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DICHLORVOS INSECTICIDE MODULATES THE HEMATOLOGICAL PARAMETERS IN JUVENILES OF AFRICAN CATFISH, *Clarias gariepinus*

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Abstract

Dichlorvos is an organophosphate used commercially for the treatment of pests. The present study was designed to investigate the effects of dichlorvos on *Clarias gariepinus* juveniles using hematological parameters. Fish were exposed to two sublethal concentrations-2.14 and 4.28mg/l corresponding to 1/10 and $1/5^{th}$ of $96LC_{50}$(21.391mg/l)of the pesticide. Blood was sampled on day1, day 5,day 10 and day 15 to estimate the hematological parameters such as white blood cells(WBC), packed cell volumes (PVC), red blood cell (RBC), hemoglobin (Hb), mean cellular hemoglobin (MCH), mean cellular hemoglobin concentration (MCHC) and mean cellular volume (MCV). Results showed concentration and time dependent values across the concentration. There was a significant decrease($p<0.05$) in PCV, RBC, Hb, MCH and MCV and MCHC when compared with the control. Meanwhile, a marked significant increase ($p< 0.05$) was observed in the values of White Blood Cells(WBC) when compared with the control. Hepatosomatic index and condition factor showed a significant difference when compared with the control. The present study revealed that dichlorvos elicits toxic stress even at sublethal concentrations resulting in aberration in the studied parameters which is very evident in the blood, serum and muscle samples.

Keywords: Toxicity, Dichlorvos, Fish, Hematology

1.0 INTRODUCTION

Environmental pollution by pesticides that flow into aquatic environment has been a subject of concern by Environmentalists. The United Nations Environment Program has defined pesticides as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating pest. Most developing nations of the world have found the use of pesticides as a necessary evil for agricultural purposes. This is predominant in underdeveloped or third world countries like Nigeria. Organophosphate pesticides are the most commonly used pesticides in the world due to its quick degradation. Unfortunately, organophosphates lack target specificity and can cause severe long lasting effects on the population. Irrespective of reports of health implications of these pesticides, most countries like ours still make use of it for their agricultural purposes. Dichlorvos (2, 2 dichlorovinyl dimethyl phosphate) was registered for use in 1948 but was introduced into the market in the year 1961. It has a molecular weight of 220.98g and the molecular formular is $C_4H_7Cl_2O_4P$. The trade name for dichlorvos include: DDVP, Dedevap, Sniper, Nuvan, Vapona,etc. Dichlorvos is one of the few organophosphates still registered for use. The World Health Organization has named dichlorvos as a highly hazardous chemical. Dichlorvos kills many organisms spiders, mites, caterpillars and trips (Lotti, 2001). It becomes poisonous when it gets to the body through any route. It serves as a contact and stomach poison for food and non-food crop pests (Suntio,1988 and Naqvi,1993). It is extremely toxic to aquatic organisms and hampers fish health through impairment of metabolism sometimes leading to death. As one of the few organophosphates still registered for use, dichlorvos has elicited worldwide concern for many reasons. It is specially used in the treatment of sea lice (*Lepophtheinus salmonis* and *Caligus elogatus*) on commercial salmon farms. But this pesticide usually ends up producing both lethal and sub lethal effects on the fish and even zooplanktons (Gupta,2008). At only 1mg/l or 1ppm,dichlorvos showed both acute and chronic toxicity in fish. Some other adverse effects of dichlorvos on fish have been noted by other researchers.

- Although it has been established that dichlorvos serves as a contact and stomach poison for food and non food crop pests, its toxicity to fish and other aquatic organisms need to be determined on some fish species.

Acetycholinerase in the nervous system of insects is the main target of dichlorvos. It also badly affects the genetic material of insects thereby altering the gene. The safety data of dichlorvos has been severally reviewed by United

States Environmental Protection Agency (USEPA, 1995). Since dichlorvos is an acetylcholinerase inhibitor, symptoms include weakness, headache, vomiting, hypotension, diarrhea, abdominal cramps, eye and skin irritation, atazia, convulsions, etc. Several researchers have been carried out to determine the effects of dichlorvos on fish. For this study, *Clarias gariepinus* was used. Fish as vertebrates contain a low-fat high quality protein. Several vitamins and elements have been identified in fish. This include riboflavin, phosphorus, omega-3-fatty acids, potassium, calcium, etc. Fish protein can be used to complement amino acid pattern and improve the overall protein quality of a mixed diet (FAO, 2005).

Ayanda and Egbamuno (2005) reported that most fish contain significant amount of all amino acids. The African Catfish is generally accepted by many people as a source of meat (Prusynski, 2003) and it is mostly smoked and used in soups throughout Africa and beyond. Streams, ponds, swamps, rivers and lakes are aquatic habits of African catfish (Ayanda and Egbamuno, 2012). According to Nwamba (2008), catfish is recognized by its long dorsal and anal fins, has a slender body, a flat bony head, and a broad terminal mouth.
Lakra and Nagpure (2009) reported that catfish is at the risk of being contaminated in the environment through the use of pesticides in agricultural areas and linear food relationship among organisms.

2.0 MATERIALS AND METHOD

The materials used for this analytical work include the test samples, equipments and various chemicals.

2.1 COLLECTION AND TRANSPORT OF EXPERIMENTAL FISH AND CHEMICALS:

The juveniles of African catfish, *Clarias gariepinus* were collected from Rogeny Game Village Idemiri LGA of Anambra State. The fish were transported in a 500 litres capacity of aquaria tank to the experimental site-Heldin Fishery Unit,Old airport road, thinkers corner. A total 300 juveniles of *Clarias gariepinus* of average weight 200$\pm$0.70g and length of 27$\pm$12cm were used for the experiment. The experimental chemical-dichlorvos (1000EC) was purchased from Ogbete market, Enugu.

2.2ACCLIMITATION OF EXPERIMENTAL FISH

The acclimatization of the fish was done for two weeks (14 days) under laboratory conditions. They were placed in a fifteen acclimatization tank of 25 litres and filled

with 10 litres of tap water. The fish were fed daily at 3% body weight during the period of acclimatization. The tanks were checked daily for fish mortality at time intervals as recommended by Sprague (1975).

Range finding test was carried out to determine the concentration of the test solution for definitive test.

2.3 ACUTE TOXICITY BIOASSAY

Acute 96h bioassay was conducted in the laboratory as described by Sprague (1973) and APHA(1985) to determine the toxicity of dichlorvos to *Clarias gariepinus*. The fish were divided into experimental and control groups. The control group had no treatment while the experimental group had six different concentrations. The experiment was carried out according to the guideline of U.S EPA (1994) and replicated thrice for the purpose of accuracy. The six different concentrations were 18mg/l, 20mg/l,22mg/l, 24mg/l, 26mg/l, 28mg/l and the control. A total of 10 fish were used per replicate. The acute toxicity test lasted for four days (96h) in which the mortality rate as well as survival of the fresh water fish under each concentration of dichlorvos was recorded. The readings were taken after 24h, 48h,72h and 96h exposure time. The water was not changed (static system) until after 96h but dead fish were removed to avoid the pollution of the water. Death rate was not recorded in the control.

2.4 SUBLETHAL CONCENTRATIONS

Two sublethal concentrations were determined from 96h LC_{50} following the result obtained from probit analysis method as described by Finney (1971). The 96h LC_{50} value from probit is 21.4mg/l. The first sublethal concentration I(SL-I) was divided by 5 ($1/5^{th}$ of 96h LC_{50}=4.278) while the second sublethal concentration II(SL-II) was divided by 10($1/10^{th}$ of 96h LC_{50} =2.139). Fish are then exposed to these two sublethal concentrations and a control. Each treatment group was further randomized into three replicates (ten fish per replicate) in 10 litres of water. The exposure period was 15 days during which the fish was fed with small quantity of food approximately 1% of total body weight about an hour before the test solution was recommended to avoid catabolism and subsequent mortality. One fish from each replicate was sacrificed to get blood, liver and muscles. To avoid stress, the fish were anaesthetized using tricainemethana sulfonate. Other morphological indices such as weight of fish, fork length, total length, weight of liver were determined before the carcass was finally disposed.

2.5 HAEMATOLOGICAL ASSAY:

For hematological assay, blood samples were collected from each concentration from the head and the caudal fin region which was cut with a scissors. EDTA

containers were used to collect the samples to avoid coagulation. Several hematological parameters analyzed include-PCV,RBC, WBC and Hb

2.5.1 PACKED CELL VOLUME:

Packed cell volume(PCV) was determined using the method as described by Nelson and Morris(1989).

2.5.2 Method:

Capillary tube was used to collect the blood sample and sealed at one end wit plasticine. It was then spinned in the hematocrit centrifuge at 10,000r/min for 5 minutes and read with hematocrit reader to determine the blood plasma percentage.

2.5.2 HAEMOGLOBIN (Hb):

Hemaglobin was gotten by dividing the PCV values by 3 for each concentration.

2.5.3 WHITE BLOOD CELL:

Neubauer haemocytometer was used to determine the WBC as described by Rusia and Sood (1992).

Method:

380ul of Turk's solution was added into the test tubes. This was followed by adding 20ul of blood sample which was mixed together and allowed to stand for two minutes. The counting chamber was observed using X10 objective lens of the microscope.

2.5.4 RED BLOOD CELL COUNT:

Neubauer hemocytometer was used to determine the RBC as described by Rusia and Sood(1992).

Method:

4ml of 100% Na_2CO_3 was added into the test tubes. This was followed by addition of 20ul of blood sample that was mixed and allowed to stand for three minutes. The counting chamber was observed using X10 objective lens of the microscope.
Other hematological parameters were calculated using the formular as described by Yekeen and Fawole (2012). The derived parameters are:

Mean Cellular Volume (MCV) = PCV/RBC * by 10

Mean CellulaHaemglobin (MCH)= Hb/PCV * by 10Mean Cellular

HaemoglobinConcentration (McHc)= Hb/PCV * by 100

3.0 RESULT

3.1 Effect of Dichlorvos on Condition Factor (CF) and Hepatosomatic Index (HSI)

Table 1 and 2 showed the hepatosomatic index and condition factor of *Clarias gariepinus* exposed to dichlorvos. On day 1, the lowest value of HSI was observed at 4.28(a)mg/l while the highest value was observed at 2.14(a)mg/l. The lowest value of HSI in day 5 was observed at 2.14(b)mg/l while the highest value was observed at 4.14(a)mg/l. On day 10, the highest value of HSI was obtained at 2.14(a)mg/l while the lowest value was observed at 4.28(a)mg/l. When compared with the control, 4.28(a)mg/l recorded the lowest value of HSI while the highest value was observed at 4.28(b)mg/l.

Table 1: Record of Hepatosomatic Index at Different Concentrations.

Parameter	Conc.	Day 1	Day 5	Day 10	Day 15
Hapatosomatic index	0.00	1.23 ± 0.29^{b_1}	0.97 ± 0.25^{b_1}	0.92 ± 1.04^{b_1}	0.87 ± 0.07^{b_1}
	2 .14	0.68 ± 0.41^{a_1}	0.55 ± 0.24^{b_2}	$0.41\pm.0.08^{b_2}$	0.32 ± 0.07^{b_2}
	4.28	0.61 ± 0.08^{a_1}	0.47 ± 0.19^{b_2}	0.38 ± 0.46^{b_2}	0.28 ± 0.28^{b_2}

Means with different alphabetical superscripts differ significantly across the duration while means with different numerical figures differ significantly across the concentration ($p< 0.05$).

Table 2: Record of Condition Factor at Different Concentrations.

	Conc.	Day 1	Day 5	Day 10	Day 15
Condition factor	0.00	1.87 ± 0.40^{b_1}	1.83 ± 0.00^{b_1}	1.77 ± 0.03^{b_1}	1.72 ± 0.31^{b_1}
	2 .14	1.47 ± 0.60^{b_2}	1.26 ± 0.02^{b_2}	1.07 ± 0.65^{b_2}	0.73 ± 0.00^{b2}
	4.28	1.31 ± 0.33^{b_2}	1.14 ± 0.41^{b_2}	0.99 ± 0.22^{b_2}	0.66 ± 0.12^{b_2}

Means with different alphabetical superscripts differ significantly across the duration while means with different numerical figures differ significantly across the concentration ($p< 0.05$).

3.2 Record of Mortality After Exposure to Dichlorvos

Table 3 clearly showed that there was increase in mortality with a corresponding increase in concentrations. No mortality was observed in the control group after 96h of the test. In 18mg/l concentration, 3 fishes died out of the exposed 30 after 96h showing that the concentration was mild. The case was different when there was an increase in the concentrations. At 20mg/l, 9 fish died out of the exposed 30. There was a double increase in the death of fish from 20mg/l to 22mg/l. 24, 27 and 30 fish died at 24mg/l, 26mg/l and 28 mg/l respectively. This showed that these concentration became very toxic to the fish. The highest level of death was recorded at 28mg/l where all the fish died.

Table 3: Cumulative Mortality of *Clarias gariepinus* Exposed to Different Concentrations of Dichlorvos

		No of deaths					
Conc.	**No exposed**	**24**	**48**	**72**	**96**	**% Mortality**	**% Survival**
Control	30	0	0	0	0	0	100
18	30	0	0	1	3	10	90
20	30	0	3	6	9	30	70
22	30	2	6	10	18	60	40
24	30	4	10	18	24	80	20
26	30	5	10	17	27	90	10
28	30	7	15	27	30	100	0

3.3 Effects of Different Concentrations of Dichlorvos on Hematological Parameters in *Clarias gariepinus* at Different Exposure Periods.

Table 4 shows the hematological parameters of *Clarias gariepinus* exposed to different concentrations of dichlorvos. On day 1, 5 and 15, the lowest values of Hb were obtained at 4.28mg/l while the highest value was obtained at 2.14mg/l. When compared with the control, there is a significant difference ($p<0.05$) between these two values and that of the control. The case was different on day 10 where the lowest value was obtained at 2.14mg/l and highest value at 4.28mg/l. A uniform result was observed in MCH. The lowest result was obtained at 4.28mg/l across all the duration period. In MCHC day 1, the lowest values was obtained at 2.14mg/l but this was not the case for other exposure periods where the lowest values were obtained at 4.28mg/l and highest values at 2.14mg/l. For PCV, a uniform result was obtained throughout the exposure periods where the lowest values were obtained at 4.28mg/l and highest values at 2.14mg/l. The result recorded in RBC followed the same sequence with that of PCV. The lowest values were obtained at 4.28mg/l while the highest values were obtained at 2.14mg/l. In WBC, the case was different. The lowest values were obtained at 2.14mg/l across the duration period while the highest values were obtained at 4.28mg/l across the duration period.

Table 4: Summary of hematological parameters

Parameter	Concentration (mg/l)	Day 1	Day 5	Day 10	Day 15
Hb	0.00	4.15 ± 0.21^{b_1}	4.40 ± 0.41^{b_1}	4.00 ± 0.00^{b_1}	3.80 ± 0.28^{b_1}
	2.14	2.98 ± 0.00^{a_1}	2.85 ± 0.49^{a_1}	2.45 ± 0.21^{a_1}	1.75 ± 0.07^{a_1}
	4.28	1.48 ± 0.26^{a_1}	1.80 ± 0.28^{a_1}	2.00 ± 0.89^{a_1}	1.45 ± 0.21^{a_1}
MCH	0.00	3.35 ± 0.02^{b_1}	3.11 ± 0.14^{b_1}	3.33 ± 0.00^{b_1}	3.35 ± 0.02^{b_1}
	2 .14	3.09 ± 0.19^{b_2}	$3.07\pm0.02b_2$	3.24 ± 0.00^{b_2}	2.18 ± 0.01^{b_2}
	4.28	3.02 ± 0.02^{b_2}	3.05 ± 0.07^{b_2}	3.22 ± 0.01^{b_2}	2.11 ± 0.12^{b_2}
MCHC	0.00	33.80 ± 0.14^{b_1}	32.62 ± 0.67^{b_1}	33.30 ± 0.00^{b_1}	33.00 ± 0.72^{b_1}
	2 .14	32.30 ± 0.00^{b_2}	30.75 ± 0.35^{b_2}	28.65 ± 0.07^{b_2}	26.50 ± 0.14^{b_2}
	4.28	31.45 ± 0.07^{b_2}	29.15 ± 0.21^{b_2}	27.05 ± 0.06^{a1}	22.12 ± 0.11^{a1}
PCV	0.00	12.50 ± 0.70^{b_1}	13.50 ± 0.70^{b_1}	12.00 ± 0.00^{b_1}	11.50 ± 0.70^{b_1}
	2 .14	9.50 ± 0.00^{a_1}	7.50 ± 1.41^{a_1}	6.50 ± 0.70^{a_1}	5.50 ± 0.70^{a_1}
	4.28	5.50 ± 0.70^{a_1}	5.40 ± 0.70^{a_1}	5.02 ± 0.70^{a_1}	4.50 ± 0.70^{a_1}
RBC	0.00	69.00 ± 1.41^{b_1}	68.50 ± 3.53^{b_1}	64.50 ± 0.70^{b_1}	63.00 ± 1.41^{b_1}
	2 .14	59.50 ± 2.12^{a_1}	57.00 ± 1.41^{a_1}	52.00 ± 1.41^{a_1}	49.00 ± 1.41^{a_1}
	4.28	42.00 ± 1.41^{a_1}	41.0 ± 2.82^{a_1}	38.50 ± 4.94^{a_1}	38.00 ± 2.82^{a_1}
WBC	0.00	5500.00 ± 424.26^{b_1}	5950.00 ± 70.71^{b_1}	5980.00 ± 144.42^{b_1}	6200.00 ± 141.42^{b_1}
	2 .14	11200.00 ± 0.00^{a_1}	13600.00 ± 282.84^{a_1}	15800.00 ± 0.00^{a_1}	18150.00 ± 212.13^{a_1}
	4.28	12800.00 ± 282.84^{a_1}	14300.00 ± 282.84^{a_1}	17250.00 ± 358.55^{a_1}	19050.00 ± 212.13^{a_1}

Means with different alphabetical superscripts differ significantly across the duration while means with different numerical figures differ significantly across the concentration ($p< 0.05$).

4.0 Discussion

This study investigated the toxic effects of various concentrations of dichlorvos on the juveniles of African catfish, *Clarias gariepinus*. Toxicity of various toxicants are known to be dependent on various factors such as developmental stages, concentrations,sex and duration n of exposure(Pandey *et al.,* 2011). There were behavioural changes in the activities of *Clarias gariepinus* treated with different acute concentration of dichlorvos compared to the control. Among the behavioural changes were hyperactivity, equilibrium status, increased erratic swimming and jerky movement. Mucus accumulation was observed on the body surface and gill filament of dead fish during the present study. This might be as a result of increase in the activity of mucus cells due to subsequent exposure to pollutants. The tested concentrations of 2.14 and 4.28mg/l could be environmentally relevant since dichlorvos is commercially used in the control of pest. In view of the repeated application and sources from other anthropogenic agents, dichlorvos.

concentration maybe present in the environment beyond the safe level of 1ppm. This could pose potential health hazards to both vertebrate and invertebrate populations in the ecosystem. (Suzawa and Igraham, 2008).

Results from hematological parameters show a significant increase in the WBC and decrease in PCV and other parameters analyzed. This is an indication of inhibition or damage in the erythrocytes and other blood cells. Eisler(1967). The oxygen carrying capacity of the red blood cell is also inhibited because of the damage of the haemoglobin. There is a reduction in the red blood cell and Hb across the concentration.

At chronic level, dichlorvos elicited responses in the blood parameters.

4.2 Conclusion

The present findings show that fishes showed a marked change in their behaviour and mortality when exposed to various concentrations of the chemical. Mortality as observed is both time and concentration dependent**.**

The result obtained from this study shows that high concentrations of dichlorvos above its safe level of 1ppm is highly toxic to non target organisms. The application and use of dichlorvos for the control of pest should be highly monitored by relevant agencies. Further research on the genotoxic effects of dichlorvos would be promising so as to assess the molecular damage caused by dichlorvos to non target organisms.

4.3 Recommendations

- There should be urgent education of farmers and the public in general on the dangers of dichlorvos to the environment.

- Manufacturers should be compelled to categorically state the effects of dichlorvos on non target organisms.
- Manufacturing industries should brain storm and come up brilliant ideas on ways of reducing the potency of dichlorvos to non target organisms.
- The federal ministry of environment and state ministries should as a matter of urgency set up committees that would monitor and control the use of dichlorvos.

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