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RESEARCH PAPER

Effect of Short Term Exposure to Cyperdicot on Behavioural and Haematological Responses in African Catfish *Clarias Gariepinus*

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Abstract

The effects of short term exposure to cyperdicot on behavioural and haematological responses in 300 *Clarias gariepinu* swere investigated. The fish were randomly divided into three groups of 30 fish. Fish in first treatment group were exposed to tap water and served as control, while those in second and third groups were treated with 0.04 and 0.08 mg l^{-1} of Cyperdicot, respectively. The 24, 48, 72 and 96 h LC_{50} values were1.462, 1.094, 1.030 and 0.800 mg l^{-1}, respectively. The safe level for the insecticide varied from 8 x 10^{-3} to 8 x 10^{-4}mg l^{-1}. Fish exposed to sub-lethal concentrations of insecticide exhibited alterations in various blood parameters including significant reductions in RBC count, Hb and PCV. A cyperdicot– induced dose- and time-dependent significant increase in W B C count from day 10 onward was observed, while values of blood indices such as MCV, MCH and MCHC in treated fish were not significantly different from those of the control group ($P>0.05$). This study revealed that the short term exposure to cyperdicot on behavioural and haematological responses in *Clarias gariepinus* elicited reduction of RBC, Hb and PCV values while MCV and MCH caused both macrocytic and microcytic anemia in the fish.

Keywords: Cyperdicot, *Clarias gariepinus*, haematology, behaviour parameters.

Introduction

Cyperdicot, a commercially formulated agrochemical insecticide, is known for its action against a wide range of insects. It is a synthetic pesticide composed of cypermethrin (50 mg l^{-1}) and dimethoate (250 mg l^{-1}) a synthetic pyrethroid and organophosphate derivatives (Agwu *et al*., 2016).It is a contact insecticide which kills target organisms by altering normal neurotransmission within the nervous system of the organisms by inhibiting the enzyme acetyl cholinesterase (ACHE), which hydrolyses the neurotransmitter acetylcholine (ACH) in cholinergic synapses and neuromuscular junctions. Non-target organisms can be exposed to Cyperdicot by inhalation, ingestion and/or dermal exposure (Glen *et al*.,2014). It enters the aquatic environment because of its proximity to the agricultural activities along water bodies and has been detected in many rivers in both urban and agricultural regions (Ayoola and Ajani, 2007). Furthermore, the indiscriminate or misuse of the insecticide or discharge of untreated effluents into natural water ways, have harmful effects on the fish populations and other aquatic organisms and may contribute to long term ecotoxicological effects in resident aquatic organisms (Leilan*et al*.2015).

In the water, the molecules of these contaminants may bind to the materials in suspension, accumulate in the sediment or can be absorbed by the aquatic organisms with attendant physiological responses including effect on behavior and haematology (Jordan *et al.,* 2013).As result of high water solubility, low persistence and extensive usage of the insecticide in the environment, exposure to non-target aquatic organisms is a source of concern. Changes in enzyme activity and other biomarkers have been studied as possible tools for aquatic toxicological research (Moore and Simpson, 1992; Abuo *et al.,* 2001).

Sub-lethal effects are biochemical in origin, exerting their effects at basic levels of the organisms by reacting with enzymes or metabolites and other functional components of the cell. Transminase enzymes play vital roles in carbohydrate-protein metabolism in fish and other organism's tissues (Eze, 1983).

The indigenous African catfish, *C. gariepinus* was selected for the bioassay experiments because it can be found in other tropical countries of the world.

It is also an aquaculture candidate that can narrow the gap between the demand for and supply of animal protein in developing countries. The species is also an attractive model for toxicity studies because of its availability throughout the year, voracious feeding habit, prolific reproduction and general hardness in culture environments. The adverse effects of agrochemicals and their residues on non-target organisms have not been seriously considered in Nigeria (Ayoola and Ajani, 2007). Despite a number of researches carried out on the toxicity effects (Fazio *et al.*, 2014, Naccari *et al*., 2015; Di Bella *et al*., 2015) of agrochemical insecticide on the haematology of *Clarias gariepinus*, little is known about the lethal toxicity and haematological changes that *Clarias gariepinus* may undergo on exposure to Cyperdicot. The purpose of this study was to investigate the effects of short term exposure to cyperdicot on behavioural and haematological responses in African catfish, *Clarias gariepinus.*

Materials and Methods

Experimental Fish

Three hundred *C.gariepinus* juveniles, mean weight 150 + 5.20 g, length= 35.00 + 2.50 cm, from Sacen Fish Farm, were treated with 0.05% potassium permanganate to avoid possible dermal infections. They were acclimatised for 20 days in a 1000 l plastic tank, fed 3% body weight (BW) in divided rations twice daily (7.00 am and 7.00 pm) with a laboratory-prepared pelleted diet containing 35% crude protein (Eyo*et al*,. 2013). Feeding was terminated 24 h prior to the range -finding and toxicity test, to reduce ammonia content in the water (Ward and Parrish 1982, Reishand and Oshida, 1987). The ethical guidelines of the Animal Care Committee (UNN-EGACC, protocol no. 0430/2013) of the University of Nigeria, Nsukka were strictly followed.

The pH of water and sediment samples was measured in the laboratory using the Hanna pH meter (Hi-1922 model) according to APHA (1992).

The conductivity of water was determined using the Hanna 911 conductivity meter which was standardized with 0.01N potassium chloride (KCl) solution (APHA, 1992). The readings were taken from the display on the meter and values were recorded in micro Siemens per centimeter (µS/cm) (APHA, 1992).

Alkalinity Mg calcium carbonate

$$(CaCo_3)/L = \frac{A \times N \times 5000}{\text{Volume of Sample}}$$

Where:
A = Volume of acid used.
N = Normality of standard acid used
Hardness, mg equivalent CaCO3/L = 2.497 [Ca, mg/L] + 4.118 [Mg, mg/L]

Pesticide

Cyperdicot is composed of cypermethrin and dimethoate. Cypermethrin is an insecticide in the synthetic pyrethroid family, first marketed in 1977. The primary manufacturers in the U.S. are Zeneca Inc., FMC Corp., and American Cyanamid Co. Common brand names are Demon, Cymbush, Ammo and Cynoff. Dimethoate first marketed in 2001 by FAO, is an organ phosphorus and systemic pesticide with stomach and cholinesterase inhibition actions.

The trade names are Danadim, Rogo, and Roxion. The primary manufacturers in Denmark and Italy are Cheminouta.

Determination of LC_{50} Concentration

A toxicity assay to determine the 96 h LC_{50} values of Cyperdicot was conducted with a definitive test in a semi-static system in the laboratory following standard methods (APHA, 2005). A range-finding tests (5, 4, 3.5, 3 and 2.5mg L^{-1}) was carried out to determine the concentrations of the test solution for the definitive test. The experiment was conducted in 60 x 30 x 30 cm glass aquaria containing 40 L of de-chlorinated aerated water. The test solution was changed on every alternate day to counter-balance the decreasing pesticide concentrations. To prevent oxygen depletion, experimental tanks were continuously oxygenated using an air pump. Dead fish were immediately removed to avoid possible deterioration of the water quality. Behavioural changes in fin and opercular movements, equilibrium status, swimming rate, air gulping and skin coloration during the test period were observed.

In the definitive test a set of 10 fish specimens was randomly exposed to Cyperdicot at 5, 4, 3.5, 3 and 2.5 m g^{-1} concentrations. Another set of 10 fish specimens was simultaneously maintained in tap water, without test chemical, and considered as control. The experiment was set in triplicate to obtain LC_{50} values of the test chemical under a photoperiod of 12 hour light and 12 hour dark. The LC_{50} values (95 % confidence limits) of different concentrations of Cyperdicot in *C. gariepinus* were found to be 1.462^{a} (1.290-3.289) 1.094^{a} (1.180-1.328) 1.030^{b} (0.875-1.100) 0.800^{c} (0.734-0.980), respectively for 24, 48, 72 and 96 h exposure time. Probit analysis (Finney, 1971) was used to determine the concentration at which 50% mortality (LC_{50}) occurred using SPSS version 17.0.

The 96 h LC_{50} was calculated to be 0.08 mg l^{-1}. The safe level of the test pesticide was estimated by multiplying the 96 h LC_{50} with different application factors (AF) as suggested by the international Joint Commission (IJC, 1977).The mean water quality of the test solution determined in the experimental tanks following the standard method (APHA, 2005) were

(Mean ± SE): dissolved oxygen 7.02 ± 0.46 mg/l, temperature 25.70 ± 0.86°C, pH 7.04 ± 0.34, conductivity 275 ± 2.30 Scm-^{1} and total hardness 202.5 ± 4.45 mg/l as $CaCO_3$. The experiment was conducted following the OECD 173 guidelines for semi-static test conditions (OECD, 1992).

Determination of Sub-lethal Concentrations

The 96 h LC_{50} value of Cyperdicot on *C. gariepinus* was found to be 0.80 mg l-^{1}. Based on this value, two sub-lethal concentrations of 0.04 and 0.08 mg l-^{1} corresponding to 1/20th and 1/10th of the 96 h LC_{50} of the pesticide, respectively, were prepared by serial dilution of the stock solution with dechlorinated water and used for the *in vivo* exposure. A total of 90 fish from the acclimatised batch were used during the *in vivo* experiment. The fish were randomly divided into three groups of 30 fish, without regard to sex . Fish in the first treatment group were exposed to tap water and served as control, while those in second and third groups were treated with 0.04 and 0.08 mg L-^{1}of Cyperdicot, respectively. Each treatment group was further randomised into three replicates of 10 fish per replicate in 40 L (60 x 30 x 30 cm) glass aquaria. The exposure lasted for a period of 15 days during which the fish were fed daily small quantity of food approximately 1% of total body weight about an hour before the test solution was renewed, to avoid catabolism and subsequent mortality.

Estimation of Haematological Parameters

The total red cell count /cu.mm of blood (RBC) and the total leukocyte count (WBC) were determined using a Neubauer-type hemocytometer with Toisson's solution as the diluting fluid for RBC, and Turk's solution for WBC (Rusia and Sood, 1992). The haemoglobin level of blood was estimated following the cyanmethemoglobin method (Blaxhall and Daisley, 1973) with some modifications. Each 0.02 ml blood sample was mixed with 4 ml Drabkin's solution and allowed tostand for 10 minutes for proper color development, after which absorbance was read at 540 nm in a Unican spectrophotometer against a blank. Hematocrit (PCV) was analysed by centrifugation of the blood for five minutes at 14,000 × g in heparinised glass capillaries using a microhaematocrit centrifuge (Hawkesley& sons, Lancing, UK) at room temperature (Nelson and Morris, 1989). The haematocrit was read after centrifugation using the microhaematocrit reader and the result expressed as the percentage of the whole blood. Haematological indices such as MCHC, MCH and MCV were calculated according to the formula proposed by Dacie and Lewis (2001):

$$\text{MCHC (g dl-1)} = \frac{\text{Hb (g dl-1)} \times 100}{\text{PCV (\%)}}$$

$$\text{MCH (pg cell-1)} = \frac{\text{Hb (g dl-1)} \times 10}{\text{RBC count in millions mm}^{-3}}$$

$$\text{MCV (fl cell-1)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC count in millions mm}^{-3}}$$

Statistical Analysis

The data obtained, expressed as means SE, were analysed using the statistical package SPSS 17.0 (SPSS, Chicago). The data were subjected to one-way analysis of variance (ANOVA) of and Duncan's multiple range tests to determine the significance difference at the 5% probability level. A p-value less than 0.05 were considered statistically significant.

Results

Physico-Chemical Parameters of the Test Water

During the experimental period the test water pH ranged from 6.89 to 7.16, temperature ranged from 25.10 to 27.0 °C, dissolved oxygen varied from 6.61 to7.82 mg l-^{1}, conductivity ranged from 68.33-71.00 µM cm-^{1}, and total hardness and alkalinity varied from 5.99 to 6.28 mg l-^{1} and 136.5 to 180.5 mg l-^{1} as $CaCO_3$, respectively (Table 1).

Toxicity bioassay, safe level and behavioural characteristics

In the toxicity bioassay, a concentration-dependent increase and time-dependent decrease were observed in the death rate, to the extent that exposure duration time increased from 24 to 96 h, the concentration of Cyperdicot required to kill the fish was reduced. The LC_{50} values with 95% confidence limits of different concentrations of Cyperdicot in *C. gariepinus* were 1.462^{a} (1.290-3.289) , 1.094^{a} (1.180-1.328), 1.030^{b} (0.875-1.100) and 0.800^{c} (0.734-0.980) mg l-^{1} for 24,48,72 and 96 h exposure times, respectively (Table 2).The estimated safe levels of

Table1: Physico-chemical parameters of the test water used for lethal concentrations on *C. gariepinus*

Characteristics	Unit	Mean	Range
pH	-	6.98	6.89-7.16
Temperature	^{0}C	26.90	25.10-27.0
Conductivity	µM cm^{-1}	69.80	68.33-71.00
Dissolved oxygen	mg l^{-1}	6.85	6.61-7.82
Alkalinity	mg l^{-1}	24.16	25-27
Total hardness	mg l^{-1}	6.04	5.99-6.28

Table 2. Lethal concentrations of Cyperdicot (mg l^{-1}) and 95% confidence intervals (in parentheses) for *C. gariepinus*depending on exposure time (n = 10) in three replicates. Each value is the mean ± SE of 10 identical observations. Values in rows with different superscript letters differ significantly (P<0.05)

Lethal	Exposure time (h)			
Concentration	24	48	72	96
LC10	0.897^{a} (0.960-1.231)	0.635^{a} (0.716-0.10)	0.506^{b} (0.615-0.770)	0.510^{c} (0.389-0.720)
LC20	1.065^{a} (1.142-1.650)	0.871^{a} (0.885-1.046)	0.705^{b} (0.730-0.861)	0.690^{c} (0.492-0.790)
LC30	1.202^{a} (1.044-2.127)	1.080^{a} (1.005-1.174)	0.780^{b} (0.820-0.930)	0.750^{c} (0.571-0.850)
LC40	1.430^{a} (1.220-2.660)	1.083^{a} (1.099-1.340)	0.859^{b} (0.900-1.010)	0.805^{c} (0.659-0.910)
LC50	1.462^{a} (1.290-3.289)	1.094^{a} (1.180-1.328)	1.030^{b} (0.875-1.100)	0.800^{c} (0.734-0.980)
LC60	1.704^{a} (1.274-3.070)	1.312^{a} (1.166-1.641)	1.015^{b} (1.046-1.210)	0.913^{c} (0.805-1.081)
LC70	1.762^{a} (1.459-4.110)	1.549^{a} (1.362-1.010)	1.207^{b} (1.123-1.243)	0.987^{c} (0.872-1.222)
LC80	2.172^{a} (1.564-5.690)	1.620^{b} (1.370-2.287)	1.220^{b} (1.217-1.420)	1.062^{c} (0.943-1.224)
LC90	2.410^{a} (1.720-9.724)	2.105^{a} (1.554-3.027)	1.413^{b} (1.357-1.710)	1.203^{c} (1.036-1.798)

Table 3. Estimates of safe levels of Cyperdicot pesticide at 96 h exposure time

Chemical safe level (mg l^{-1}) Cyperdicot	96 h LC_{50} (mg l^{-1})	Method	Application Factor
2.30×10^{-2}	0.800	Hart et al. (1948)*	-
8×10^{-3}		Sprague (1971)	0.1
8×10^{-4}		CWQC (1972)	0.01
8×10^{-3} - 8×10^{-7}		NAS/NAE (1973)	0.01-0.00001
4×10^{-3}	0.05	CCREM (1991)	
4×10^{-3}	5% LC_{50}	IJC (1977)	

*C=48 h LC_{50} x $0.03S^{2}$, where C is the presumably harmless concentration and S = 24 h LC_{50} / 48 h LC_{50}

Cyperdicot in *C. gariepinus* varied from 8 x 10-3 to 8 x 10-4 mg l^{-1} (Table 3). Behavioural responses of the fish to Cyperdicot were observed in the exposed fish as well as in the control, in both the toxicity and sub-lethal concentrations. Normal swimming behaviour was observed in the control throughout the exposure period. In tanks with the test chemical, the fish swam erratically with jerky movements and hyperactivity. Faster opercular movement, surfacing and swallowing of air were observed. With increase in duration of the exposure, swimming and body movements were retarded and copious mucus was secreted and deposited in the buccal cavity and on the gills. The fish subsequently lost balance, became exhausted owing to respiratory difficulties, and finally settled on the bottom and died. In the sub-lethal concentration similar abnormal behaviour was exhibited, but no mortality was recorded (Table 4). Studies on toxicity with *C. gariepinus* indicate variations in LC_{50} values depending on the pesticide type, duration of exposure and stage of maturity (Table 5).

Haematological Parameters

The red blood cell count (RBC) and hemoglobin in the experimental group were not significantly different from those of the control (P>0.05) throughout the duration of the experiment except on day 15,when they were significantly reduced (P< 0.05) Table 6. There was no significant difference in PCV values between the control and exposed fish on day 1 (P>0.05), but PCV was significantly reduced (P<0.05) from day 5 of exposure. A Cyperdicot–induced dose- and time-dependent significant increase in W B C count from day 10 onward (P<0.05) were observed, while values of blood indices (MCV, MCH and MCHC) in the experimental fish were not significantly different (P>0.05) from the control group throughout the duration of the experiment.

There were dose- and time-dependent significant decreases (P<0.05) in the levels of neutrophils compared to the control throughout the experimental duration Table 7. The lymphocyte levels were significantly elevated (P<0.05) from day 5 onward, but the values of the monocytes, basophils and eosinophils were not significantly different from the control.

Discussions

The abnormal behavioural alterations in Cyperdicot-exposed fish may indicate disturbance in the internal physiology of the fish, which may be attributed to the neurotoxic property of the drug. Studies by Bull *et al*. (2007) indicated that Cyperdicot interferes with signaling at the neuromuscular junction, thereby disrupting the Ca+2 voltage-gated channels. This will result in conformational changes in membrane lipid and membrane fluidity, which is a further indication of a neuro-pharmacological effect of Cyperdicot on the fish (Wilson *et al*.,2003). This, according to Sarai *et al.* (2013) would result in prolonged neuromuscular depolarisation, culminating in the observed uncoordinated and jerky movement that was noticed in Cyperdicot-exposed fish. Similar behavioural responses have been observed in fish

Table 4. Behavioural and dermatological changes of *C.gariepinus* juveniles exposed to various concentrations of Cyperdicot

Toxicity Test																								
Exposure time(h)	24						48						72						96					
Concentration (mg/l)	0	5.0	4.0	3.5	3.0	2.5	0	5.0	4.0	3.5	3.0	2.5	0	5.0	4.0	3.5	3.0	2.5	0	5.0	4.0	3.5	3.0	2.5
Behavioural changes																								
Loss of reflex	-	+	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Air gulping	-	-	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Erratic swimming	-	-	-	-	-	+	-	-	+	-	-	+	-	+	++	-	+	++	-	++	++	-	++	+++
Dermatological changes																								
Discoloration	-	+	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	++	++	-	++	++
Sub-lethal test																								
Exposure time(h)	24						48						72						96					
Behavioural changes																								
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
Air gulping	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	++	-	++	+++
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	++	++
Dermatological changes																								
Discoloration	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	++	+	-	++	++	-	++	+++
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

= no significant, +=low severity, ++, = moderate severity, and +++= high severity

exposed to chemotherapeutic compounds (Obiekezie and Okafor, 2005; Mitchell and Hobbs 2007, Wang *et al.,* 2009; Nwani *et al.,* 2013).Also, similar observations have been reported in *Clariasal bopunctatus* after exposure to monocrotophos (Mgbenka *et al*., 2005). Moreover, hypoxic conditions also contributed to the increase surfacing and gulping in surface water, which could also have been an attempt by the fish to avoid breathing in the poisoned water. Hypoxic conditions arise primarily due to damage of gills of fish exposed to pesticide, which hampers oxygen uptake. The increased mucus secretion by the fish after Cyperdicot exposure is probably an adaptive response to counter the irritating effects of the pesticide on body surface and mucus membrane. The observed abnormal behaviour alterations in cyperdicot-exposed fish are consistent with previous reports on organophosphate-based pesticides (Adhikari *et al.,* 2004;Yaji, 2011) and other pesticides such as malathion, profenofos, praziquantel (Pandey *et al.* 2011, Nwani *et al*., 2014) and atrazine (Nwani *et al*,. 2012).

In this study, the toxicity level of Cyperdicot on *Clarias gariepinus* was found to be 0.800 mg l^{-1}, based on the 96 hr LC_{50} value. The LC_{50} value reported in the present study for commercial formulation of Cyperdicot is lower than the 1.05 mg l^{-1} and 13.6 mg l^{-1} reported by Usmani and Knowles (2001) and Das and Murkherjee (2003) when organophosphate derivatives-based pesticide were exposed to *Labeorohita* fingerlings and larvae and adults of *Helicoerpazea* and *Agrotisipsolon* respectively. The L_{C50} obtained in our present study for commercial formulation of Cyperdicot is also lower than the 3.74 mg l^{-1} obtained by Dixon and Dick (1985) when *Cyprinuscapio* were exposed to pyrenoid-based pesticide for 48 h as well as the observations made by Fazio *et al*. 2014, Arukwe *et al*. 2014, Wågbø *et al*. 2012, Cangialosi *et al*. 2012. Our LC_{50} value 0.800 mgl^{-1}, however, is higher than the0.620 mg l^{-1} and .0750 mg l^{-1} 96 h LC_{50} reported by Nath and Banerjee (1996) and Yaji *et al,*. (2011) when *Channa punctatus* and *Oreochromis niloticus* were exposed to organophosphate commercial formulation pesticide.

Toxicity tests in fish are useful in assessing possible eco-toxicological risks of contaminants (Prusty *et al*., 2011). The literature indicated that the toxicity of organophosphate-based herbicides varies from one species to another, and even in strains of the same species. Toxicity of chemicals to aquatic organisms has been reported to be affected by temperature, pH, dissolved oxygen, size and age, type of species, water quality, concentration and formulation of test chemicals (Young, 2000). Toxicity of compounds to organisms has however been known to be dependent on concentration, age, sex and exposure period (Saravanan *et al.,* 2012). The safe level obtained for Cyperdicot in the present study varied from 8 x 10-3 to 8 x 10-4 mg l^{-1}. However, due to large variation in safe levels as determined by different methods, the estimates of safe levels cannot be guaranteed (Buikem *et al*., 1982). Extrapolation of laboratory data to the field is not always meaningful value, and hence it is difficult to determine acceptable concentration based on laboratory experiments that may be considered' safe' in the field (Abuo *et al*., 2001; Pandey *et al.,* 2005). Similar observations were recorded by Sampath *et al.* (1993) in *Oreochromis mossambics e*xposed to organ phosphorous, Omoregie *et al.,* (1994) in *Orechromis niloticus* exposed to formalin, Svoboda *et al.* (2001) in *Cyprinus carpio exposed* to diazinon and

Gabriel *et al.* (2007) in *C. gariepinus* exposed to refined crude oil product kerosene.

Exposure of C. *gariepinus* to sub-lethal

Table 5. Results of various toxicity studies of some pesticides on *C. gariepinus*

Pesticide	Assay	Result	Reference
Diazinon	96 h LC50	11.80 mg1^{-1} in juvenile	Nwani et al. (2011)
Diazinon	96 h LC50	6.60 mg l^{-1} in adult	Adedeji et al. (2008)
Endosulfan	96 h LC50	8.8 ppm in juvenile	Agbohessi et al. (2013
Gammalin20	96 h LC50	30 ppb in fingerlings	Ezemonye and Ogbomida (2010
Glyphosate	96 h LC50	211.80 mg l^{-1} in juvenile	Nwani et al. (2013)
Lambdacyhalothrin	96 h LC50	0.325 ppm in juvenile	Yekeen, Fawole, and Bakare (2013)
Lindane	96 h LC50	1.29 ppm in juvenile	Lawson et al. (2011)
Cyperdicot	96 h LC50	0.80 mg l-1 in juveniles	This study

Table 6. Effects of exposure to various sub-lethal levels of Cyperdicot on RBC parameters in *C. gariepinus*

Parameters/(s)	Concentration (mg/l)	Duration (days)			
		1	5	10	15
RBC (×106 cells/mm^3	Control	7.61 ± 0.81^{a1}	8.22 ± 08.4^{a1}	7.86 ± 0.06^{a1}	89.73 ± 0.64^{a1}
	0.04	7.71 ± 0.86^{a1}	6.90 ± 0.93^{a1}	6.95 ± 0.63^{a1}	4.65 ± 0.60^{b2}
	0.08	7.15 ± 0.77^{a1}	7.14 ± 0.96^{a1}	6.87 ± 0.71^{a1}	4.57 ± 0.58^{b2}
PVC (%)	Control	26.00 ± 0.77^{a1}	29.50 ± 0.71^{a1}	30.50 ± 0.72^{a1}	27.50 ± 1.83^{a1}
	0.04	26.50 ± 0.81^{a1}	23.00 ± 0.83^{b2}	23.50 ± 0.73^{b2}	16.00 ± 0.68^{c2}
	0.08	27.00 ± 0.62^{a1}	25.00 ± 0.49^{a2}	24.00 ± 0.65^{a2}	14.50 ± 0.59^{b2}
WBC (×104	Control	6323 ± 2.63^{a1}	8024 ± 6.41^{a1}	8100 ± 7.72^{a1}	7650 ± 8.41^{a1}
cells/mm3)	0.04	6474 ± 7.82^{a1}	8124 ± 6.01^{a1}	8350 ± 6.44^{b1}	7930 ± 7.91^{b2}
	0.08	6451 ± 5.41^{a1}	8201 ± 5.63^{a1}	9000 ± 6.76^{c2}	9050 ± 6.77^{c2}
Hb (g/dL)	Control	7.65 ± 0.66^{a1}	8.80 ± 0.71^{a1}	9.45 ± 0.66^{a1}	8.45 ± 0.81^{a1}
	0.04	6.10 ± 0.54^{a1}	6.95 ± 0.82^{a1}	7.15 ± 0.71^{a1}	5.60 ± 0.71^{b2}
	0.08	6.30 ± 0.63^{a1}	7.65 ± 0.54^{a1}	7.30 ± 0.46^{a1}	5.15 ± 0.62^{b2}
	Control	8.82 ± 0.83^{a1}	9.52 ± 0.67^{a1}	10.78 ± 0.92^{a1}	9.52 ± 1.01^{a1}
MCH (pg/cell)	0.04	9.45 ± 0.66^{a1}	9.06 ± 0.71^{a1}	9.25 ± 0.94^{a1}	9.91 ± 0.83^{a1}
	0.08	10.41 ± 0.45^{a1}	9.63 ± 0.83^{a1}	18.55 ± 1.16^{a1}	9.24 ± 0.46^{a1}
	Control	32.27 ± 1.45^{a1}	32.22 ± 1.94^{a1}	32.18 ± 1.09^{a1}	32.16 ± 2.11^{a1}
MCHC (g/dL)	0.04	32.09 ± 1.16^{a1}	32.13 ± 1.86^{a1}	32.27 ± 3.04^{a1}	31.94 ± 1.64^{a1}
	0.08	32.21 ± 1.84^{a1}	32.27 ± 2.35^{a1}	32.20 ± 2.06^{a1}	32.23 ± 3.09^{a1}
	Control	28.51 ± 1.86^{a1}	30.65 ± 1.86^{a1}	34.52 ± 1.86^{a1}	27.70 ± 2.06^{a1}
	0.08	30.57 ± 2.45^{a1}	29.38 ± 2.09^{a1}	$29.82 \pm 1.54_{a1}$	29.09 ± 1.07^{a1}
MCV (fl/cell)	0.04	33.36 ± 2.11^{a1}	30.94 ± 1.16^{a1}	30.77 ± 1.11^{a1}	26.83 ± 1.08^{a1}

Values with different alphabetic (lowercase) superscripts differ significantly ($p<0.05$) between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($p < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean standard error of the mean.

concentrations of Cyperdicot elicited changes in some haematological parameters. The RBC, Hb and PCV values were appreciably reduced at higher concentrations of Cyperdicot. The reduction in these parameters may be attributed to haemolysis caused by the drugs action on the fish. The decrease may also be attributed to the limit in erythrocyte synthesis due to impaired osmoregulation across the gill epithelium and accumulation of the toxicant in the gill region (Saravanan *et al*., 2011; Pereira *et al*,. 2013). Furthermore, blood indices are often subjected to variations depending upon stress and environmental factors (Goel *et al.,* 1981; Hlavova, 1993;Marsalek*et al.* 2014).The observed leukocytosis from day 10 of exposure indicated an immune protective response against the stress imposed by the drug (Davis *et al*., 2008). Exposure to toxicants such as Cyperdicot stimulated the lymphocyte cells in the lymphomyeliod tissue as defense mechanism against the stressor, hence the observed proliferation of WBC in the peripheral blood (Campbell, 1996). The data on MCV and MCH showed that Cyperdicot caused both macrocytic and microcytic anemia on day 1, and between days 5 and 15, respectively. Microcytic anemia was reported in *O. mykiss* treated with sulfadiazine and trimethoprin (Lunden and Bylund, 2002) and sulfamerazine (Saglam and Yonar, 2009;Šišperová *et al*.,2015).There was no significant change in the values of MCHC in Cyperdicot-treated fish compared to the control. Li *et al.* (2011c) obtained similar results for the red blood cell indices of *O. mykiss* exposed to the pharmaceutical drug carbamazepine, (Serckova *et al*., 2016). Changes in leukocyte differentials have been used as good indicators of stress (Cole *et al.,* 2001). Neutrophils and lymphocytes make up the majority of WBC and proliferate in circulation in response to stress (Jain 1993, Thrall, 2004). The observed significant increase in lymphocytes from day 5, and of neutrophils throughout the duration of exposure, may have been provoked by the stress imposed by Cyperdicot on the fish. Stress-induced lymphopenia and neutrophilia have been shown to be related to elevated glucocorticoids secretion which acts to increase their

Table 7. Effects of exposure to various sub-lethal levels of Cyperdicot on differential WBC counts (percentage) in *C gariepinus*

Parameter(s)	Concentrations (mg/l)	Duration (Days)			
		1	5	10	15
Neutrophils	Control	12.00 ± 1.11^{a1}	13.00 ± 1.93^{a1}	13.60 ± 1.13^{a1}	13.8 ± 1.09^{a1}
	0.4	16.50 ± 1.86^{a2}	17.00 ± 2.06^{a2}	23.50 ± 1.41^{b2}	25.00 ± 2.11^{b2}
	0.8	20.3 ± 1.14^{a3}	19.50 ± 1.81^{a2}	24.11 ± 1.63^{b2}	27.50 ± 3.06^{c2}
Lymphocytes	Control	16.50 ± 1.16^{a1}	22.00 ± 1.14^{a1}	18.00 ± 1.10^{a1}	16.00 ± 1.66^{a1}
	0.4	29.50 ± 1.09^{a1}	23.00 ± 0.83^{b1}	23.50 ± 0.93^{b2}	23.00 ± 1.41^{b2}
	0.8	33.00 ± 0.93^{a2}	25.50 ± 0.71^{b2}	26.00 ± 0.71^{b3}	30.50 ± 0.89^{c3}
Monocytes	Control	0.40 ± 0.06^{a1}	0.50 ± 0.01^{a1}	0.50 ± 0.01^{a1}	1.00 ± 0.02^{a1}
	0.4	0.40 ± 0.06^{a1}	0.30 ± 0.01^{a1}	1.00 ± 0.02^{a1}	0.60 ± 0.03^{a1}
	0.8	2.00 ± 0.06^{a1}	1.00 ± 0.04^{a1}	1.50 ± 0.11^{a1}	0.50 ± 0.07^{a1}
Basophils	Control	0.04 ± 0.00^{a1}	0.04 ± 0.01^{a1}	0.04 ± 0.00^{a1}	0.04 ± 0.00^{a1}
	0.4	0.04 ± 0.00^{a1}	0.04 ± 0.01^{a1}	0.03 ± 0.00^{a1}	0.01 ± 0.00^{a1}
	0.8	0.03 ± 0.01^{a1}	0.03 ± 0.01^{a1}	0.04 ± 0.01^{a1}	0.05 ± 0.00^{a1}
Eosinophils	Control	0.40 ± 0.01^{a1}	0.10 ± 0.01^{a1}	0.40 ± 0.01^{a1}	0.30 ± 0.01^{a1}
	0.4	0.40 ± 0.02^{a1}	0.50 ± 0.03^{a1}	0.10 ± 0.04^{a1}	0.30 ± 0.02^{a1}
	0.8	0.40 ± 0.01^{a1}	0.50 ± 0.02^{a1}	0.10 ± 0.02^{a1}	0.35 ± 0.03^{a1}

Values with different alphabetic (lowercase) superscripts differ significantly ($P<0.05$) between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly ($P < 0.05$) between different exposure periods within the same concentration. Results are expressed as mean standard error of the mean.

percentage levels (Davis *et al*., 2008). Similar results have been reported in fishes exposed to different concentrations of pharmaceuticals (Kreutzmann, 1977; Lunden *et al*., 1999, Li *et al.,* 2011). Other leukocyte differentials such as monocytes, basophils and eosinophil were comparable to the control throughout the experimental period. Similar observations have been also reported in other fishes treated with different toxicants (Velisek *et al.,* 2009, Mohammad *et al*., 2012).

Conclusion

This study showed that both behavioural and sub-lethal concentrations of Cyperdicot affected both the behavioural responses and the haemotological parameters of juveniles of *Clarias gariepinus.* It is shown that long-term exposure to Cyperdicot at sub-lethal concentrations induced haematological alterations in *Clarias gariepinus*, and that the use of haematology is adequate in detecting and monitoring possible effects of sub-lethal dose of pesticide in the environment. From the present study it could be concluded that, when fishes are exposed to fourth generation pesticides such as Cyperdicot, they have various haematoxic effects on the survival of the fish. This in turn also will affect the fecundity of the fish population and other non-targeted organisms such as man through the food chain. Therefore the levels in the aquatic environment should not be higher than the sub-lethal exposure levels. Action is needed when the levels increase markedly.

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