiscriminately employed, mostly by rural and urban farmers and sometimes members of the society. Thus, they are potential environmental contaminants, and pose threat to the wellbeing of man and animals especially aquatic organisms. To determine their impacts in the environment, their respective acute toxicity tests were carried out according to the static non-renewable bioassay procedure. The experimental design consisted of a set of five concentrations [100, 200, 300, 400, 500 mg/L, and a control set up (0 mg/L) of pesticides A, B, and C (dragon, sniper, and kartodim 315ec respectively] with two extra replicate concentration and control for each set; in separate 30 L capacity calibrated rectangular tanks, each filled up to the 15 L mark. Each tank was distinctively labeled and loaded with 10 tests organism, making a total of 540-fish. The 96 h  $LC_{50}$  of pesticide B, (that is sniper) was found to be 27.0 mg/l. The 96 h  $LC_{50}$  of A and C are less toxic as their respective 96 h  $LC_{50}$  was found to be 53.8 and 41.4 mg/l. The parameters considered include cumulative average value of operculum movement and tail beat frequency, and cumulative number of discoloration, erratic swimming, and mortality for each set of concentrations of the test substance: dragon, sniper, and kartodim 315ec respectively. The result shows that the lethal effect of the pesticides A, B, and C on the fish depends on concentration and duration of exposure to the substances as observation shows that the cumulative average number of discoloration, erratic swimming, and mortality increases with increasing concentration and exposure time, while the cumulative average of operculum movement and tail beat frequency decreases with increasing concentration and exposure time.

**Key words:** 96 h  $LC_{50}$ , *Clarias gariepinus*, dragon, sniper, kartodim 315ec.

### INTRODUCTION

Pesticides and herbicides at high concentration are known to reduce the survival, growth and reproduction of fish, and produce many visible effects on fish (Rahman et al., 2002).

Examples of harmful practices include uncontrolled application of herbicide, insecticide and pesticides amongst other factors. Terrestrial and aquatic environmental contaminations often result from diverse activities of man in the environment. The Food and Agricultural Organisation (FAO) estimates that about one

billion people world-wide rely on fish as their primary source of animal protein (FAO, 2000). Fish culture is one of the fastest growing sectors of the world's animal production with an annual increase of about 10% (FAO, 1997). *Clarias gariepinus*, also known as African mud catfish, is the most popularly cultured fish in Nigeria (Sogbesan and Ugwumba, 2006). However the healthiness and continued existence of fish, and other aquatic organisms are constantly threatened by man's indiscriminate use of harmful substance such as herbicide and pesticides, etc. on their habitat. Herbicides are

widely used for the control of water plants which may impede the flow of aquatic life and may contribute long term effects in the environment (Annue et al., 1994). The constant flow of agricultural effluents into fresh water often leads to a variety of pollutant accumulation, which becomes apparent when considering toxic pollution (Mason, 1991). Surface runoff of pesticides into rivers and streams can be highly lethal to aquatic life.

Herbicides can accumulate in bodies of water to levels that kill off zooplankton, the main source of food for young fish.

Accidental spills and dumpsites also account for a part of the environmental pesticide input. In contrast to many other man-made chemicals present in the environment, pesticides is deliberately spread into the environment.

Pesticides are a group of toxic compounds used by humans that have a profound effect on aquatic life and water quality (Luskova et al., 2002). They are beneficial chemicals in that they can be used to protect against forest and farm crop losses and can aid more efficient food production. They are used to prevent or stop the spread of destructive and nuisance organisms. The disadvantages of pesticides include their toxicity to humans, animals, useful plants and their persistence in the environment.

To sustain a substantial rate of increased production, a matching increase in habitat and environmental protection and toxicant monitory, regulation and control guided by a proper probit analysis and bioassay result is imperative.

Bioassay is the estimation or determination of concentration or potency of physical, chemical, or biological substance (agent) by means of measuring and comparing the magnitude of the response of the organisms with that of standard over a suitable biological system under standard set of conditions. It is the determination of the relative toxicity of toxicants by studying and examining their effects on living organisms. It involves the application of stimulus to a subject and the observation of the subjects' response to the stimulus. The stimulus may be a chemical, fungicide, or any substance having the potential to harm the living organism (subject) being

studied. The intensity of the stimulus may be varied by varying the dose which the organisms are subjected to. The dosage can be measured in terms of weight, volume, or concentration. The subject may be a plant, an animal, insects, a bacteria culture, etc.

Probit analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. This is done by testing the response of an organism under various concentrations of each of the chemicals in question and then comparing the concentrations at which one encounters a response.

Contamination of water bodies often result from direct use of harmful substance on aquatic environment, and run-offs from land into water bodies hence, acute toxicity test, a test method used in determining the concentration of substance that produces a toxic effect on a specified percentage of test organisms in a stipulated period of time was considered in this work. Death and some other factors were used as a measure of toxicity.

Uncontrolled and indiscriminate application of pesticides tends to contaminate the environment via run offs from urban/rural agricultural lands and coastal shores into water bodies. Thus, the potential accumulation, resuspension and bioavailability of toxicant in run offs which may be taken up by fishes and other aquatic organisms necessitates the need to assess potential source and adverse effects of aquatic contamination and control or address such.

Generally, in order to determine and evaluate the effects of potential toxic chemicals on aquatic organisms aquatic toxicity tests are usually carried out. Because chemicals have varying degree of toxicity on organisms, often times the objective of toxicity tests is to identify chemicals and their threshold value at which notable adverse effects on aquatic organisms is recorded. These tests provide a database that can be used to assess the risk associated with a situation in which the chemical agent, the organism, and exposure conditions are defined, and assist in monitoring and control of potential toxicants. During toxicity tests, certain factors of interest are usually monitored as bio-indicators of harmful effects of the chemical substance on

the aquatic organisms.

According to Kock et al. (1996), fishes are widely used to evaluate the health of aquatic ecosystem and their physiological changes serves as biomarkers of environmental pollution. *C. gariepinus* is the most widely used owing to its hardiness and ability to tolerate both well and poorly oxygenated waters. The main objective of the article is to investigate the toxic effects of three different pesticides commercially known as Dragon (A), Sniper (B) and Kartodim 315ec (C) on *C. gariepinus* a fish that is commonly consumed in Africa by determining their respective 96-h LC<sub>50</sub> values.

## MATERIALS AND METHODS

Six hundred healthy specimens of catfish (*C. gariepinus*) of either sex were purchased on order from African Regional Aquaculture Centre, (ARAC) at Allu, Port Harcourt, Rivers State and stored in a large aquarium in ARAC. The fish were kept three weeks for proper acclimatization, and unfed two days prior to exposure to the pesticides.

Then, about 100 fish specimens were randomly selected, and their weight and length measured and recorded in order for determination of the average weight and length of the fishes, which were  $56.4 \pm 31.1$  g and  $18.5 \pm 3.50$  cm respectively. After this, 540 experimental fish and 10 extra fish were selected at random and kept in a static system of water.

For dose-response test of dragon (that is pesticide A) on the fish specimens, ten fish each were randomly selected and placed in six separate 30 L capacity calibrated rectangular tanks, with two replicates per tank, and, each filled up to the 15 L mark. Each tank was distinctively labeled to represent a set of five concentrations (100, 200, 300, 400, 500 mg/l, and a control set up (0 mg/l) of substance A for each tank and their respective replicates. Total of 180 fish were loaded at ten per tank, and exposed to different concentrations of contaminants, labeled as described below (page 4).

For dose-response test of pesticide B, and C on the fish specimens, same loading, and

set of concentration and control as in A was carried out.

In all cases, the feeding was stopped two days prior to the exposure of the fish to pesticides A, B, and C respectively and the fish were not fed throughout the test.

The acute toxicity tests were performed according to the static non-renewal bioassay procedure.

The experimental design consisted of a control and a set of five concentrations (100, 200, 300, 400, 500 mg/L) of pesticide A, two replicates per set with ten fish per tank in each replicate. Same design set up as for substance A was adopted for substance B, and C, each set up having a total number of 180 fish, making 540 fish in all. It is noteworthy to mention that in this study, highest concentration that is 500 mg/l stock concentration =  $0.5 \text{ g/l} = (100/3) \text{ mg/l}$  tank solution which represents 100% concentration.

For treatment with dragon (Pesticide A), we had: ( $A_1R_{1-3}$ , to  $A_6R_{1-3}$ ); with sniper, we had: ( $B_1R_{1-3}$  to  $B_6R_{1-3}$ ); and kartodim 315ec had: ( $C_1R_{1-3}$  to  $C_6R_{1-3}$ ) where ( $A_1R_2$ ), and ( $A_1R_3$ ) are the replicates of ( $A_1R_1$ ) having concentration of contaminant, i.e. dragon of  $A = 100 \text{ mg/l} = 0.1 \text{ g/l}$  each.

Also, ( $A_2R_2$ ) and ( $A_2R_3$ ) are the replicates of ( $A_2R_1$ ) each having concentration of contaminant  $A = 200 \text{ mg/l} = 0.2 \text{ g/l}$ . ( $A_6R_2$ ) and ( $A_6R_3$ ) are the replicates of ( $A_6R_1$ ) being the control tank of treatment (A). Same form of identification applies for treatment B, C and their replicates.

Each set up was regularly monitored at 12 h interval, and observations recorded throughout a period of 96 h. During the monitoring, fish showing no respiratory movement and response to touch (tactile stimulus) were considered as dead and removed immediately. Also, during the test, the fish exhibit behavioral changes as well as mortality which were regularly monitored and recorded cumulatively from 12 h throughout a period of 96 h. Some observed behavioral changes prior to death of the fish include low operculum movement rate, low tail beat frequency, as well as erratic swimming.

For each test, the 96 h LC<sub>50</sub> was

determined with probit analysis using the IBM-SPSS-20 statistical package.

**RESULTS AND DISCUSSION**

In order to compare the relative acute lethal toxicity of certain toxicants to organisms, the 96 h LC<sub>50</sub> values of the toxicants having often been used as a measure of the various toxicity of toxicants has proven useful. During each tests, observation shows that at onset, on exposing the fish to different concentrations of the toxicants A, B, and C, the fish exhibited anomalous behaviors in form of restlessness as well as rapid opercula and body movement. However, as the test progressed, observation reveals gradual discoloration, accumulation of mucus on the body surface of the fish, reduced opercula movement rate, loss of stability (erratic swimming), and finally the reduction in number (death) of the fish especially in tanks

having high dosage of the toxicants. At the end of the experiment, that is after 96 h, the cumulative mortality in each tank and the corresponding two replicates per level of concentration (L.O.C), per treatment, that is say contaminant-A was observed, recorded, and summed-up to represent the aggregate or cumulative mortality across the three tanks (L.O.C) per treatment with each contaminant. Thus, since the loading per tank or replicate is uniform that is (10 per tank or replicate), the aggregate or cumulative response (mortality) represents the total number of mortality recorded per population sample of 30 specimens. The mortality per population sample could be seen from Table 2. Table 1 represents the number of mortality per tank, or replicate per L.O.C. In the table, each entry in the separate brackets represents the mortality per tank or replicate having a loading of ten each.

Tables 2, 3, and 4 show their respective

**Table 1.** 96 h Cumulative mortality in tank replicates for each treatment.

Concentration (g/l)	Treatments for each set of concentration		
	Mortality in replicate treatment with A	Mortality in replicate treatment with B	Mortality in replicate treatment with C
0.0	(0, 0, 1)	(0, 0, 0)	(0, 0, 0)
0.1	(0, 0, 0)	(0, 1, 1)	(0, 0, 0)
0.2	(1, 0, 1)	(2, 2, 1)	(1, 1, 1)
0.3	(1, 1, 1)	(3, 4, 3)	(2, 2, 2)
0.4	(2, 2, 1)	(5, 6, 5)	(3, 4, 2)
0.5	(3, 3, 3)	(6, 7, 5)	(4, 3, 4)

**Table 2.** 96 h response of *C. gariepinus* on treatment with different dosage of pesticide A.

Stock conc. (mg/l)	Efflt conc. (mg/l)	Log <sub>10</sub> conc (mg/l)	Number of Subjects exposed	Cumulative Responses (mortality)	Expected responses	Residual	Probability
100	6.67	0.824	30	0	0.156	-0.156	0.005
200	13.33	1.125	30	2	1.303	0.697	0.043
300	20.00	1.301	30	3	3.369	-0.369	0.112
400	26.67	1.426	30	5	5.837	-0.837	0.195
500	33.33	1.523	30	9	8.351	0.649	0.278

**Table 3.** 96 h response of *C. gariepinus* on treatment with different dosage of pesticide B.

Stock conc. (mg/l)	Efflt conc. (mg/l)	Log <sub>10</sub> conc (mg/l)	Number of subjects exposed	Cumulative Responses	Expected responses	Residual	Probability
100	6.67	0.824	30	2	1.454	0.546	0.048
200	13.33	1.125	30	5	6.031	-1.039	0.201
300	20.00	1.301	30	10	10.823	-0.823	0.361
400	26.67	1.426	30	16	14.822	1.178	0.494
500	33.33	1.523	30	18	17.958	0.042	0.599

respective probit (probability unit) from the probit analysis of mortality-concentration data from each test.

The LC<sub>50</sub> and the corresponding 95%

confidence limits of Table 5 was extracted from the SPSS probit analysis as shown in Table 6. Table 5 is an edited extract imported from the SPSS probit analysis of

**Table 4.** 96 h response of *Clarias gariepinus* on treatment with different dosage of pesticide C.

Stock Concentration (mg/l)	Efflt Concentration (mg/l)	Log <sub>10</sub> Concentration (mg/l)	Number of subjects exposed	Cumulative Responses	Expected Responses	Residual	Probability
100	6.67	0.824	30	0	0.365	-0.365	0.012
200	13.33	1.125	30	3	2.430	0.570	0.081
300	20.00	1.301	30	6	5.537	0.463	0.185
400	26.67	1.426	30	9	8.805	0.195	0.294
500	33.33	1.523	30	11	11.827	-0.827	0.94

**Table 5.** Result of LC<sub>50</sub> including 95% confidence interval for treatment with A, B, and C.

Treatment with pesticide	LC <sub>50</sub> (mg/l)	95% Confidence limits		Chi <sup>2</sup>
		Lower	Upper	
A (Dragon)	53.80	37.50	218.0	0.811
B (Sniper)	27.01	22.30	36.30	0.719
C (Kartodim 315ec)	41.40	31.80	83.90	0.664

**Table 6.** LC<sub>50</sub> and the corresponding 95% Confidence interval for pesticide A, B, and C.

Probabiity	A Concentration (mg/l)			B Concentration (mg/l)			C Concentration (mg/l)		
	LC <sub>50</sub> Estimate	Lower Bound	Upper Bound	LC <sub>50</sub> Estimate	Lower Bound	Upper Bound	LC <sub>50</sub> Estimate	Lower Bound	Upper Bound
0.1	18.934	11.224	23.748	9.173	5.126	12.135	14.654	8.380	18.457
0.15	23.121	16.919	30.329	11.277	7.120	14.277	17.877	12.168	21.914
0.2	27.099	21.388	40.375	13.289	9.182	16.356	20.937	15.871	25.901
0.3	35.094	27.517	73.249	17.361	13.529	20.957	27.079	22.100	37.637
0.35	39.306	30.056	97.241	19.519	15.751	23.784	30.313	24.661	45.943
0.4	43.769	32.508	127.920	21.815	17.954	27.181	33.737	27.053	56.149
0.5	<b>53.807</b>	<b>37.484</b>	<b>218.309</b>	<b>27.006</b>	<b>22.305</b>	<b>36.346</b>	<b>41.432</b>	<b>31.774</b>	<b>83.938</b>
0.6	66.147	42.972	374.728	33.432	26.891	50.080	50.882	36.903	126.890
0.7	82.499	49.571	670.221	42.010	32.314	71.733	63.392	43.059	198.598
0.8	106.839	58.444	1326.801	54.882	39.664	110.332	81.990	51.376	336.802
0.9	152.911	73.256	3429.303	79.507	52.275	202.099	117.141	65.394	703.237
0.92	169.106	78.027	4478.594	88.227	56.436	239.759	129.484	69.945	865.115
0.95	205.603	88.166	7521.924	107.980	65.433	334.313	157.278	79.672	1293.900
0.99	358.294	124.586	32883.551	191.741	99.302	862.837	273.336	115.133	4070.057

toxicity of pesticide A, B, and C.

The study reveals that *C. gariepinus* is susceptible to lethal effects of each test contaminants A, B, and C, and that its susceptibility depends on concentration of the contaminants and time of exposure of the fish to each of the contaminants. Thus, there was a

corresponding increase in mortality, discoloration, and number of fish exhibiting erratic swimming. This trend is represented in Figure 1,2 and 3 from values presented in Tables 6 and 7.

In the table, the average value of operculum movement rate (per minute), and tail

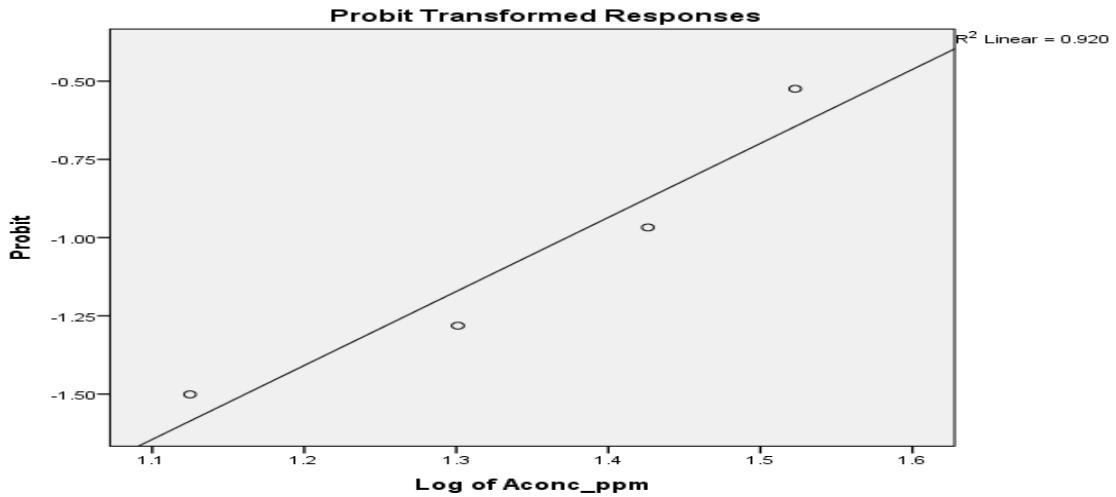


Figure 1. Plot of log<sub>10</sub> Conc. vs. probit of mortality for pesticide A.

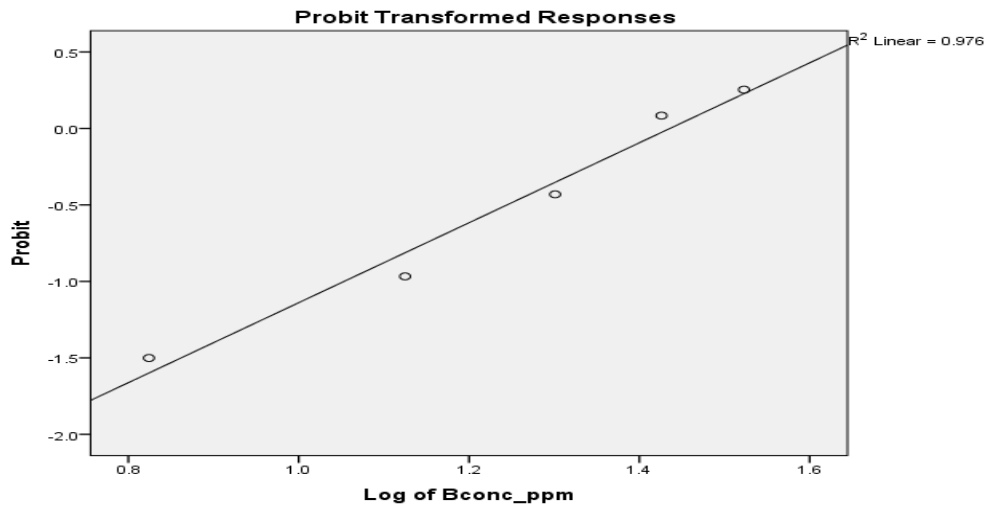


Figure 2. Plot of log<sub>10</sub> Conc. vs. probit of mortality for pesticide B.

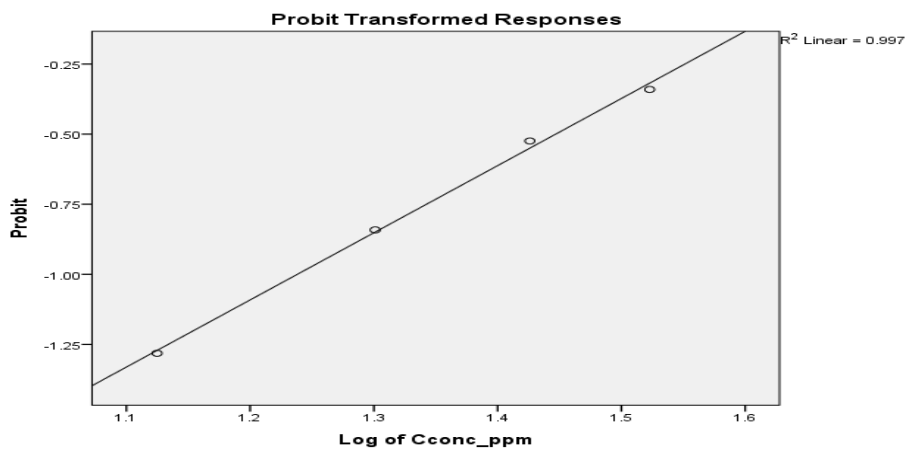


Figure 3. Plot of log<sub>10</sub> Conc. vs. probit of mortality for pesticide C.

**Table 7.** Numerical indices showing variation of mortality and other parameters with Concentration of pesticide A, B, and C respectively.

Parameters	Numerical indices of monitored parameters in A						Numerical indices of monitored parameters in B						Numerical indices of monitored parameters in C					
	A <sub>1</sub> (0.1 g/l)	A <sub>2</sub> (0.2 g/l)	A <sub>3</sub> (0.3 g/l)	A <sub>4</sub> (0.4 g/l)	A <sub>5</sub> (0.5 g/l)	A <sub>6</sub>	B <sub>1</sub> (0.1 g/l)	B <sub>2</sub> (0.2 g/l)	B <sub>3</sub> (0.3 g/l)	B <sub>4</sub> (0.4 g/l)	B <sub>5</sub> (0.5 g/l)	B <sub>6</sub>	C <sub>1</sub> (0.1 g/l)	C <sub>2</sub> (0.2 g/l)	C <sub>3</sub> (0.3 g/l)	C <sub>4</sub> (0.4 g/l)	C <sub>5</sub> (0.5 g/l)	C <sub>6</sub>
O.B.F	63.3	61.4	58.8	57.4	56.5	67.7	75.8	71.7	69.2	66.9	63.8	78.5	64.2	62.5	59.6	57.4	57.4	70.7
Discoloration	10	16	18	26	27	0	10	12	13	18	18	0	6	10	11	19	25	1
T.B.F	64.3	60.1	58.4	55.6	53.4	72.9	75.3	74.3	72.2	69.1	68.3	78.1	68.7	66.4	63.7	61.3	58.7	76.2
Erratic swimming	6	10	12	21	22	0	13	14	16	19	21	0	8	10	15	20	22	1
Mortality	0	2	3	5	9	1	2	5	10	16	18	0	0	3	6	9	11	0

beat frequency of the specimens were recorded. However, for observed number of discoloration, erratic swimming, and mortality, the cumulative value in the three replicates was recorded.

The number of subjects exhibiting discoloration and erratic swimming in the control tanks is zero, except in few cases like (tank C<sub>6</sub>; Figure 4) where one is observed. This could be attributed to the stress resulting from handling, pre-loading steps and thus eventual weakness of this subject. Also the common increased or rapid operculum movement rate as well as rapid tail beat frequency noticed at onset in all the treatments could be attributed to the survival instincts of the subjects in response to the stimuli which they are exposed to.

However, the resistance of the fishes begins to drop, such that the specimens become easily susceptible to the influence/effect of the toxicants, hence the

progressive fall in operculum movement rate and tail beat frequency. This trend finally results in the death that is mortality of the specimens. It would be observed as shown from Figure 5 that the rate of operculum movement and tail beat frequency in all the control treatments are higher than the rest at every instance. Also, the observation of gradual drop in rate of operculum movement and tail beat frequency with increasing concentration in all treatments shows that the toxicants are responsible for the observed trend of inactivity, and hence final death of the specimens.

Since discoloration and erratic swimming often precede mortality, the observed trend of increasing number of specimen exhibiting discoloration and erratic swimming with increasing concentration point to the fact that the toxicant is responsible for the mortality of the specimen.

It would be observed from Table 5 that

the contaminants has wide ranging confidence limit, hence in an attempt to further compare the lethality, or toxicity of the pesticides (A, B, and C), taking the mean of the values of LC<sub>50</sub>, lower and upper 95% confidence interval from Table 4 provides different index for each substance. These indices are represented in the following table.

Since mortality is a function of dosage or concentration of lethal substance that is mortality increases with increasing dosage or level of contaminant, it follows that the lower the index, the higher the lethality of the substance. Hence from the Table 5 the order of lethality of the three contaminants can be represented as: B > C > A. This means that a fixed dosage of substance B on a given population of organism (*C. gariepinus*) will kill more of the organism than the number which will be killed by same dosage of pesticide C, and D on the same population of organism under the same condition.

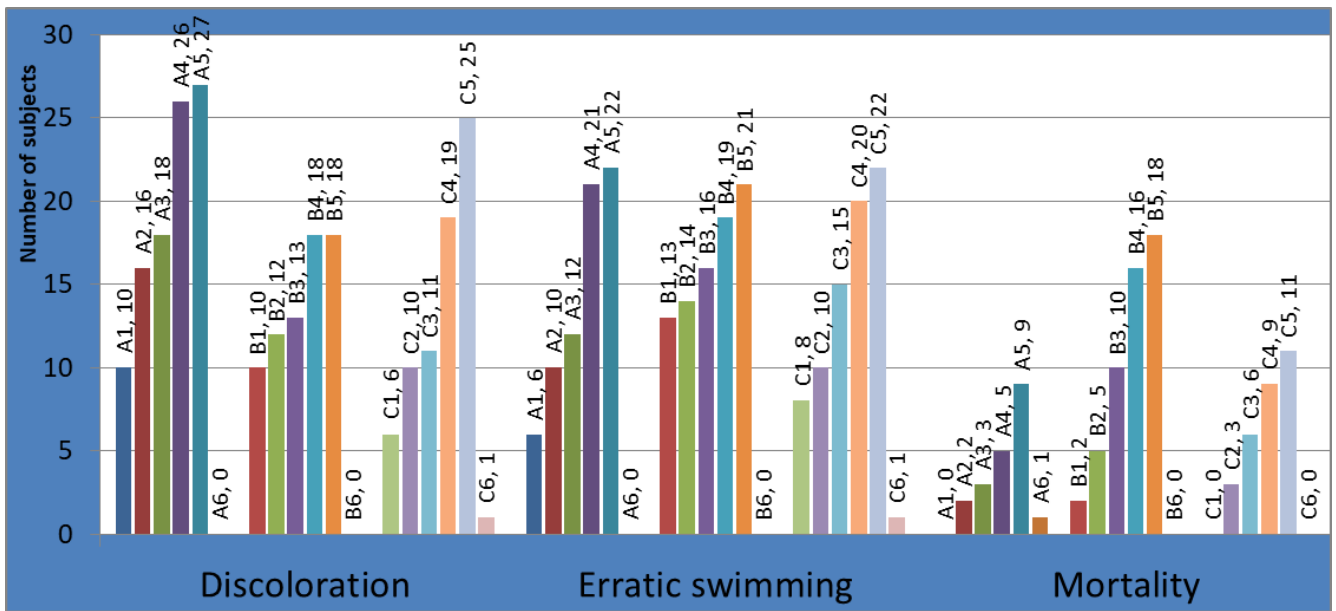


Figure 4. Chart showing variation of cumulative discoloration, erratic swimming, and mortality with concentration of pesticide A, B, and C respectively.

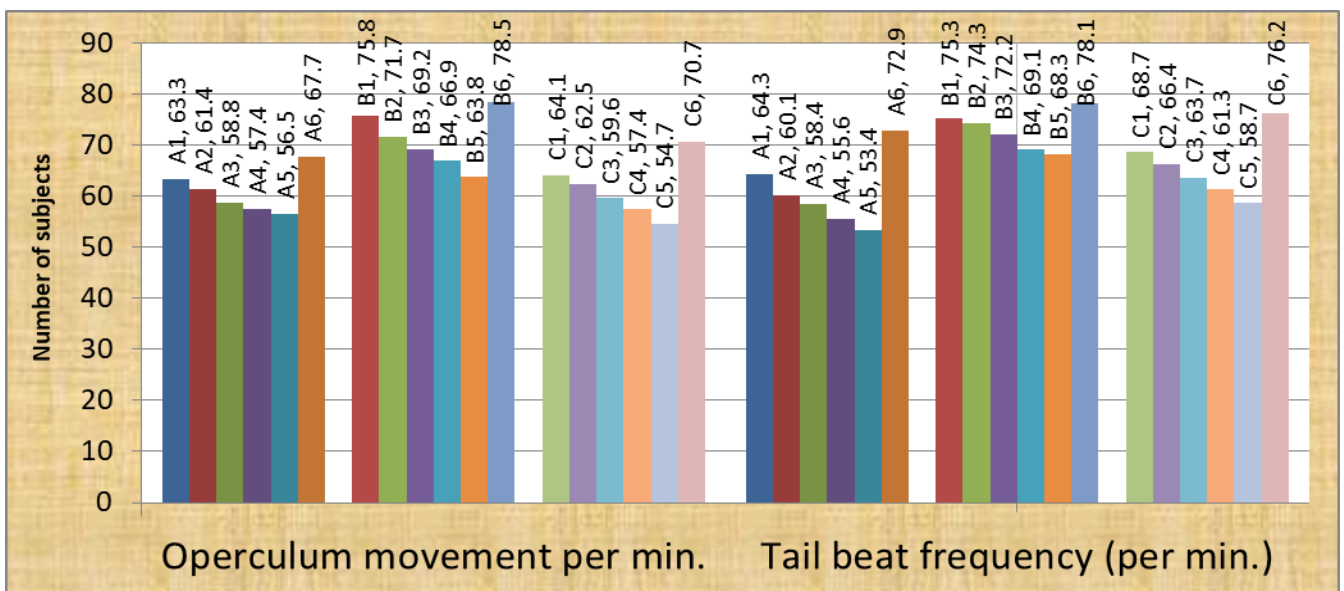


Figure 5. Chart showing variation of operculum movement rate ( $\text{min}^{-1}$ ) and tail beat frequency with concentration of pesticide A, B, and C. respectively.

**Conclusion**

From the results obtained, it can be concluded that each of the pesticides dragon, sniper and kartodim 315ec (A, B, and C respectively) is quite toxic to *C. gariepinus* at different dosage. This study provides findings that can be used by institution, government, and agency concerned with toxicant monitory and control in formulating policy, health regulations and laws for environmental protection, in land as well as water bodies. This is so because there

is a connection between land terrestrial and aquatic environment. The findings can be used by aqua-culturists to formulate guide on ‘safe-level’ or safe limit application of each substance for the purpose of parasite and pest control in aquatic environment.

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