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Original Research Article

Effects of sub-lethal toxicity of chlorpyrifos and DDforce pesticides on haematological parameters of *Clarias gariepinus*

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The injudicious and unregulated discharge of agricultural chemicals especially pesticides into aquatic bodies have caused ecological problems to all classes of aquatic organisms including fish. In this study, an attempt was made in assessing the toxic effects of sublethal concentrations of chlorpyrifos and DDforce on the haematological parameters of *Clarias gariepinus* juveniles. The range-finding tests for chlorpyrifos (0.40, 0.55, 0.70 and 0.85mg/l) and DDforce (0.15, 0.20, 0.25 and 0.30mg/l) was carried-out to determine the concentrations of the test solution for the definitive test. The 96h LC_{50} value was found to be 0.30mg/l chlorpyrifos and 0.18mg/l DDforce. Thereafter, $1/10^{th}$, $1/50^{th}$ and $1/100^{th}$ of LC_{50} was taken and the experiment continued for four weeks (28 days). The blood sample was collected on days 14 and 28. Standard haematological procedures were adopted to evaluate the blood parameters. There were significant reduction ($p<0.05$) in packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), lymphocytes and monocytes at days 14 and 28 while white blood cell (WBC), neutrophils and mean cell haemoglobin concentration (MCHC) values showed significant increase ($P<0.05$) at days 14 and 28. However, there were variations in mean values of mean cell volume (MCV) and mean cell haemoglobin (MCH) at days 14 and 28 respectively. Results obtained in this study suggest that exposure to low concentrations of chlorpyrifos and DDforce induced stress and altered the haematological parameters of *Clarias gariepinus* juveniles.

Key words: Acute toxicity, chronic toxicity, chlorpyrifos, *C. gariepinus*, DDforce, haematological parameters.

INTRODUCTION

Injudicious and unselective use of agrochemicals has caused great distress among health and environmental scientists. Accounts of field application of pesticides in developed countries revealed that less than 0.1% of pesticides applied to crops reach target pest, thus over 99% moves into an environment to pollute the land, water and air (Pimental, 2005). Among these pesticides are Chlorpyrifos (0, 0-Diethyl – 0 – 3, 5, 6 – trichloro-2-pyridylphosphorothioate) and DDforce (2, 3-dichlorovinyl dimethyl phosphate), an organophosphate (OP) insecticides commonly used in agriculture and houses to control the variety of insects are highly toxic to fish and aquatic invertebrates. Exposure to the low level of pesticides has attested to cause profound effects on non-target organisms.

Pesticides may also find its way into the food chain and cause functional damage (Waliszewski et al., 2003) and affect and alter the performance of the organism (Silva and Gammon, 2009). Their residues often sink to the bottom of the water body where they exert effects on aquatic lives, particularly fish. They generate a main menace because of their injuriousness persistency and propensity to gather in the organism (Joseph and Raj, 2010).

Blood is the most essential and abundant body fluid and is a vehicle for quickly mobilizing defense against trauma and ailments. Its composition often reflects the overall physiological disorder and extensively used in Ichthyology research, aquaculture research as well as toxicology and biological monitoring (Adedeji et al., 2008; Adeyemo, 2008). The blood parameters have been considered as diagnostic guides of the pathological condition and are significant for the assessment of systemic functions and general health of animals. Changes in haematology also aid in diagnosing the structural and functional status of faunas exposed to the toxicants (Suvetha et al., 2010; Chaudhary et al., 2015; Prasad et al., 2015). Fishes are known to be in close relationship with the aquatic environment, hence, the blood will divulge conditions within the body of the fish long before there is any visible manifestation of disease (Okechukwu and Auta, 2007).

Studies have revealed that when the aquatic quality is affected by contaminants, any physiological variations will be revealed in values of one or more haematological parameters of aquatic animals (Akinrotimi et al., 2007b; Gabriel et al*.,* 2007c). Penalties of toxicants or pesticides on haematological parameters of a number of fish species have been examined in several fish species: in *Cyprinus carpio* (Satyanarayan et al., 2004; Salvo et al., 2008); in *Clarias batrachus* (Summarwar and Verma, 2012); in *Oreochromis mossambicus* (Ali and Rani, 2009; Desai and Parikh, 2012); in *Heteropneustes fossilis* (Chaudhary et al., 2015; Prasad et al., 2015), and in *Puntius mesopotamicus* (Carraschi et al., 2012). However, limited information is available on the effects of DDforce and chlorpyrifos, particularly with reference to the sub-lethal concentration on the haematological modulation in *Clarias gariepinus*. Hence, this study was carried-out to assess and contribute to knowledge on the haematological alterations in *Clarias gariepinus* at different concentrations of DDforce and chlorpyrifos.

MATERIALS AND METHODS

Sample Collection, Examination, and Preparation

Two hundred and fifty (250) healthy and active *C. gariepinus* juveniles (15-21cm in length; 58-75g in weight) were obtained from Federal Department of Fisheries, Alagbaka, Akure, Ondo State, Nigeria; and transported in a plastic container filled with pond water to the Environmental Biology and Public Health Laboratory of the Federal University of Technology, Akure, Ondo State, Nigeria. The health status of selected fish was assessed based on the presence or absence of physical injuries and other morphological deformations. The fish was certified healthy by assessment before the commencement of the study. They were acclimatized under laboratory conditions (27°C Temperature, 42% Relative Humidity) for three weeks (21 days) prior to the commencement of the experiment. They were fed to satiety daily (7:00 am and 7:00 pm) with Durante floating pellets containing 65% crude protein. Feeding was terminated 24h prior to the range-finding and toxicity test, to reduce ammonia content in the water.

Water Quality Parameter Measurements

Some water quality parameters were measured during and before the acute toxicity test. A hygrothermometer (HH439 model) was used to measure the water temperature (°C). pH and Dissolved oxygen (mg/l) level were measured using Hanna pH meter (HI 96107 model) and a dissolved oxygen meter (DO- 970 model) according to APHA (1992).

Test Chemicals

Chlorpyrifos 480EC (0, 0-Diethyl – 0 – 3, 5, 6 – trichloro- 2-pyridyl phosphorothioate), Batch No: 20140620, NAFDAC Reg: A5-0714 and DDforce 1000EC (2, 3-dichlorovinyl dimethyl phosphate), Batch No: 20141212, NAFDAC Reg: A5-0107 an organophosphate (OP) insecticide were purchased from an agrochemical shop in Akure, Ondo State, Nigeria; and stored at ambient temperature (27°C). The test concentrations were prepared as described by Food and Agricultural Organization (1977) manual of Aquatic Science Research.

Acute Toxicity Test

The acute toxicity test to know the 96h LC_{50} values of DDforce and Chlorpyrifos was accompanied with a definitive test in a semi-static system in the laboratory following standard methods (APHA, 2005). A range-finding tests for Chlorpyrifos (0.40, 0.55, 0.70 and 0.85mg/l) and DDforce (0.15, 0.20, 0.25 and 0.30mg/l) was carried out to determine the concentrations of the pesticides solution for the definitive test. The fish were starved for 24 hours prior to acute toxicity tests. The experiment was conducted in 40 x 20 x 20 cm plastic tank containing 25L of well water which was continuously aerated in the laboratory. Dead fish were immediately removed to avoid possible deterioration of the water quality. In the definitive test, a set of 10 fish specimens were randomly exposed to Chlorpyrifos and DDforce for 96 hours at 0.40, 0.55, 0.70, 0.85mg/l; and 0.15, 0.20, 0.25, 0.30mg/l concentrations in triplicates. Another set of 10 fish specimens was simultaneously maintained in

water, without test chemical, and considered as control. Behavioral changes in the fish during the test period were observed. The temperature (24.00±0.00, 23.54±0.26, 26.33±0.27), pH (5.27±0.03, 5.29±0.08, 6.67±0.07) and dissolved oxygen (3.83±0.03, 3.87±0.09, 5.87±0.03) for Chlorpyrifos and DDforce were monitored during and before the experiment.

Sublethal Toxicity Test

For chronic toxicity study, the 96h LC_{50} value of Chlorpyrifos and DDforce on *C. gariepinus* was found to be 0.30mg/l Chlorpyrifos and 0.18mg/l DDforce, $1/10^{th}$, $1/50^{th}$ and $1/100^{th}$ of LC_{50} was taken and the experiment was continued for four weeks (28days). Fish were fed once a day and water exchange was made daily with fresh test solutions in each experimental container. The fish were randomly divided into three groups without regard the sex. Fish in the first treatment group were not exposed to treatment and served as control, while those in second and third groups were treated with 0.03, 0.006 and 0.003mg/l of Chlorpyrifos and 0.018, 0.0036 and 0.0018 mg/l of DDforce respectively. Each treatment group was further randomized into three replicates of 10 fish per replicate in 25L (40 × 20 × 20 cm) plastic tanks.

Blood Collection and Analysis

Samples from both the control and test fish were sacrificed for haematological study by puncturing the heart with a heparinized plastic syringe fitted with 21 gauge hypodermic needle and preserved in disodium salt of Ethylene Diamine Tetraacetic Acid (EDTA) bottles. The Blaxhall and Daisley (1973), and Wedemeyer et al. (1983) haematological methods were adopted for this study. The cyano-haemoglobin method was used to determine haemoglobin (Hb) using diagnostic kits from Sigma diagnostics USA and packed cell volume (PCV) was determined by the microhaematocrit method. Red blood cell (RBC), leucocrit (LCT) and thrombocyte count were determined with the improved Neubauer haemocytometer according to (Dacie and Lewis, 1991). White blood cells (WBC) was determined with the improved Neubauer counter, while differential counts such as neutrophils, lymphocytes, and monocytes were determined on blood film stained with May-Grunwald-Giemsa stain (Mirale, 1982).

Derived Parameters

Haematological parameters were further used to evaluate the effects of the pesticides on the fish blood. The calculation for each of the derived parameters used is as shown below:

MCV: The average volume of a single cell expressed in femtolitre (fl) or mm^3;

$$\text{MCV (fl)} = \frac{\text{PCV (\%)}}{\text{RBC } (10^6)} \times 10$$

MCH: This expresses the average Hb content in picorgramme of a single RBC;

$$\text{MCH (pg)} = \frac{\text{Hb}}{\text{RBC } (10^6)} \times 10$$

MCHC: This refers to the percentage haemoglobin in 1dl of packed RBC.

$$\text{MCHC (g/dl)} = \frac{\text{Hb}}{\text{PCV (\%)}} \times 100$$

Statistical Analysis

Data obtained for haematological parameters of *C. gariepinus* were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21 to generate the mean and standard error. Mean generated were separated and compared by Duncan's New Multiple Range Test (DNMRT). The mean mortality percentage of *C. gariepinus* treated with different concentrations of Chlorpyrifos and DDforce for 96 h were subjected to straight line graph using GraphPad prism version 7. Probit analysis was used to determine the concentration at which 50% mortality (LC_{50}) occurred using SPSS version 21.0.

RESULTS

The 96h (Acute toxicity test) percentage mortality of *C. gariepinus* treated with different concentrations of Chlorpyrifos and DDforce are presented in Figure 1 and 2. The percentage mortality was found increasing with an increase in the concentrations. The lethal concentrations at different levels of percentages were obtained using probit analysis. The LC50 for Chlorpyrifos and DDforce was 0.30mg/l and 0.18mg/l; the lower and upper limit are 0.210 and 0.392; 0.115 and 0.224. The least and highest mortality rate responses were observed at 0.40mg/l (30%) and 0.85mg/l (85%) of Chlorpyrifos; 0.18mg/l (30%) and 0.30mg/l (90%) of DDforce respectively.

The haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Chlorpyrifos and DDforce for 14 days are presented in Table 1 and 2. The Packed Cell Volume (28.33 ± 0.89^b, 25.33 ± 0.76^a, 24.43 ± 0.67^a, 24.00 ± 0.33^a), Red Blood Cells (77.67 ± 3.93^b, 72.67 ± 0.88^a,

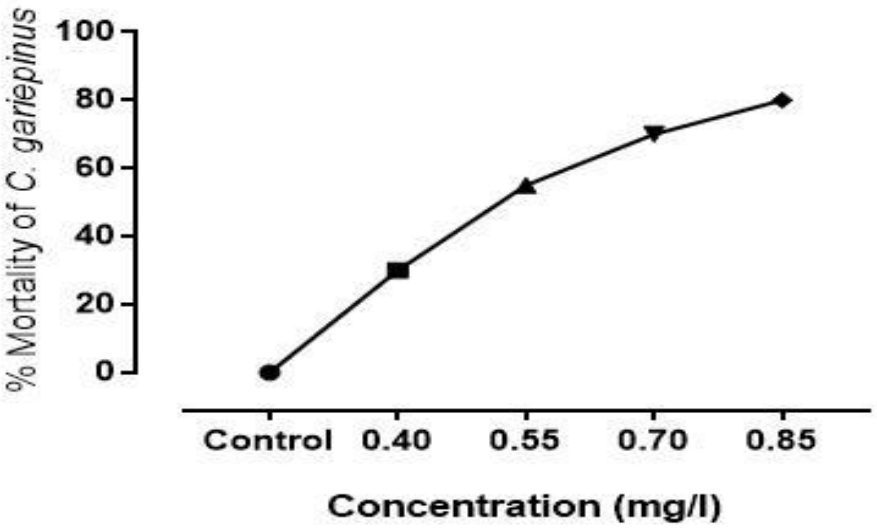

Figure 1: Mean mortality (%) of *C. gariepinus* treated with different concentrations of Chlorpyrifos at 96 hours.

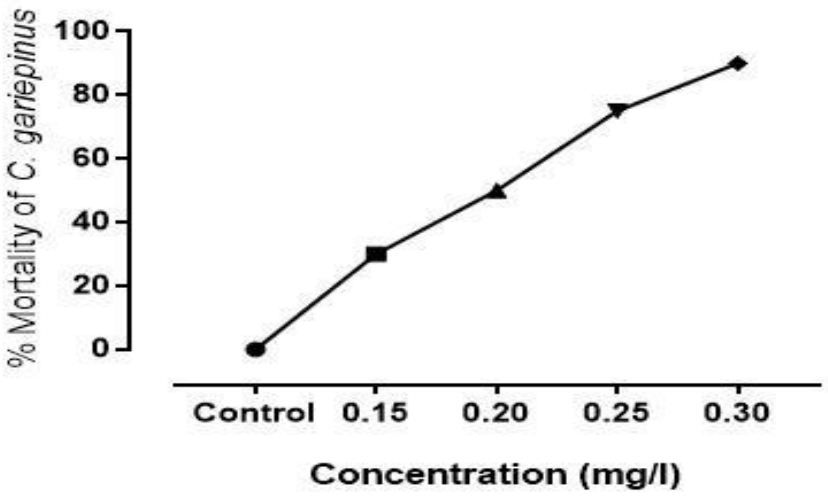

Figure 2: Mean mortality (%) of *C. gariepinus* treated with different concentrations of DDforce at 96 hours

70.33±0.67[a], 68.33±0.56[a]), haemoglobin (9.30±0.20[b], 7.43±0.15[a],6.80±0.21[a], 6.12±0.34[a]) and lymphocytes (67.67±0.33[c], 62.33±1.45[b], 61.67±0.33[b], 55.67±1.09[a]) decreased significantly (P<0.05) from lowest concentration to the highest concentration compared to the control. While White Blood Cells (56.33±2.73[a], 60.34±1.45[b], 62.83±3.18[b], 63.42±3.89[b]) neutrophil (18.33±0.33[a], 19.67±1.20[a], 22.00±0.58[b], 23.67±0.78[b]) and Mean Corpuscular Haemoglobin Concentration (29.40±1.86[a], 30.37±1.33[a], 35.50±0.61[b], 35.76±1.57[b]) increase significantly (P<0.05) from lowest concentration to the highest concentration compared to the control. There was a significant difference (P<0.05) in the PCV, RBC, WBC, Hb, lymphocytes, neutrophil and MCHC except for 1/100[th] (0.003 mg/l, 0.0018 mg/l) of the Chlorpyrifos and DDforce that shows no significant difference (P>0.05) compared to the control. Also, there is a significant difference (P<0.05) in the neutrophil except for 1/100[th] (0.003 mg/l) of the Chlorpyrifos that shows no significant difference (P>0.05) compared to the control. There is no significant difference (P>0.05) in the monocytes, basophil, eosinophil, MCV and MCH compared to the control.

Table 1. Haematological parameters of *Clarias gariepinus* exposed to different concentrations of Chorpyrifos for 14 days

Parameters	0.00mg/l	0.003mg/l	0.006mg/l	0.03mg/l
PCV (%)	28.33±0.89^{d}	25.33±0.76^{a}	24.43±0.67^{a}	24.00±0.33^{a}
RBC (10^{9}/l)	77.67±3.93^{c}	72.67±0.88^{a}	70.33±0.67^{a}	68.33±0.56^{a}
WBC (10^{8}/l)	56.33±2.73^{a}	60.34±1.45^{b}	62.83±3.18^{b}	63.42±3.89^{b}
Hb (g/dl)	9.30±0.20^{b}	7.43±0.15^{a}	6.80±0.21^{a}	6.12±0.34^{a}
Lymphocytes (%)	67.67±0.33^{c}	62.33±1.45^{b}	61.67±0.33^{b}	55.67±1.09^{a}
Neutrophil (%)	18.33±0.33^{a}	19.67±1.20^{a}	22.00±0.58^{b}	23.67±0.78^{b}
Monocytes (%)	12.00±0.58^{a}	11.33±0.67^{a}	10.98±0.58^{a}	10.00±0.78^{a}
Basophil (%)	1.67±0.33^{a}	2.67±0.67^{a}	2.67±0.33^{a}	2.65±0.33^{a}
Eosinophil (%)	1.33±0.33^{a}	2.33±0.33^{a}	2.31±0.33^{a}	2.24±0.18^{a}
MCV (10^{-12}fl)	2.81±0.30^{a}	3.35±0.14^{a}	3.16±0.06^{a}	3.28±0.78^{a}
MCH (10^{-12}pg)	0.99±0.08^{a}	1.11±0.01^{a}	1.18±0.04^{a}	1.32±0.07^{a}
MCHC (g/dl)	29.40±1.86^{a}	30.37±1.33^{a}	35.50±0.61^{b}	35.76±1.57^{b}

Note: Means values with the same superscript alphabets in the rows are not significantly different (P<0.05) from each other using Duncan's New Multiple Range Test (DNMRT).

Table 2. Haematological parameters of *Clarias gariepinus* exposed to different concentrations of DDforce for 14 days

Parameters	0.00mg/l	0.0018mg/l	0.0036mg/l	0.018mg/l
PCV (%)	28.33±0.89^{d}	24.48±0.86^{c}	20.44±0.64^{b}	17.83±0.53^{a}
RBC (10^{9}/l)	77.67±3.93^{c}	72.73±0.67^{b}	69.58±0.87^{b}	63.75±0.78^{a}
WBC (10^{8}/l)	56.33±2.73^{a}	60.37±1.78^{b}	61.88±3.28^{b}	63.72±3.19^{b}
Hb (g/dl)	9.30±0.20^{b}	7.10±0.35^{a}	6.77±0.41^{a}	6.09±0.67^{a}
Lymphocytes (%)	67.67±0.33^{c}	62.25±1.20^{b}	60.73±0.82^{b}	55.69±1.45^{a}
Neutrophil (%)	18.33±0.33^{a}	20.77±1.11^{b}	23.34±0.78^{b}	24.05±0.56^{b}
Monocytes (%)	12.00±0.58^{a}	11.00±0.00^{a}	10.67±0.67^{a}	10.45±0.64^{a}
Basophil (%)	1.67±0.33^{a}	2.33±0.33^{a}	2.67±0.33^{a}	2.68±0.34^{a}
Eosinophil (%)	1.33±0.33^{a}	1.67.0.33^{a}	1.56±0.18^{a}	1.45±0.21^{a}
MCV (10^{-12}fl)	2.81±0.30^{a}	2.93±0.06^{a}	3.13±0.07^{a}	3.10±0.26^{a}
MCH (10^{-12}pg)	0.99±0.08^{a}	0.94±0.03^{a}	1.07±0.04^{a}	1.95±0.07^{a}
MCHC (g/dl)	29.40±1.86^{a}	31.37±0.50^{a}	34.13±1.18^{b}	34.78±1.27^{b}

Note: Means values with the same superscript alphabets in the rows are not significantly different (P<0.05) from each other using Duncan's New Multiple Range Test (DNMRT).

The haematological parameters of *C. gariepinus* exposed to sub-lethal concentrations of Chlorpyrifos and DDforce for 28 days are presented in Table 3 and 4. There is a significant reduction (P<0.05) in the Packed Cell Volume (29.97±1.39^{d}, 19.97±0.68^{c}, 17.23±0.17^{b}, 12.97±0.97^{a}), Red Blood Cells (86.76±2.83^{d}, 65.73±0.73^{c}, 59.73±0.87^{b}, 51.85±0.77^{a}), haemoglobin ((11.27±0.67^{b}, 4.98±0.30^{a}, 4.04±0.11^{a}, 3.86±0.24^{a}) lymphocytes (70.09±1.22^{c}, 60.77±0.97^{b}, 54.53±0.21^{a}, 53.72±1.02^{a}) and monocytes (14.00±0.00^{b}, 11.67±0.13^{a}, 11.23±0.33^{a}, 10.89±0.23^{a}) from lowest concentration to the highest concentration compared to the control. While White Blood Cells (60.92±2.97^{a}, 67.56±1.78^{b}, 71.54±4.21^{c}, 72.99±4.29^{c}) neutrophil (22.57±0.11^{a}, 24.03±1.56^{b}, 24.98±0.96^{b}, 25.71±1.45^{b}) and Mean Corpuscular Haemoglobin Concentration (31.17±0.93^{a}, 36.43±3.43^{b}, 37.23±1.65b, 39.98±2.56^{c}) increase significantly (P<0.05) from lowest concentration to the highest concentration compared to the control. Also, eosinophil of the Chlorpyrifos and Mean Corpuscular Volume (MCV) of the DDforce increase significantly (P<0.05) compared to the control. There was a significant difference (P<0.05) in the PCV, RBC, WBC, Hb, lymphocytes, neutrophil, monocytes, and MCHC compared to the control. While basophil, eosinophil, MCV, and MCH show no significant difference (P>0.05) compared to the control.

DISCUSSION

Acute and sublethal toxicity tests are commonly used to assess the toxicity of chemicals on non-target animals (Santos et al*.,* 2013). The 96h LC_{50} is one of the most important factors for evaluating the toxic effects of contaminants. The 96h LC_{50} value of Chlorpyrifos and DDforce in this study was found to be 0.30mg/l and 0.18mg/l which suggest that the pesticides are toxic to fish. A steady trend was generally observed in the mortality rate of *C. gariepinus* which increases with an increase in concentrations. At the early stage of the toxicant

Table 3: Haematological parameters of *Clarias gariepinus* exposed to different concentrations of Chorpyrifos for 28 days

Parameters	0.00mg/l	0.003mg/l	0.006mg/l	0.03mg/l
PCV (%)	29.97±1.39^{d}	19.97±0.68^{c}	17.23±0.17^{b}	12.97±0.97^{a}
RBC (10^{9}/l)	86.76±2.83^{d}	65.73±0.73^{c}	59.73±0.87^{b}	51.85±0.77^{a}
WBC (10^{8}/l)	60.92±2.97^{a}	67.56±1.78^{b}	71.54±4.21^{c}	72.99±4.29^{c}
Hb (g/dl)	11.27±0.67^{b}	4.98±0.30^{a}	4.04±0.11^{a}	3.86±0.24^{a}
Lymphocytes (%)	70.09±1.22^{c}	60.77±0.97^{b}	54.53±0.21^{a}	53.72±1.02^{a}
Neutrophil (%)	22.57±0.11^{a}	24.03±1.56^{b}	24.98±0.96^{b}	25.71±1.45^{b}
Monocytes (%)	14.00±0.00^{b}	11.67±0.13^{a}	11.23±0.33^{a}	10.89±0.23^{a}
Basophil (%)	2.00±0.00^{a}	2.00±0.10^{a}	2.67±0.33^{a}	2.23±0.26^{a}
Eosinophil (%)	1.33±0.33^{a}	1.85±0.00^{a}	2.00±0.30^{a}	2.27±0.34^{a}
MCV (10^{-12}fl)	2.73±0.08^{a}	3.32±0.23^{a}	2.71±0.10^{a}	2.82±0.24^{a}
MCH (10^{-12}pg)	0.87±0.04^{a}	1.20±0.02^{a}	1.11±0.02^{a}	1.58±0.07^{a}
MCHC (g/dl)	31.17±0.93^{a}	36.43±3.43^{b}	37.23±1.65^{b}	39.98±2.56^{c}

Note: Means values with the same superscript alphabets in the rows are not significantly different (P<0.05) from each other using Duncan's New Multiple Range Test (DNMRT).

Table 4. Haematological parameters of *Clarias gariepinus* exposed to different concentrations of DDforce for 28 days

Parameters	0.00mg/l	0.0018mg/l	0.0036mg/l	0.018mg/l
PCV (%)	29.97±1.45^{c}	18.48±1.56^{b}	15.74±0.84^{a}	14.09±0.77^{a}
RBC (10^{9}/l)	86.76±2.83^{c}	55.39±0.89^{b}	51.33±0.34^{a}	49.29±0.14^{a}
WBC (10^{8}/l)	60.92±2.97^{a}	64.19±2.28^{b}	64.98±3.23^{b}	66.24±3.67^{b}
Hb (g/dl)	11.27±0.67^{b}	4.73±0.45^{a}	3.92±0.22^{a}	3.07±0.33^{a}
Lymphocytes (%)	70.09±1.22^{c}	58.79±1.23^{b}	52.78±0.09^{a}	50.09±0.45^{a}
Neutrophil (%)	22.57±0.11^{a}	24.09±1.78^{b}	24.18±0.78^{b}	24.67±1.25^{b}
Monocytes (%)	14.00±0.00^{c}	11.33±0.03^{ab}	24.18±0.78^{b}	9.87±0.25^{a}
Basophil (%)	2.00±0.00^{a}	2.32±0.33^{a}	2.33±0.33^{a}	2.14±0.11^{a}
Eosinophil (%)	1.33±0.33^{a}	2.33±0.33^{a}	2.33±0.53^{a}	2.17±0.13^{a}
MCV (10^{-12}fl)	2.43±0.08^{a}	2.50±0.09^{a}	2.57±0.15^{a}	2.98±0.13^{a}
MCH (10^{-12}pg)	0.87±0.04^{a}	0.84±0.04^{a}	0.90±0.02^{a}	1.05±0.23^{a}
MCHC (g/dl)	31.17±0.93^{a}	35.09±0.55^{b}	35.60±1.24^{b}	35.98±1.45^{b}

Note: Means values with the same superscript alphabets in the rows are not significantly different (P<0.05) from each other using Duncan's New Multiple Range Test (DNMRT).

introduction, all the fish survived the initial attack. This may be owing to their defensive adaptations as the respiratory mucosa on the inner walls of the air-sacs is thrown into folds and ridges for increasing the surface area for gas exchange. In contact with low oxygen level, *C. gariepinus* breathe through their skin and even use their air bladder as an emergency lung by gulping surface air. During the 48, 72, and 96h of exposure, the fish displayed physiological malfunctions such as hyperventilation, motionless State, increase opercular ventilation, general body weakness, skin discoloration, loss of reflex, erratic swimming which were noticeable particularly among some fish in the highest concentrations of Chlorpyrifos (0.85mg/l) and DDforce (0.30mg/l) in which 80% and 90% mortality was recorded. The physiological malfunctions are believed to weaken the organism's resistance to toxins and consequently resulting in the significant death of almost 50% at the highest concentration. With progressive exposure, deaths become inevitable even at a lower concentration. This could be owing to stress and the cumulative impact of Chlorpyrifos and DDforce toxicity. The mortality pattern recorded in this study agrees with that observed by Rand and Pectrocelli (1985) which stated that there should be less than 35% mortality in one of the concentrations and at least more than 65% mortality in the highest concentration. The mortality observed in the study was considered a result of stress-induced on the immune system of fish. Thus, slow toxic progress and long continuance can result in a chronic toxic response.

The haematological parameters have been used as a sensitive indicator of stress in fish exposed to different aquatic contaminants and toxins of various types. Sublethal concentrations of toxicants in the aquatic ecosystem will not certainly result in the outright death of aquatic organisms. However, the bioaccumulation of these contaminants over an era of time may create potential health threats not only to the aquatic animals like fish but also on higher trophic level particularly man. Contaminants

can result in numerous physiological dysfunctions in fish which induce alterations in haematological parameters as a result of blood water interaction.

The packed cell volume (PCV), haemoglobin (Hb) and Red Blood Cell counts (RBC) are vital indicators of oxygen conveyance capacity of fish thus making it possible to create a relationship with the oxygen concentration present in the habitat and the health status of the fish (Lamas et al., 1994). On the other hand, the white blood cells confer protection or defense against infectious agent caused by microbial and chemical factors (Gusmao et al., 2007).

The significant reduction in PCV, Hb, and RBCs with increasing concentration and exposure time of *C. gariepinus* to Chlorpyrifos and DDforce indicating a condition of erythropenia and haemolysis resulting from impaired osmoregulation across the gill epithelium as there was a significant decline in dissolved oxygen level. It may also be due to the disruption of the iron synthesis or owing to the inhibitory effect of the toxic substance on the enzyme system accountable for the production of haemoglobin caused by exposure of the Chlorpyrifos and DDforce. Also, decrease in RBCs count, Hb concentration and PCV values apparently reflected erythrocyte haemolysis and/or irreversible impairment of kidney functions.

Adamu and Audu (2008) also reported that the significant decrease in PCV may be ascribed to gill damage and/or damaged osmoregulation leading to anaemia and haemodilution. The declined in RBCs might be attributed to the declined erythropoietic activity. In most vertebrates, including fishes, erythropoietic activity is controlled by erythropoietin produced in the kidney (Gluszak et al., 2006). This erythropoietin further helps erythropoiesis by inducing haemopoietic stem cells to differentiate into erythroblasts which form RBCs. Erythropoietin also triggers pyridoxal phosphate in development of RBCs, inducing haemoglobin production (Oruc 2010). The decline in Hb concentration might be owing to an increase in the rate at which Hb is damaged or a decrease in the rate of its production (Ural, 2013).

Similar to this finding, a decrease in the number of RBCs, Hb and PCV values of fish exposed to diazinon was stated by Banaee et al. (2008, 2011) and correlated it to destruction of cells and/or reduction in the size of cells owing to the adverse effects of the pesticide. Zaki et al. (2009) stated that RBCs count, Hb concentration, and PCV values were reduced in the fish exposed to malathion. Similar observations were reported for juvenile *C. gariepinus* separately treated with Lambda-cyhalothrin, Cypermethrin and Deltamethrin pesticides (Yekeen, 2009). A similar decrease in RBCs has stated for Cypermethrin treated *Labeo rhoita* (Das and Mukherjee, 2003), fresh water common carp (*Cyprinus carpio*) treated with diazinon (Svoboda et al., 2001) and *C. gariepinus* treated with diazinon (Adedeji et al., 2009). Reduction in Hb content observed in this finding may also be an indication of decline in haemoglobin synthesis as well as a decrease in oxygen carrying capacity which may perhaps be as a result of interference of Chlorpyrifos and DDforce with haemoglobin synthesis pathway. Ramaswamy et al. (1996) reported a significant decrease in Hb content in the blood of a fresh water fish, *Sarotherodon mossambicus,* on exposure to dimecron and carbamate.

Increase in WBC content with increase in concentrations observed in this finding may be due to the activation of the animal's defense mechanism and the immune system. Several chemical compounds including insecticides, generate antibodies owing to their interference with the immune system which could be the cause for an increase in WBC (Muralidharan, 2012). WBC is involved in the control of immunological function and the changes in the WBC counts after exposure to various toxins may indicate a reduction in non-specific immunity of the fish (Saha and Kaviraj, 2009) and the substantial increase in the WBC count may be a protective response in fish under stress. In general, increased WBC count in fish exposed to the chronic doses indicates leukocytosis such as lymphophenia and heterophilia which are characteristic of leucocytic response in animals exhibiting stress (Ahmad 2012). Stimulation of lymphopoiesis and/or improved release of lymphocytes from lymphomyeloid tissue under poisonous stress may lead to an increase in WBC count (Svobodova et al., 1997). In the present investigation, the substantial increase in WBC count may have caused from the excitation of the defense mechanism of the fish to stand the effect of pesticide (Gabriel et al., 2009). The increase in the number of WBC is a protective reaction against pesticide stress. These changes are probably the result of the initiation of the immune system in the presence of a pesticide, which in turn may be an adaptive response of the fish resulting in a more effective immune defense (Modesto and Martinez, 2010). This finding is in agreement with earlier investigators who have observed similar results after exposure of some fish to pesticides such as diazinon (Svobodova et al., 2003; Padash-Barmchi et al., 2010), malathion (Thenmozhi et al., 2011), paraquet (Safahieh et al., 2012) and curzate (Desai and Parikh, 2012).

The erythrocyte indices like mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) seems to change which are more sensitive and can cause reversible alterations in the homeostatic system of fish. Fluctuations in these indices directly correspond with the values of RBC count, Hb concentration, and PCV. A significant increase in MCHC was observed. However, slight fluctuations were recorded in the MCH and MCV as compared to the control. Olanike and Adeyemo (2007) found an increase in MCV, MCH, and MCHC on exposure of experimental fish to lead.

As regards the differential count of white blood cells, the lymphocytes and monocytes decreased significantly with an increase in the concentrations while the neutrophils increase significantly as compared to the control. The decrease and increase in the lymphocytes, monocytes, and

neutrophils may be associated with the nature of the immunological challenge to which the fish was exposed at a particular period of time and in the various sub-lethal concentrations of Chlorpyrifos and DDforce. The decreased number of monocytes in *C. gariepinus* with exposure to Chlorpyrifos and DDforce agreed with the report of Nussey et al. (1995) on *Oreochromis mossambicus* treated with Copper. Monocytopenia has been reported in *C. albopunctatus* exposed to gammalin 20 (Mgbenka et al., 2003). Akinrotimi et al. (2012) also stated decrease in lymphocytes and increase in neutrophils during exposure of *C. gariepinus* juveniles to cypermethrin. Impact of toxic substances on neutrophil number has been reported by Velisek et al. (2009) in air-breathing fish *Anabas testudinetis* exposed to detergent. The basophils and eosinophils were reported to be the least values of the differential count of white blood cell in *C. gariepinus* juveniles with exposure to Chlorpyrifos and DDforce. Modra et al. (1998) observed the low concentration of eosinophils and the absence of basophils in several fish species, such as *Cyprinus carpio, Tinca tinca, Siluris glanis* and *Oncorhynchus mykiss*. In this finding, the observed low percentage of basophils and eosinophils supports the findings of Srivastava and Narain, A. S. (1982) who observed very low percentage of basophils and eosinophils in *Clarias batrachus*, *Hetropneustes fossils,* and *Amphipnous Cuchia.*

CONCLUSION

It can be deduced from this finding that chlorpyrifos and DDforce have the potential to damage the physiological activities of the fish which led to alterations observed in haematological parameters. The alterations in these parameters may provide early warning signals for the determination of acute and chronic toxic levels of pesticides used in the field and its effects on the aquatic organisms. Hence, discharge of these pesticides in the aquatic environment should be restricted in order to reduce its potential risk to fishes as well as humans.

Conflict of interest

No conflict of interest exits in the submission of this manuscript.

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