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Full Length Research Paper

Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*)

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The aim of this study was to assess the effect of diazinon [0,0-diethyl 0-(2-isopropyl-6-methylpyrimidin-4yl) phosphorothioate] on cultured and wild African catfish (*Clarias gariepinus*). The effect was assessed based on results of acute toxicity tests and on a comparison of results of haematological examination of a control and an experimental group exposed to Diazintol^R pesticide preparation (active substance 162 mg.l^{-1} of diazinon). The acute toxicity test lasting 96 h was performed semi-statically on the African catfish of mean weight 350 ± 15 g, and mean total length of 35 ± 2.0 cm. Examination of erythocyte and leucocyte profile was performed on 16 control and 20 experimental specimens of the catfish after 96 h of exposure to Diazintol^R in concentration of 9.4 mgl^{-1}. The 96 h LC_{50} value of Diazintol^R for the African catfish is 6.6 mgl^{-1}. The experimental group of the African catfish showed significantly lower values ($p < 0.05$) of erythocyte count (RBC), haemoglobin content (Hb) and haematocrit (PCV) compared to the control group. Values of MCV, MCH and MCHC were comparable in both groups during the study. On the contrary, there was a significant decrease in leucocyte count ($p < 0.05$), as well as in both the relative and absolute lymphocyte count ($p < 0.05$) and a significant increase in both the relative and absolute count of developmental forms of neutrophile granulocytes: myelocytes ($p < 0.05$) and metamyelocytes ($p < 0.05$) in the experimental group. Relative and absolute count of monocytes and both the band- and segmented neutrophile granulocytes was comparable in both groups during the study. Changes in values of both the erythocyte and leucocyte profile after exposure to diazinon-based preparation may be referred to disruption of haematopoiesis as well as to a decrease on non-specific immunity of the fish.

Key words: Pesticide, diazinon acute toxicity, erythocyte profile, leucocyte profile, catfish.

INTRODUCTION

Pesticide use is known to cause serious environmental problems, especially in the dry season, because during this period the dilution capacity of the water systems is low, thus increasing the risk of high concentrations of toxic chemicals. Moreover, the dry season is often the critical period for many animals, especially fish and birds. Fish stocks suffer from natural mortality and high fishing pressure at the end of the dry season. Contamination of water by pesticides either directly or indirectly can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans eating these fishes.

Blood analysis is crucial in many fields of ichthyological research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation (Adedeji et al., 2000). Haematological indices are very important parameters for the evaluation of fish physiological status. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners, and diseases (Luskova, 1997; Golovina, 1996; Zhiteneva et al., 1989). In warm-blooded animals, changes in the blood parameters of fish, which occur because of injuries or infections of some tissues or organs, can be used to determine and confirm the dysfunction or injuries of the latter (organs or tissues). However in fish, these parameters are more related to the response of the whole organism, that is, to the effect on

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fish survival, reproduction and growth. Although the mechanisms of fish physiological and biochemical reaction to xenobiotics has not been enough investigated, it is obvious that species differences of these mechanisms exist. Thus, a significant number of data on the changes of haematological indices of salmonid fish under the influence of various environmental factors (chemicals as well), which allow certain conclusions to be drawn, can be applied to other fish species only with much care (Folmar, 1993). On the basis of studies of warm blooded animals, specific indices reflecting the effect of some substances on the organism (e.g. changes in the blood serum cholinesterase under the influence of insecticides or changes in methaemoglobin under the influence of nitrites) have been determined, however the complex of unspecified biochemical indicators of blood reveals more fully the general effect of pollutants on fish and makes possible to forecast the consequences of long-term exposure to chemical pollutants.

Many workers have assessed the effect of various pesticides on the behaviors and haematological responses of various species of fish, (Anees, 1978; Benarji and Rajendranath, 1990; Svoboda et al., 2001) and have found varying responses after exposing the fish to varying sublethal concentrations using the 96 h acute toxicity tests. The purpose of this study is to assess and contributes to knowledge on the heamatological changes in cultured and wild African catfish following the exposure of the fish to diazinon.

MATERIALS AND METHODS

Acute toxicity test and blood collection

The acute toxicity tests lasting 96 h was performed semi-statically on the African catfish of mean weight 350 ± 15 g, and mean total length of 35 ± 2.0 cm according to Adedeji (2008). At the end of 96 h blood samples were collected from the remnant fish. Examination of erythocyte and leucocytes profile was carried out on 16 catfish (control) and 20 catfish (experimental). For blood collection 0.2 mg benzocaine dissolved in 5 ml acetone in 4 liters of water was used as an anesthetic agent. Blood was drawn from the posterior caudal vein according to Schmit et al. (1999) with a 22 G hypodermic needle and 2 ml was decanted into plastic tubes containing the sodium salt of ethylene diamine tetra acetic acid (Na-EDTA) as an anticoagulant. Whole blood (50 µl) was stained for enumeration of red blood cells (Shaw, 1930). Blood smears were air dried for 5 min, fixed in absolute methanol, and stained for 60 seconds in giemsa stain.

Haematological analyses

Red blood cell (RBC), total white blood cell (WBC) and platelet counts were done using the Neubauer haemocytometer. The haematocrit or packed cell volume (PCV) and haemoglobin (Hb) concentration values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods (Hesser, 1960), respectively. The mean corpuscular volume haemoglobin (MCV), mean corpuscular haemoglobin (MCH) and mean haemoglobin concentration (MCHC) were calculated from the data using standard formulae:

Blood smears made and stained with Giemsa, were used to determine WBC, thombocyte count and differential WBC counts. Percentage of RBC, WBC and thombocytes were determined by counting 1,500 cells. The WBC and thombocyte percentage was multiplied by the RBC count from the haemocytometer to determine the WBC and thombocyte absolute count. For the differential count, WBC and thombocytes were counted until 200 WBC were enumerated on blood smears, and the percentage of each WBC type and of thombocyte were multiplied by the total WBC and thombocyte count to obtain absolute differential cell counts. This method of manually determining total WBC and differential count has been recommended for avian (Zinkl, 1986) and fish (Stoskopf, 1993) blood, because nucleated RBC prevent accurate enumeration using automated analysis (Huffman et al., 1997). The majority of blood values determined for fishes have been reported as mean ± SEM (Hubec et al., 2000), the data generated in this study are thus presented as mean ± SEM.

Statistical analyses of data

The data obtained from this study were subjected to various statistical tools. The differences in the means (± SEM) between groups were assessed using students' t-test, Pearson's correlation and Levene's test for equality of variance (SAS, 1988). A P-value of $P < 0.05$ was taken as significant.

RESULTS

Fish after an acute exposure to diazinon had significantly lower erythocyte count ($p < 0.05$) when compared with the control specimens (cultured and wild catfish), as shown in Table 1 and Figure 1. There was a significant decreased ($p < 0.05$) in WBC count in the test groups (cultured and wild catfish) following the exposure to acute effect of diazinon (Table 1 and Figure 4). The PCV value was significantly ($p < 0.05$) decreased in the test group (cultured and wild catfish) exposed to the acute effect of the diazinon (Table 1 and Figure 2). At ($p < 0.05$) there was a significant decrease in the haemoglobin content of the exposed group (cultured and wild catfish) compared with the control groups (Table 1 and Figure 3).

The absolute and relative values of the differential WBC counts are as shown in Table 1.There was a decrease in both the absolute and the relative lymphocyte counts in the experimental group ($p < 0.05$) following the acute effect of diazinon on the experimental groups. There were significant increases in thombocyte count in the experimental group following exposure to acute effect of diazinon ($p > 0.05$) (Table 1 and Figure 5). The values of MCV in the experimental groups (cultured and wild catfish) showed significant decrease ($p < 0.05$) following the acute effect of diazinon (Table 2 and Figure 6, in the test treatment). MCH values in the experimental groups (cultured and wild catfish) showed significant decrease ($p < 0.05$) following the acute effect of diazinon (Table 2 and Figure 7).

DISSCUSION

Haematological indices are of different sensitivity to

Table 1. Mean values of haematological analysis control and exposed group.

Indices	Cultured						WILD					
	Control			Test Group			Control			Test Group		
	Values	SD	SEM	Values	SD	SEM	Values	SD	SEM	Values	SD	SEM
PCV %	37.25	2.12	0.75	25.75	2.59	0.75	31.17	1.47	0.42	26.250	3.327	1.174
HB g/100 ml	10.10	0.214	7.56E-2	6.967	0.577	0.17	8.40	3.94	1.14	7.075	0.623	0.220
RBC x 10 E 6	2.4563	0.168	5.93E-2	1.4433	0.255	7.351E-2	2.145	1.01	0.29	14575	0.2400	8.487E-2
WBC x 10 E 3	40250.0	1966.7	695.33	20691.7	3294.5	951.03	32983.3	15836.3	4571.6	20631.2	3726.4	1317.49
THOM	382500.0	43803.95	15487	410416.7	50563.1	14596.3	361800	37243.3	11777.4	418750	53768	19010.1
LYMPH	90.00	2.1381	1.0522	66.2455	3.3421	0.9474	90.00	2.1082	0.6667	67.663	3.925	1.3879
HETER	6.00	2.9761	1.0522	29.7500	1.8153	0.5240	6.100	2.271	0.718	28.50	2.0702	0.7319
MONO	3.050	1.1650	0.4119	2.833	1.7495	0.5050	1.214	0.7559	0.286	1.667	0.5164	0.2108
EOSIN	1.033	0.5774	0.3333	1.250	0.5	0.25	2.467	1.4142	0.4714	3.00	1.8257	0.6901

Table 2. Derived haematological parameters wild and cultured catfish (control and test).

	Wild catfish						Cultured catfish					
	Control (No = 8)			Test (No = 10)			Control (No = 8)			Test (No = 10)		
	MCV (fl)	MCH (pg)	MCHC (g/l)	MCV(fl)	MCH (pg)	MCHC (g/l)	MCV(fl)	MCH (pg)	MCHC (g/l)	MCV(fl)	MCH (pg)	MCHC (g/l)
MEAN	146.935	3.930	27.027	183.605	4.9461	27.219	152.633	4.1320	27.2035	183.1395	4.9373	27.2573
SEM±	4.85	9.9 E-2	0.518	12.0816	0.2496	1.0905	6.3522	0.1219	0.6605	9.8297	0.2128	0.9274
SD±	15.323	0.3125	1.6384	34.1718	0.7061	3.0845	17.9668	0.348	1.8682	34.0512	0.7372	3.2125

various environmental factors and chemicals (Vosyliene, 1999). Haematology and clinical chemistry analysis, although not often used in fish medicine, can provide substantial diagnostic information. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology, since they are sensitive to slight fluctuation that may occur within their internal milieu (Fernades and Mazon, 2003). On the basis of haematological studies, it would be possi-ble to predict the physiological state of fish in natural water bodies.

Haematology studies in teleosts have indicated that haematocrit values might be useful as a general indicator of fish health, since fish given iron deficient diets, or those exhibiting anaemia; all possess reduced haematocrit (PCV) values (Gatlin and Wilson, 1986). Previous haematological studies of nutritional effects (Rehulka, 1989, 2000), infectious diseases (Rehulka, 2002a) and pollutants (Rehulka, 2002b) brought knowledge that erythocytes are a major and reliable indicator of various sources of stress (Rainza-Paiva et al., 2000). Erythocytes reflect the state of the organism over a prolonged period of time (Sniezsko, 1961; Haley and Weiser, 1985). High concentration of pesticides or long term exposure of fish to their sublethal concentration usually decreases erythocyte indices.

In this study, the main haematological response of catfish (cultured and wild) to the acute exposure to diazinon based organophosphorous

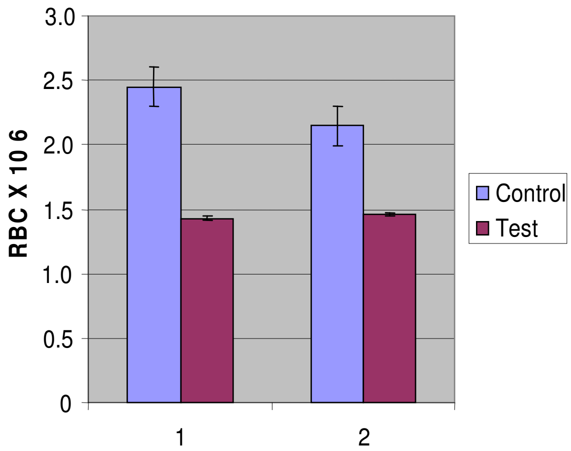

Figure 1. Red blood cell count (RBC) of control and experimental catfish exposed to acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) with different letters are significantly different ($p < 0.05$).

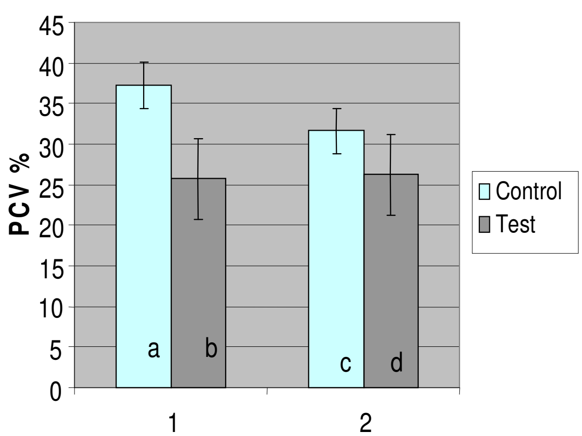

Figure 2. Packed cell volume (PCV) of control and experimental catfish exposed following acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

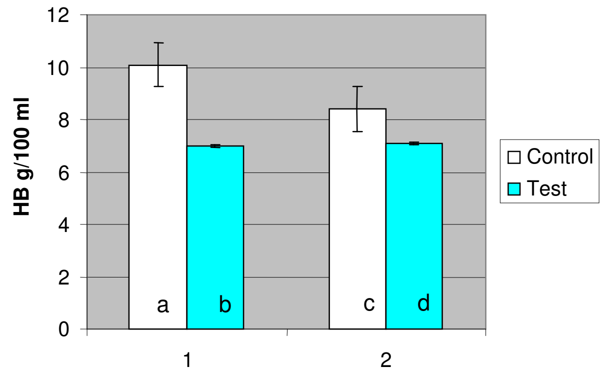

Figure 3. Haemoglobin concentration (HB) of control and experimental catfish exposed to acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

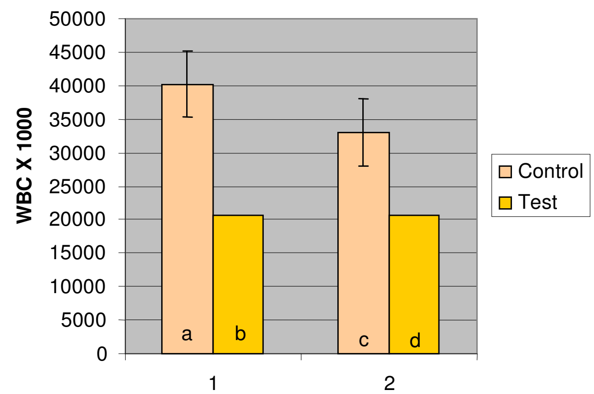

Figure 4. White blood cell count (WBC) of control and experimental catfish exposed to acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

pesticide LC_{50} 6.6 ppm was a significant decrease ($p < 0.05$) of erythocyte count, haematocrit value and haemoglobin content compared to the control groups (Table 1 and Figures 1, 2 and 3).

Decreased erythocyte count and haemoglobin content in freshwater fish *Cyprinus carpio* after acute exposure to diazinon was also reported by Svoboda et al., (2001). Other effective substances of organophosphorous pesticides also induce changes which give evidence for decreased haematopoiesis followed by anemia induction in fish. Changes in erythocyte profile induced by acute effect of dichlorvos in *Clarias batrachus* (Benarji and Rajendranath, 1990), trichlorphon in *Piaractus mesopotamicus* (Tavares et al., 1999), malathion in *Cyprinion wabsoni* (Khattak and Hafeez, 1996), formothion in *Heteropneustes fossilis* (Singh and Srivastava, 1994) and of Ekolux organophosphorous preparation in *Oreochomis mossambicus* (Sampath et al., 1993).

There was a significant decreased in WBC count as well as in both the relative and absolute lymphocyte counts in the test group cultured and wild catfish ($p < 0.05$) (Table 1 and Figures 4 and 5). Significant decrease of white blood cell count and absolute lymphopenia and granulocytosis characterize the white blood cell profile of common carp after the acute exposure to diazinon-based pesticide. Lymphopenia as a consequence of methylparathion based pesticide was reported by Nath and Banerjee (1996) in *H. fossilis* and also by Siwicki et al.

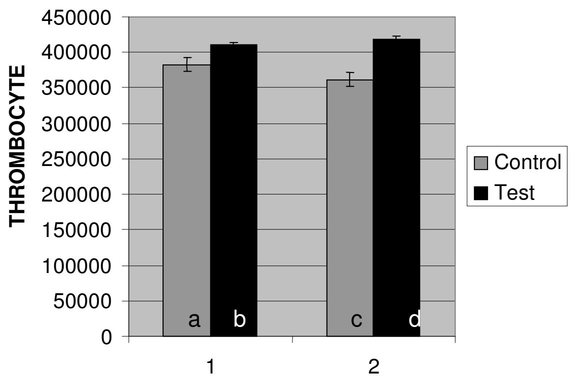

Figure 5. Thombocyte count of control and experimental catfish exposed to acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

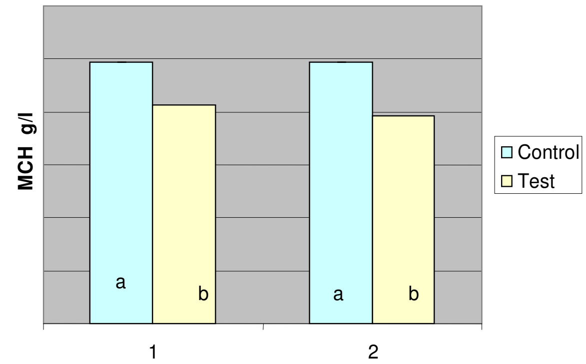

Figure 7. Mean corpuscular haemoglobin (MCH) of control and experimental catfish exposed to acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

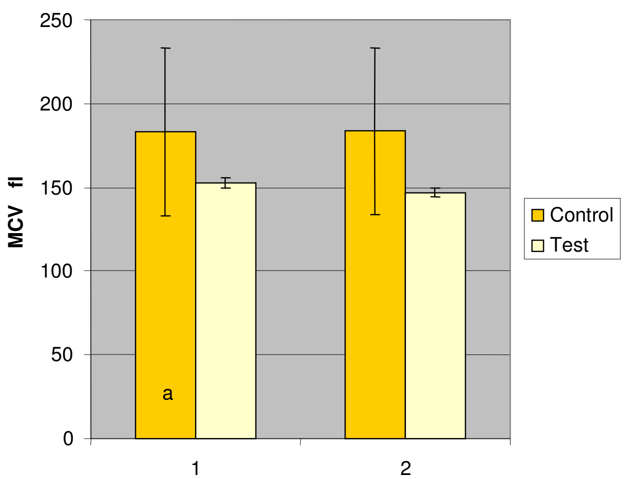

Figure 6. Mean corpuscular volume (MCV) of control and experimental catfish exposed to acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

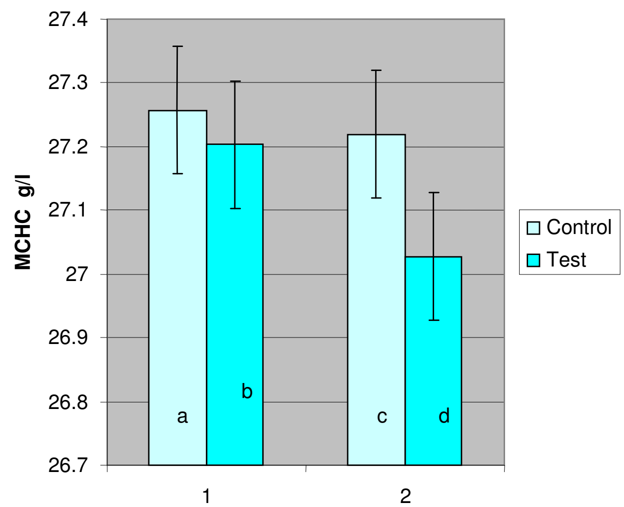

Figure 8. Mean corpuscular haemoglobin concentration (MCHC) of control and experimental catfish exposed to acute effect of diazinon. **1** Cultured catfish, **2** Wild catfish. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

(1990) in common carp after an acute effect of trichlorfon. Ghosh and Banerjee (1993) reported lymphopenia and both neutrophile and eosinophile granulocytosis in *H. fossilis* after an effect of dimethoate in 96h LC_{50} concentration. A decreased non-specific immunity in fish can be expected after acute exposure to organophosphorous pesticides due to decreased white blood cell count, lymphopenia and granulocytosis. Lymphopenia and granulocytosis have been reported after exposure to many pollutants (Svobodova et al., 1996). These changes in differential WBC count also give evidence for decreased level of nonspecific immunity in fish after acute exposure to toxic substances.

Diazinon induced a significant increase in thombocyte count in the experimental group. Thombocytes are comparable to mammalian blood platelets and play an important role in blood clotting, which prevents blood loss from hemorrhage. A high number of thombocytes reduce clotting time (Srivastava, 1969); by as much as 50% in cases where the number of circulating thombocytes was found to be one to two times higher than normal (Cassilas and Smith, 1977). MCV and MCH values showed significant decrease in the experimental group following acute effect of diazinon (Table 2, Figures 6 and 7). Another type of haematological response to the effect of organophosphorous compounds was a significant increment of MCV associated with increment of PCV value and drop of MCHC. This response was reported in Common *carp* after acute effect of phenitrothion, imidan and dichlorvos (Svobodova 1971, 1975).

Conclusion

The role of blood parameters in the assessment of the health status of fish is emphasized by the observations of Cyriac et al. (1989) and Omoregie (1998) who noted the possibility that changes in the blood will reveal conditions within the body of the fish long before any outward manifestation of diseases. Contrary to the submission of Moiseenko (1998), it is very difficult to forecast changes in fish of natural water bodies on the basis of haematological indices due to age, seasonal, and sexual fluctuations of these indices (Luskova, 1997). However detailed studies of the diversity of pathological changes in blood indices is required in order to find general regulations of transformation in the blood system of fish caused by various pollutants as well as pesticides with the possibility of finding critical point, below which irreversible changes in the blood circulation, blood formation and immune system, which lead to the increased mortality of fish, will go a long way in validating the use of haematological parameters as a useful biomarker to measure the exposure of fish to pesticides in natural waters.

This study has enhanced knowledge of physiological values of RBC and WBC indices including differential counts in the African catfish (*C. gariepinus*) cultured and wild exposed to various concentrations of diazinon. It has also been able to contribute to the applied and basic research needs of aquatic toxicology. It is important to know that erythopoiesis is influenced by a number of environmental factors and must be taken into account when haematological indices are evaluated.

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