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Acute Toxicity of Dichlorvos to *Oreochromis Niloticus* and Its Haematological Effects

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Abstract: **The acute toxicity of Insecticide dichlorvos to juveniles of Oreochromic niloticus of mean standard length of juveniles of Oreochromic niloticus 6.0± 21cm and weight of 9.20± 13g body weights were investigated under laboratory condition at the concentrations of 0.00uL^{-1}, 0.50uL^{-1}, 1.00uL^{-1},1.50uL^{-1} and 2.00uL^{-1}. At Organ level Oreochromis niloticus exposed to acute concentrations of herbicide propanil exhibited agitated swimming, loss of equilibrum, air gulping, period of quiescence, and the fish turned on its flank and swarm in circles and finally died Accumulation of mucus also was observed on the gill filaments and body surface of the dead fish after their exposure to the lethal concentration of Dichlorvos.. In conclusion, acute concentrations of dichlorvos are harmful to Oreochromic niloticus a nontarget organism. It is recommended that manufacturers should be compelled to state categorically the effect of dichlorvos to nontarget organisms (or aquatic organisms).**

Keywords: **Acute toxicity, dichlorvosl, behaviour, Clarias gariepinus.**

1. INTRODUCTION

Dichlorvos is one of the few organophosphates still registered for use. Although it serves as a contact and stomach poison for food and non-food crop pests, toxicity to fish and other aquatic organisms need to be determined on some fish species. (Suntio *et al* 1988, Naqvi *et al* 1993, Toledo *et al* 1992.). Varo *et al*, (2003), stated that Dichlorvos is also commonly used in shell fish farming to eradicate crustacean ectoparasites. It is specially used in the treatment of sea lice (*Lepophtheirus salmonis* and *Caligus elogatus*) on commercial salmon farms. This pesticide however often ends up producing both lethal and sub-lethal effects on the fish and even zooplankton (Gupta *et al*., 2008.) At only 1.00 ppm, dichlorvos, showed both acute and chronic toxicity in fish (Gupta *et al*., 2008). Dichlorvos (2,3-dichlorovinyl- dimethyl phosphate) is used to control households and stored products insects. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies in greenhouse, outdoor fruits, and vegetable crops (Lotti, 2001).

In Nigeria, especially the Northern part, dichlorvos is traded under different names such as Nuvan, Sniper, Pia-pia (Hausa) and is handled and used as a household insecticide indiscriminately. Dichlorvos, also known as DDVP (O.-Odimethyl-O-2, 2-dichloro-vinyl phosphate) (USEPA, 2007) is an organophosphate insecticide and has been applied in Northern Nigeria as mosquitoes insecticides over the decades, since its commercial manufacture started in 1961 (Foll *et al*., 1965; Foll and Pant., 1966; Breast Cancer and Environmental Risk Factors - BCERF., 1999). A number of evidences indicated that dichlorvos is most likely the major active pesticide ingredient of "*Ota-piapia"*. The application of *Ota-piapia*, an unspecified insecticide to prevent insect infestation of fish is still remains common practices (Eyo and Mdaihli, 1997; FAO, 2001;, Sogbesan *et al* 2012) in Nigeria.

Determination of Concentrations of Test Chemicals:

The varied concentration chosen for the exposure of *O. niloticus* was in accordance with the criteria set by the American Society for Testing and Materials (ASTM, 1977).

According to this criteria, Concentrations of the test compound used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality. For the 96hr-acute toxicity test, the concentrations of the working solutions used for the Insecticide Dichlorvus were $0.00uL^{-1}$, $0.50uL^{-1}$, $1.00uL^{-1}$,$1.50uL^{-1}$ and $2.00uL^{-1}$.

2. MATERIALS AND METHODS

Experimental Design:

Juveniles of *O. niloticus* with average length of 6.0± 21cm and weight of 9.20± 13g, were obtained locally from Amina Zira Fish Farm, Yola. 200 of the species were taken to the Fisheries Laboratory of Modibbo Adama University of Technology, Yola in a Thermo-box to prevent rapid change in temperature. The fishes were held in the Laboratory for one week prior to the commencement of the experiment. During this period, three quarters of the test water was changed daily by siphoning out the spent water. The Tanks were checked daily for fish mortality at time intervals as recommended by Sprague (1975) and dead fish removed and recorded.

Acute toxicity:

Acute 96 h static bioassays was conducted in the laboratory as described by Sprague (1973) and APHA (1985) to determine the toxicity of dichlorvos to *O. niloticus*. A total of fifty-two (52) transparent plastic tanks of size 30.5 x 30.5 x 92.5cm, 48 of them containing concentration of dichlorvos and one control (without toxicant) was used for the experiment. A total number of 260 juveniles of the specie were used for the toxicity study. Also the physico-chemical parameters of the diluting water such as temperature, pH, Dissolved oxygen, total alkalinity, and conductivity during the acute test were measured by methods described by APHA (1985). The desired propanil dichlorvos concentrations were measured and introduced into 50L of dechlorinated and aerated water in the plastic tanks. The mixtures were allowed to stand for 30 minutes before introducing test organisms *O.niloticus*. Thereafter the tanks were stocked at 10 fish per tank for the experimental run

Behavioural responses:

The behavior and general condition of the fish were observed during bioassay. Observations of the behavior were carried out at intervals of 24, 48, 72, and 96 hours. The behaviour were scored using 1-6 behavioural pattern after Auta (2001)

Respiration:

The parameters under respiration were keenly observed and recorded. These parameters included opercular and tailfin movements.

Haematology:

Two fish were randomly sampled at 24 hours interval during the 96 hours test with a small hand net from each tank and then the fishes were immediately anaesthetized in MS 222 (Tricaine methane sulphonate) for blood collection.

Collection of blood samples:

Blood was sampled as described by Blaxhall and Diasely (1973). Blood was collected by severance (2cm) of the caudal peduncle. Blood was collected with a 5mm syringe.

Haematological parameters:

Haemoglobin:

Haemaglobin determination is the quickest method for detecting anemia. The salili-Hellige haemoglobin determination was performed as follows: The sallied pipette was filled slightly above the $20mm^3$ mark, the pipette was wiped with a soft absorbent tissue to remove excess blood and the volume was adjusted to exactly $20mm^3$ by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 10.0 millilitres of 0.1N hydrochloric acid, and the pipette was rinsed several times in the acid solution. The sample was allowed to stand for not less than 3 minutes before reading the value in the colorimeter. The intensity of colour was measured at 530 to 540µm and was recorded as percent transmission.

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Determination of pack cell volume:

Pack Cell Volume (PCV) was carried out by micro-westegreen method as described by Blaxhall and Diasely (1973). The blood sample from the severed caudal peduncle was drawn into micro-haematocrit tube. The tubes were sealed with wax and centrifuged for five minutes. The PCV was measured with the aid of a microhaematocrit reader and expressed as the volume of the erythrocytes per $100cm^3$.

Red blood cell count or total erythrocyte count:

The techniques of red blood counts of fish blood are similar in most respects to those used in mammalian counts. However, the diluting fluids normally used for mammalian counts were not applicable to fish blood. Gorder's and Hayme's diluting solutions were distorted after a few minutes.

The standard RBCC diluting pipette and a 1:200 dilution were used for the red blood cell count. Blood was drawn just beyond the 0.5 mark on the pipette. The tip of the pipette was wiped with a soft absorbent tissue to adjust the volume to exactly the 0.5 mark. The pipette was immediately filled to the 101 mark with Hendricks diluting fluid. Partial rotation of the pipette while being filled assured the complete mixing of the blood and diluting fluid, and prevented clotting. With its ends griped between the thumb and second finger, the pipette was then shaken for 30 to 60 seconds. After the pipette had been shaken, a few drops of the diluted blood were expelled from it. Control of over flow of fluid was maintained by replacing the index finger over the bulb end of the pipette. The haemocytometer which was the counting chamber was used by using the pipette which was held to the edge between the cover slip and the chamber, and capillary action drew the diluted suspension of cells into the chamber. The haemocytometer was then placed under the light microscope, and the cells were counted. The haemocytometer is divided into ruled areas $1mm^2$, with the centre square millimeter divided into 25 groups of 16 small squares. The cells within the boundaries of five of these small squares were counted. Each corner plus the center group were counted when the red blood cell count was computed, the number of cell counted in all five squares was multiplied by 10^6, this gave the total number of cells per cubic millimeter (mm^3) of blood (Hesser, 1960).

Total leucocytes count:

Shaw's solutions A and B allowed differentiation between leucocytes, erythrocytes and thrombocytes. Both solutions were filtered just prior to use. Solution A was made fresh each day, solution B was stable for several days. Leucocytes were counted using Shaw's solution A and B. The blood was drawn up to the 0.5 mark, solution A was added to fill the bulb of the pipette approximately half filled, and mixed. Then, the pipette was removed from solution A and filled to the mark 101 with solution B. The pipette was then shaken as in the erythrocyte count. A few drops were expelled and the haemocytometer was filled in the manner described previously. For comparison of the total number of leucocytes, the cells in the four large squares noted by the large cycle were counted. The total number of cells counted multiplied by 500, determined the total number of leucocytes per cubic millimeter (mm^3) of blood (Hesser, 1960).

Analysis of Mean Corpuscular Volume (MCV):

Blood samples were analysed immediately after collection. The parameters analysed for were the Pack Cell Volume (PCV) also referred to as Haematocrit. The values for Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Heamoglobin Concentration (MCHC) were calculated according to the formulae below.

$$MCV(um3) = \frac{Ht}{RBC\ (Cells\ mm^{-}3)} x10$$

$$MCH\ (pg\ cell^{-1}) = \frac{Hb(g100ml)^{1}}{RBC\ (cells\ mm^{-}3} \times 10$$

$$MCHC\ (g\ 100ml^{-1}) = \frac{Hb\ (g\ 100ml)}{Ht\ (\%)} \times 10$$

Mortality of the juveniles:

Observations to determine the mortality of *C. gariepinus* and *O. niloticus* were carried out at 24, 48, 72, and 96 hours.

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Statistical Analysis:

Analysis of variance (ANOVA) and Duncan multiple range tests were employed to test for differences between treatments (CRD). Correlation coefficient (r) was used to determine the relationship between the various parameters. Regression coefficient between the probit kill and log concentration of the toxicant were determined after the acute toxicity bioassay.

3. RESULTS

Acute Toxicity:

Behavioural responses of O.niloticus to dichlorvos:

As can be seen in Table 1, '1-6' represents different behavioral reactions in order of their appearances. Agitated movement occurs across all the concentrations from 0.5uL^{-1} to 2.00.00uL^{-1}. Loss of equilibrium was dose dependent as it was the next behavioral reaction after agitated movement. It occurred from 1.00 to 2.00uL^{-1}. Period of quiescence started at 1.00uL^{-1} to 2.00uL^{-1} while in *C.gariepinus* it started at 1.50uL^{-1} to 2.00uL^{-1}. The behavioral responses of air gulping, period of quiescence and death occurred under higher doses of 1.50uL^{-1} and 2.00uL^{-1}, while copious accumulation of mucus and blood on gill filaments finally occurred only in the highest dose 2.00uL^{-1}.

Table 1: Behavioral Responses of *Oreochromis niloticus* to Different Doses of dichlorvos at 96hrs

Behavioral Responses	Doses of Dichlorvos in uL^{-1}					
	0.00	0.50	1.00	1.50	2.00	
Agitated movement		1	1	1	1	
Loss of equilibrium			2	2	2	
Air gulping			3	3	3	
Period of quiescence			4	4	4	
Death				5	5	
Copious accumulation of mucus and blood on gill filaments					6	

1-6 = Behavioral responses in different concentrations of dichlorvos

KEYS

Agitated movement = 1

Loss of equilibrium = 2

Air gulping = 3

Period of quiescence = 4

Death = 5

Copious accumulation of mucus and blood on gill filament = 6

Respiratory rates:

The values of the tail fin beats of *O, niloticus* exposed to dichlorvos in Figure 1 were dose-dependent. The tail fin beats per minute of *O. niloticus* were highest at 0 and 12 hrs in the specimens exposed to the toxicants of Contril and 0.50u/l. The result showed that the dose 2. 00uL^{-1} > 1.50µ/l > 1.00µL^{-1}. > 0.50µ/l >Control. Similarly from 24nrs to 96 hrs the tail fin beats decreased with highest concentrations at control > 0.50µ/l > 1. 00µL^{-1} > 2. 00µL^{-1}. The result showed that the tail fin beats were dose dependent.

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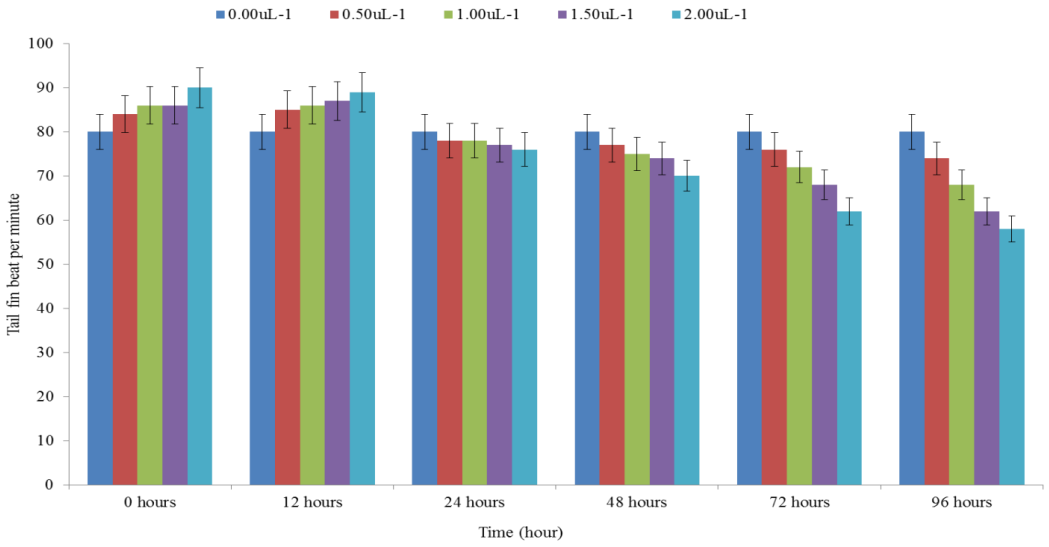

Figure 1: Tail Fin Beat per Minute of *O. niloticus* to Dichlorvos

Haematological parameters of *Oreochromis niloticus* exposed to dichlorvos:

Exposure of O. niloticus to dichlorvos for 96 hours showed significantly lower percentages of Packed cell volumes (PCV) , Haemaglobin, Red Blood Cells (RBC), Neutrophils, Monocytes and Lymphocytes than the control fish ($P<0.05$). These haematological parameters decreased with increasing concentrations of dichlorvos. In O. niloticus exposed to dichlorvos. PCV value at the lowest concentration of 0.50 μL^{-1} was 28.62%, while the highest concentration of 2.00 μL^{-1} had percentage value of 18.35 as shown in Table. 2 below.

Table 2: Haematological Responses of *Oreochromis niloticus* to Various Concentration of Dichlorvos for 96 hrs

	0.00mgL^{-1}	0.50uL^{-1}	1.00uL^{-1}	1.50uL^{-1}	2.00uL^{-1}
PCV (%)	32.50±0.75	28.62±0.65	25.25±0.56	22.05±0.46	18.35±0.33
Haemoglobin (%)	8.42±0.07	5.14±0.06	4.50±0.04	4.22±0.04	2.53±0.02
WBC (x10^9)	4.86±0.06	5.26±0.06	5.80±0.08	6.24±0.08	8.63±0.09
RBC	7.23±0.08	6.86±0.06	5.85±0.06	4.25±0.05	2.48±0.03
Neutrophils%	20.32±0.23	22.62±0.25	22.70±0.32	23.56±0.41	23.90±0.45
Lymphocytes%	76.24±0.68	70.36±0.64	66.24±0.56	62.35±0.54	58.76±0.46
Eosinophils%	9.64±0.56	8.96±0.54	7.23±0.43	6.85±0.32	5.56±0.23
Monocytes%	4.15±0.08	3.23±0.07	3.10±0.04	2.86±0.03	2.05±0.01
ESR(mm/h)	3.86±0.07	5.05±0.08	5.85±0.08	6.46±0.09	6.86±0.09
Thrombocytes%	150.32±0.98	132.06±0.87	120.58±0.78	115.88±0.65	92.73±0.58

4. DISCUSSION

Behavior:

Oreochromis niloticus exposed to acute concentrations of dichlorvos exhibited agitated swimming, loss of equilibrum, air gulping, period of quiescence, and the fish turned on its flank and swarm in circles and finally died. Hyper activities were the most common responses effects on *O niloticus* and were dose dependent. Such activity was also reported by Matsumura (1975) to be the primary and principal sign of nervous system failure due to pesticide poisoning which affects physiological and biochemical activities in non target organisms. Pal and Koner (1987) opined that disruption of the functioning of the nervous system of fish might be the cause of slow and agitated swimming, erratic movement and loss of equilibrum.

Accumulation of mucus also was observed on the gill filaments and body surface of the dead fish after their exposure to the lethal concentration of dichlorvos. Hossain *et al*. (1987) stated that increase in production of mucus over the body as a result of toxicant may interfere with the gaseous exchange, secretion and waste products and osmoregulation. Similar observations were made by Shafiel and Costa (1990), Babatunde (1997) and Auta (2001), who studied the effects of pesticides on different species of fish. The accumulation of mucus may result from an increase in the activity of mucus cells subsequent to exposure to pesticides. This resulted in increase in the production of mucus over the body of the fish. It seems that the solution of the pesticides tend to precipitate or coagulate muco protein on the gill epithelium. This may interfere with the gaseous exchange, secretion of waste products and osmoregulation. The toxic action of the toxicants appeared combine effects of precipitation of mucus on the gills and osmoregulatory stress with death resulting from suffocation. Blood was also observed around the gill coverings of the dead fishes. This suggests that the fishes might have suffered from gill haemorrhage. Similar findings were reported by Shafiel and Costa (1990) when fry and fingerlings of *Oreochromis mossambicus* (Peters) were exposed to the pesticides, Ronstar, Elsan, Endosulfan, Basfapon, Rogor 40 (dimethoate) and Azodrin 60.

The results of the tail fin beats also showed that increase in time of exposure resulted in decrease of tail fin beats in *O.niloticus*, this suggests decreased oxygen consumption and reduced energy. The reduction of respiratory rate implies that the fish had become fatigued due to several attempts to escape from the toxic medium to facilitate more oxygen intake. These behavioural patterns are indicative of respiratory impairment, due to the effect of the toxicant on the gills and general metabolism. (Chindah *et al*., 2004).

Haematology:

The examination of haematological parameters in *O.niloticus* indicated that the dichlorvos at acute levels elicited response which involved a decrease in the percentages of Packed Cells Volume (PCV), Haemaglobin, Red Blood Cells (RBC), Neutrophils, Monocytes and Lymphocytes, indicating severe anaemia in the exposed fish The anaemic effect could be due to destruction of or inhibition in erythrocyte production. Eisler (1967) reported that erythropenia (deficiency in the number of red blood cells) in fish exposed to methoxychlor and methylparathion had low haemoglobin and haematocrit content value as well as low erythrocyte sedimentation rate (ESR). Similarly, anaemia associated with erythropenia was reported by Srivastava and Mishra (1979) in *Colisa fasciatus* after acute exposure to lead. Leucocytosis was evidenced by the increase in Total Leucocytes Count (TLC) with increased concentration of dichlorvos in the present study.

This examination of haematological parameters in *O. niloticus* indicated that dichlorvos, at chronic level elicited responses in the blood parameters. The results obtained showed that when the fish species were exposed to different concentrations of the toxicant the haemoglobin concentration, haematocrit, erythrocyte counts and elevated mean corpuscular haemoglobin were lowered. All these indicated that the toxicant exerted effects similar to that elicited anaemia. Similar reduction of these parameter in fish exposed to various pesticides have been documented by Anees (1978), Omoregie, *et al* (1990) and Gill, *et al* (1991). The reduction of haematological variables related to oxygen transport (RBCC, Hb, Ht) tend to suggest a reduction of the oxygen carrying capacity of the blood, The implication of these findings is that the oxyphoretic capacity of the erythrocytes in this species might have been interfered with. Also, taking into account that the toxicants may have induced hypoxic stress in the two fish species and the fact that fish generally respond to low environmental oxygen by an increase in the red blood concentration count (Lloyd, 1992). It was expected that blood values after these exposures would change accordingly. Since this was the case, it is possible that the toxicants did not interfere with the oxyphoretic capacity of the erythrocytes in these species. In addition to these, the growth reduction observed in this study could have been due to the reduced oxygen carrying capacity of the blood resulting in inefficient utilization of assimilated food or to inhibition of certain enzymes of the metabolic pathways.

White blood cells count (WBCC) in fish blood exhibits a direct correlation with feeding status as reported by Smirnova (1965). The results of this study showed that acute exposure of dichlorvos to *O. niloticus*, there was a reduction in WBCC. This finding suggests that the reduction of WBCC might have been due to poor feeding in the fish species and hence the reduction in weight.

The decrease in the Mean Corpuscular Haemoglobin Concentration (MCHC) in the test groups suggests that the primary effect of the toxicant on the blood may be a reduction in haemoglobin. In their studies, Oladimeji and Ologunmeta (1987) reported similar reduction in the haematocrit and haemoglobin in fish exposed to sub-lethal concentration of lead.

4. CONCLUSION

The results of this study clearly showed that the behavioural and haematological responses of this freshwater fish species were dose dependant. These findings are in line with that of Tripathi, (1992) and Alam and Maughan (1993). Similar observations were made by Lloyd (1992). Several workers have reported that fish have a broad range of sensitivities to pesticides toxicity (Ufodike and Omoregie, 1990; Tripathi, 1992;).

5. RECOMMENDATIONS

a. It is recommended that attempts should be made to monitor and control the usage of dichlorvos.

b. Manufacturing industries should look into ways of reducing the potency of dichlorvos to non-target organisms such as fish, while maintaining its effectiveness as rice herbicide.

c. Manufacturers should also be compelled to state categorically the effect of dichlorvos and other chemicals, to non-targeted (or aquatic organisms).

d. Proper education of farmers on the danger of dichlorvos to the environment is urgently required.

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