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EFFECTS OF NICOSULFURON HERBICIDE ON THE HAEMATOLOGICAL PROFILE AND BEHAVIOUR OF JUVENILE *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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ABSTRACT
The challenges faced by Freshwater fish are enormous especially those that occur as a result of Agricultural intensification, through constant discharge of wastes into aquatic environment which led to accumulation of heavy chemicals and other variety of pollutants. Herbicides present in these wastes are washed down, carried by rains and flood to nearby aquatic environment. This study has determined the hematological effect of exposure of the juvenile African Catfish (*Clarias gariepinus*) to varying acute concentrations of Nicosulfuron herbicide for 96hr using static bioassay. Healthy180 pieces active fish of mean weight 13.6-46.1g and11.00-20.00 cm length were randomly distributed into five concentrations of Nicosulfuron herbicide and control (0.0, 1.5, 1.625, 1.75, 1.875 and 2.0 ml) in triplicates of ten fish per tank. Data obtained were analyzed for descriptive statistics (mean and standard error of means) and further subjected to analysis of variance (ANOVA) at $P< 0.05$ using Minitab. There was an increase in WBC (6.00 ± 2.83 - 8.55 ± 7.78 ($10^3/mm^3$), PCV (27.00 ± 2.00 - 36.67 ± 1.53 %), Hb (9.03 ± 0.75 - 12.23 ± 0.60 g/d), MCV (108.57 ± 3.39 - 128.47 ± 6.00 FL) and MCH (42.53 ± 1.11 - 48.83 ± 4.91 pg) with increase in concentration while MCHC decreased with increase in concentration. The result shows no significant difference in RBC and MCHC. The study therefore, concluded that toxicity could be of low impact when compared to other toxicants but the bioaccumulation effect can be hazardous and this finding can serve as baseline information to develop models on effects of Nicosulfuron herbicides on ecological characteristics of aquatic environment. It can also be as an index of toxicity in water to determine the health of an aquatic organism.

Keywords: *Clarias gariepinus*, Nicosulfuron, Haematology, Behaviour

INTRODUCTION
Fish is an important source of protein to large teaming population of Nigeria. It provides 40% of the dietary intake of animal protein of the average Nigerian. Fish and fish products constitute more than 60 % of the total protein intake in adults especially in rural areas (Adeleke *et al.,* 2011). Most animal protein contains high cholesterol, which can induce some health disorder unlike fish. The importance of fish in human nutrition include a nutrient profile superior to all terrestrial meats (beef, pork, and chicken, etc) being an excellent source of high quality animal protein and highly digestible energy; a good source of sulphur and essential amino acids such as lysine, leucine, valine, and arginine (Akinwole and Adedayo, 2014) also, sources of essential micronutrients such as calcium, iron, vitamin A and zinc (Allison, 2011).

Clarias gariepinus is an important fish in Nigerian Aquaculture, and the second most culture fish in Nigeria (Offem *et al.,* 2010).

Clarias gariepinus is famous in Aquaculture because of its taste, high price command in the market and availability of seeds since it can reproduce or at least initiate reproduction in captivity. This places it in Nigerian Aquaculture higher than its relative, *Heterobranchus* species that cannot reproduce in captivity (Freund *et al.,* 1995; Offem *et al.*, 2008; Anetekhai, 2013). This fish is remarkable fish species in Nigeria where it is a leading aquatic crop. It has credentials of fast growth, resistance to disease and handling stress. It has air-breathing structure and therefore tolerates very low oxygen levels in any aquatic environment as well as on land (FAO, 2012).

Pesticides are known to be toxic by design, and it is designed to kill, repel and reduce pests, unwanted herbs, rodents, fungi or other organisms which impart threat to crop plants. So, they are extensively being used by farmers in modern agricultural practices to increase crop production in other to sustain the human population. However, lack of knowledge and injudicious use of the pesticides leads to lethal effects on organisms. After surface runoff, these toxicants enter aquatic system and impart hazardous effect on non-target organisms specially fishes. These toxic chemicals change the quality of water and thus, affect health of fish and other aquatic organisms (Dhasarathan *et al.*, 2000).

Nicosulfuron is a member of the sulfonylurea family of herbicides. It controls weeds by inhibiting the plant enzyme acetolactate synthase, or ALS. This enzyme is not found in livestock, fish or man. Inhibiting the ALS enzyme system blocks the production of the amino acids, valine and isoleucine, essential building blocks of proteins and other plant components (Wilmington, 1988). Nicosulfuron is postemergence herbicide with a non-ionic surfactant which is applied when weeds are 4-12 inches tall and actively growing. Rain within two hours of application will not decrease its effectiveness (Thomson, 1993). Nicosulfuron is used for control of weeds such as Johnsongrass, quackgrass, foxtails, shattercane, panicums, barnyardgrass, sandbur, pigweed, morning glory and others. Crops include field corn and popcorn (OHS Database, 1994).

Fish blood gives the possibility of knowing physiological conditions within the fish long before there is an outward manifestation of diseases. This is because under stressful conditions as well as environmental imbalances, some parameters in the blood change in response to reflect the change (Shah and Atindag, 2004). The potential utility of biomarker for monitoring both environmental quality and health of organisms inhabiting polluted aquatic ecosystems has received increasing attention in recent times (Lopes *et al.*, 2001). According to Railo *et al.* (1995), blood parameters of diagnostic importance are electrolytes, leucocytes, haemoglobin, haematocrit and leucocytes differential count would readily respond to incidental factor such as physical and environmental stress due to xenobiotics in water.

Among different aquatic organisms, fish are highly sensitive to the environmental contamination of water (Amal *et al.,* 2020). Hence, pollutants such as pesticides may affect significantly certain physiological processes like biochemical and hematological parameters, alteration in genetic and protein level, changes in histology and particularly oxidative stress in fishes. Thus, impart serious impairment to health status of fishes. As fish is the mostly consumed aquatic food providing high protein in diet, it also affects human health (Shah and Atindag *et al.,* 2004).

Despite the rangy use of Nicosulfuron herbicide, determining its effects on haematological parameters of *C. gariepinus* is pertinent for exploitation towards ascertaining its hazardous effect on the aquatic system. It is assumed that the residue might affect the fish from runoffs into the aquatic system, even some farmers intentionally wash the equipment used for spraying the chemicals into some nearby rivers which eventually flow into any close ponds. Although the work done on terrestrial organisms shown not to be very toxic to rat and Mice with the LD_{50} values: 30 mg/kg (OHS Database, 1994), it was however, slightly toxic to bird on an acute and dietary basis with the LD_{50} value of 2,250 mg/kg (mallard ducks); 5,620 mg/kg (Wilmington, 1988). The present study therefore, investigated the effects of Nicosulfuron herbicide on some selected blood parameters of *C. gariepinus* under laboratory condition, so as to ascertain their level of tolerance and their suitability as bio-indicator in freshwater ecosystems. This research aimed at studying the acute toxicity (LC_{50}) of Nicosulfuron herbicide to juvenile *C. gariepinus*, its effect on haematological parameters and behavior of the fish exposed to the toxicant.

MATERIALS AND METHODS

The experiment was carried out in the fisheries laboratory of Adekunle Ajasin University Akungba -Akoko which is located at latitude 7.4696° and longitude 5.7362°. Plastic tanks of 75 cm x 40 cm x 40 cm; 50L capacity were used and each tank was filled with 30 litres of unchlorinated water. Apparently 250 healthy juvenile catfish (11.00 - 20.00 cm length and 13.6-46.1 g) were collected from a private fish farm Etioro Akoko, Ondo State. The fishes were acclimated to the laboratory condition for three weeks and they were fed with commercial (Skretting) fish feed during the acclimatization period. It was fed a 35 % crude protein at 5 % total body weight. The daily ration was divided into two portions at the hour of 8am and 5pm respectively for three weeks until the experiment commenced. Feeding was discontinued 24 hours before the commencement of the experiment to minimize the production of waste in the test container. The herbicide, Nicosulfuron at 0.0, 1.5, 1.625, 1.75, 1.875 and 2.0 ml replicated six times was applied using 5ml syringe (Adene *et al.*, 2017).

Toxicity Test

Range finding test: Preliminary 24 h range finding test was conducted to determine the toxic range of Nicosulforon to juveniles of *C. gariepinus,* following static bioassay procedure according to The Organization for Economic Co-operation and Development's (OECD) guidelines (OECD, 1992). The fish were batch weighed and distributed into a set of 18 rectangular plastic tanks (75 × 45 ×45 cm) each filled with 30L unchlorinated water. Each of the test solutions of the selected concentrations was introduced directly into the plastic tanks in a single dose. The behavior and mortality of the test fish including the water quality in each tank were monitored and recorded every 15 min. for the first one hour, every hour for the next four hours, and every four hours for the rest of the 24 h period.

Definitive test: Based on the results from the range finding (Lethal toxicity) test described above, 96h definitive tests was carried out, following static bioassay procedures described by Parish (1985). Batches of Ten juveniles *C. gariepinus* were batch weighed and distributed into a set of 18 rectangular plastic tanks (75 × 45 × 45 cm) each filled with 30 L of Unchlorinated water.

Five test solutions of Nicoulfuron of 1.5, 1.625, 1.75, 1.875, 2.0 ml as earlier determined from the range finding test was introduced in a single dose directly into the plastic tanks. The test fishes were not fed throughout the 96 h test. The behavioral pattern and mortality of the test in each tank was monitored and recorded every 15 min for the first one hour, every hour for the next four hour, once every four hours for the next 24 h and once every 24 h for the rest 96 h. Dead fish were removed immediately with scoop net to avoid contamination due to rotting.

Haematological analysis: Blood (1 – 3 ml) sample from each fish was collected from the fish after 96 h of exposure. Collection of blood was done through the vertebral caudal blood vessel with the help of disposable hypodermic syringe and needle. Blood sample was emptied into 10 ml sample bottle treated containing anticoagulant, Ethylene Diamine Tetracetic Acid (EDTA). Haematological analysis of fish was conducted following the method described by Svobodova *et al.* (1991). Blood cell count (erythrocytes and leucocytes) was carried out in an improved Neubaeur haemocytometer using a modified Yokoyama diluting fluid. The basic erythrocyte indices, Mean Cell Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) were computed from hemoglobin values and erythrocyte count.

Water quality analysis: Water quality monitoring was done prior, during, and after the experiment. pH was determined using a digital pH meter (Mettle-Toledo, 320). Dissolved Oxygen (DO) was measured using a digital dissolved oxygen meter (Jenway, 9071). While, Temperature was measured using a mercury glass thermometer and the conductivity using conductometre.

Statistical analysis: All results were collated and analyzed using computerized probit and logit analysis (Lichtfield and Wilcoxon, 1949). The median lethal concentration, at selected period of exposure and an associated 95 % confidence interval for each replicate toxicity test, was subjected to logit and probit analysis (Finney, 1971) using MINITAB (version j14.) and hematological and water quality statistical analysis using SAS.

Calculation of lethal concentrations: The mortality rates observed during the stipulated exposure periods were recorded and used for calculation of 96 hLC50 (Finney, 1971; USEPA, 2000) method along with slope values. This was derived by multiplying the 96-h LC_{50} by a constant of 0.01-0.1.

RESULTS AND DISCUSSION

Lethal concentration: The acute toxicity of Nicosulfuron to *C. gariepinus* juvenile is 1.496 ml -14.962 ml. The LC_{50} represents the concentration at which 50 % of the fish population will be killed when exposed to toxicant. This is represented in Figure 1.

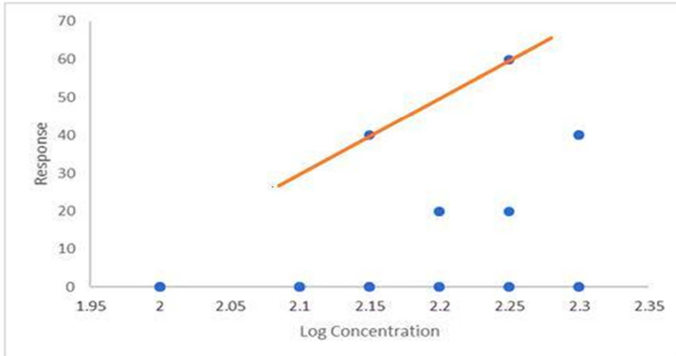

Figure 1: Log of concentration of Nicosulfuron herbicide and its probit value for juvenile *C. gariepinus*

Behavioural changes: Table 1 shows the behavioural response of juvenile *C. gariepinus* exposed to Nicosulfuron herbicide. Upon addition of toxicant, the fish showed erratic swimming, loss of reflex, air gulping, but no sign of barbel deformation. The behavioural response of *C. gariepinus* to Nicosulfuron herbicide demonstrated to be a sensitive indicator of physiological stress in fish subjected to sub-lethal concentration of pollutants (Maikai *et al*., 2008). The fish finally settled at the bottom motionless with slow opercula movement.

Heamatological studies: The result of the heamatological studies shows a significant increase ($p<0.05$) in the values of blood parameters of *C. gariepinus* after exposure to Nicosulfuron herbicide for 96 hrs (Table 2). Pack cell volume increases from 27.00 ± 2.00 % to 36.67 ± 1.53 %, heamoglobin increases from 9.03 ± 0.75 g/dl to 12.23 ± 0.60 g/dl , Red blood cell increases from 2.13 ± 0.25 (10^2/L) to 2.67 ± 0.06 (10^2/L) in the highest concentration, White blood increased from 6.00 ± 2.83 ($10^3/mm^3$) to 8.55 ± 7.78 ($10^3/mm^3$), Mean Corpuscular Volume increases from 108.57 ± 3.39 (FL) to 128.47 ± 6.00 (FL), and Mean Corpuscular Hemoglobin increases from 42.53 ± 1.11(pg) to 48.83 ± 4.91(pg) while Mean cell haemoglobin concentration decreases from 39.17 ± 0.35 (g/dl) to 35.67 ± 0.72 (g/dl).

Determination of LC_{50} is essential for acute toxicity testing and also for routine bioassay experiments. As a fishing poison, the knowledge of LC_{50} at various exposure periods would provide more provisions to the farmers in case they want to kill/eradicate the predatory fishes and weed within a convenient duration from culture ponds before stocking. The mortality rates observed in the present study suggests a clear relationship that is proportional to the mortality rate. The LC_{50} of Nicosulfuron herbicide to *C. gariepinus* juveniles is 1.496 ml which is similar to the result observed by Ayotunde *et al.* (2011) who reported that the 96 hr LC_{50} of 2.42 mg/l, for *O. niloticus* juveniles exposed to aqueous extract of *Moringa oleifera* seed powder.

The results of water quality parameters (pH, Temperature, Conductivity ($X10^4$), Dissolved oxygen and TDS) obtained for the test solutions during the experiments were not significantly different ($p>0.05$) from the control (Table 3). The result obtained for the 96 hours were found close to the physico-chemical parameter of the control. Changes in the water quality parameters showed that the concentrations affected the water quality but the values were within tolerant range.

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Table 1: Behavioural changes observed in the juvenile *C. gariepinus* exposed to different concentrations of Nicosulfuron herbicide

Behaviour/ Exposure Time	**24hrs**					**48hrs**					**72hrs**					**96hrs**				
Concentration	1.5	1.625	1.75	1.875	2.0	1.50	1.625	1.75	1.875	2.0	1.50	1.625	1.75	1.875	2.0	1.50	1.625	1.75	1.875	2.0
Loss of reflex	-	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
Air gulping	+	-	+	+	+	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Barbel deformation	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Excessive mucus	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	---
Molting	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

Keys : + means there is a reaction will – means No reaction

Table 2: Haematology Parameters of *C. gariepinus* exposed to different concentrations of Nicosulfuron herbicide

Treatment/ Parameters	T1 (0.00ml)	T2 (1.50ml)	T3 (1.625ml)	T4 (1.75ml)	T5 (1.875ml)	T6 (2.0ml)
RBC (10^2/L)	2.13 ± 0.25^b	3.00 ± 0.26^a	3.00 ± 0.10^a	2.77 ± 0.32^a	2.87 ± 0.15^a	2.67 ± 0.06^a
WBC(10^3/mm^3)	60.00 ± 10.28^b	98.07 ± 11.89^a	99.1 ± 0.62^a	88.5 ± 11.64^a	100.83 ± 8.37^a	85.73 ± 2.5^a
HB(g/dl)	9.03 ± 0.75^b	13.63 ± 1.24^a	13.50 ± 0.10^a	12.50 ± 1.47^a	13.00 ± 0.44^a	12.23 ± 0.60^a
MCV(FL)	108.57 ± 3.39^b	126.30 ± 0.92^a	126.2 ± 0.26^a	124.93 ± 0.65^a	127.77 ± 4.74^a	128.47 ± 6.0^a
MCH(pg)	42.53 ± 1.11^b	45.47 ± 0.60^{ab}	44.93 ± 0.85^{ab}	44.7 ± 0.36^{ab}	45.57 ± 2.47^{ab}	48.83 ± 4.91^a
MCHC (g/dl)	39.17 ± 0.35^a	35.97 ± 0.55^b	35.60 ± 0.61^b	35.77 ± 0.12^b	35.67 ± 0.72^b	35.67 ± 0.72^b
PCV (%)	27.00 ± 2.00^b	41.00 ± 3.46^a	40.67 ± 0.58^a	37.33 ± 4.62^a	39.33 ± 1.16^a	36.67 ± 1.53^a
N (%)	1.33 ± 0.58^a	1.33 ± 0.58^a	1.00 ± 0.00^a	1.00 ± 0.00^a	1.00 ± 0.00^a	1.00 ± 0.00^a
L (%)	95.33 ± 0.58^a	95.67 ± 0.58^a	94.00 ± 0.00^a	94.33 ± 0.58^a	94.67 ± 02.08^a	94.33 ± 0.58^a
M (%)	2.00 ± 0.00^a	1.67 ± 0.58^a	3.00 ± 0.00^a	2.67 ± 0.58^a	2.67 ± 01.53^a	2.67 ± 0.58^a
E (%)	1.00 ± 0.00^a	1.33 ± 0.58^{bc}	2.00 ± 0.00^a	2.00 ± 0.00^a	1.67 ± 0.58^{ab}	2.00 ± 0.00^a

Means values within the same row with the same letter are not significantly different from each other at P<0.05 using Duncan's New Multiple Range Test (DNMRT). RBC – Read Blood Cell; WBC – White Blood Cell; HB – Hemoglobin; MCV - Mean Cell Volume, MCH - Mean Cell Hemoglobin, MCHC - Mean Cell Hemoglobin Concentration N – Neutrophil; L – Lymphocytes; M – Monocytes; E - Eosinophils

Haematological examination shows an increase RBC, Hb, MCV, MCH, PCV, this is contrary to what was observed by Altreza *et al.* (2012) when *Mesopota michthys sharpeyi* was exposed to *Paraquat* herbicide but the decrease in MCHC (39.17 ± 0.35 to 35.67 ± 0.72) is in agreement with their work. The increase in RBC, WBC, HB, MCV, MCH and PCV correlate with the work of Adene *et al.* (2017) who investigated the acute toxicity and blood profile of adult African catfish and Ayotunde *et al.* (2011) when *O. niloticus* was exposed to aqueous extract of *moringa oleifera* seed powder. The observation was contrary to what was obtained by Adene *et al.* (2019) on haematological assessment and piscicidal effect of sodium hypochlorite on juvenile *Heterobranchus bidorsalis.*

The increase in WBC (6.00 ± 2.83 - 8.55 ± 7.78), could be as a means of fighting against the presence of the toxicant in the blood since WBC function as an Antigen that fight any unwanted microorganisms or infections in the body. This is in conformation with the observation of Ferreira *et al.* (1981), who observed an increase in haematocrit level of *Cyprinus carpio* anaesthetized with benzocaine hydrochloride.

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White blood cells are a very important blood parameter that act as a defense mechanism to the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. The function of Granulocytes and monocytes which are phagocytes is to remove debris from injured tissue and that of lymphocytes is to produce antibodies (Maheswaran, *et al.* 2008).

In this research work, the blood profile of all the fish exposed to different concentration of Nicosulfuron herbicide has higher concentrations of leucocytes than those of controls. This is in accordance with the work of Allen (1994) and Oliveira *et al.* (2006) when *Hoplias malabaricus* was exposed to sub-chronic and dietary doses of methyl mercury. A decrease in lymphocyte number is also contrary to the result of Shah and Altindağ (2004) after the exposure of *Oreochromis aureus* to mercury.

Table 3: Physicochemical parameters measured at 96h exposure of *C. gariepinus* to Metalaxyl

Time	TRT (ml)	TEMP	pH	COND	DO	TDS	SALINITY
Before	**T1**	30.10 ± 1.00^{a}	8.40 ± 1.00^{a}	172.00 ± 1.00^{c}	0.20 ±0.10^{c}	86.01 ± 1.00^{d}	0.30 ± 0.00
	T2	27.60 ± 1.00^{b}	8.03 ± 1.02^{a}	192.80 ± 1.00^{b}	0.50 ±0.10^{c}	95.89 ± 1.00^{b}	0.40 ± 0.00
Before	**T1**	31.10 ± 1.00^{a}	8.34 ± 1.00^{a}	162.40 ± 1.00^{e}	4.10 ±1.00^{a}	81.08 ± 1.00^{e}	0.30 ± 0.00
	T4	30.90 ± 1.00^{a}	7.53 ± 1.00^{a}	171.80 ± 1.00^{c}	2.80 ±1.00^{ab}	89.41 ± 1.00^{c}	0.30 ± 0.00
	T5	31.10 ± 1.00^{a}	8.42 ± 1.00^{a}	196.40 ± 1.00^{a}	1.60 ±1.00^{bc}	98.30 ± 1.00^{a}	0.40 ± 0.00
	T6	31.40 ± 1.00^{a}	7.59 ± 1.00^{a}	165.50 ± 1.00^{d}	3.10 ±1.00^{ab}	82.71 ± 1.00^{e}	0.30± 0.00
1 hr	**T1**	30.03 ± 0.12^{c}	7.96 ± 0.00^{c}	181.37 ± 0.12^{b}	3.93 ±0.06^{b}	90.65 ± 0.02^{c}	0.40 ± 0.00^{ak}
	T2	30.73 ± 0.06^{b}	7.92 ± 0.00^{e}	182.23 ± 0.12^{a}	4.30 ±0.17^{a}	91.00 ± 0.02^{a}	0.40 ± 0.00^{a}
	T3	30.57 ± 0.12^{b}	8.00 ± 0.00^{b}	181.43 ± 0.29^{b}	4.33 ±0.06^{a}	90.57 ± 0.04^{d}	0.40 ± 0.00^{a}
	T4	30.63 ± 0.06^{b}	7.90 ± 0.00^{f}	182.07 ± 0.23^{a}	3.73 ±0.06^{cd}	90.90 ± 0.01^{b}	0.40 ± 0.00^{a}
	T5	32.97 ± 0.12^{a}	8.01 ± 0.00^{a}	170.13 ± 0.29^{e}	3.83 ±0.12^{bc}	84.90 ± 0.01^{f}	0.30 ± 0.00^{a}
	T6	30.63 ± 0.12^{b}	7.93 ± 0.00^{d}	176.03 ± 0.12^{c}	3.63 ±0.12^{d}	87.92 ± 0.02^{e}	0.30 ± 0.00^{a}
2 hr	**T1**	31.97 ± 0.06^{a}	8.33 ± 0.00^{a}	189.10 ± 17^{a}	1.467 ±0.06^{d}	94.42 ± 0.01^{a}	0.40 ± 0.00^{a}
	T2	30.07 ± 0.06^{c}	8.00 ± 0.00^{c}	172.03 ± 12^{f}	2.067 ±0.06^{c}	85.93 ± 0.02^{f}	0.30 ± 0.00^{a}
	T3	30.03 ± 0.06^{c}	8.07 ± 0.00^{b}	184.23 ± 12^{b}	1.533 ±0.06^{d}	92.08 ± 0.01^{b}	0.40 ± 0.00^{a}
	T4	30.43 ± 0.12^{b}	7.90 ± 0.00^{f}	174.50 ± 17^{e}	2.133 ±0.12^{c}	87.19 ± 0.04^{e}	0.30 ± 0.00^{a}
	T5	31.97 ± 0.06^{a}	7.98 ± 0.00^{e}	180.87 ± 12^{d}	3.133 ±0.06^{a}	90.40 ± 0.01^{d}	0.40 ± 0.00^{a}
	T6	30.47 ± 0.06^{b}	7.98 ± 0.00^{d}	183.50 ± 17^{c}	2.367 ±0.12^{b}	91.70 ± 0.02^{b}	0.40 ± 0.00^{a}
3 hr	**T1**	30.60 ± 0.00^{a}	8.24 ± 0.00^{a}	183.27 ± 0.06^{a}	3.63 ±0.06^{a}	95.14 ± 0.04^{a}	0.40 ± 0.00^{a}
	T2	31.10 ± 0.00^{a}	8.23 ± 0.00^{b}	177.40 ± 0.35^{d}	3.3 ±0.12^{b}	88.56 ± 0.06^{d}	0.30 ± 0.00^{a}
	T3	31.10 ± 0.00^{a}	7.81 ± 0.00^{f}	170.50 ± 0.17^{e}	2.07 ±0.06^{c}	85.24 ± 0.03^{e}	0.30 ± 0.00^{a}
	T4	33.10 ± 3.46^{a}	7.88 ± 0.00^{c}	178.80 ± 0.50^{c}	1.07 ±0.21^{e}	89.59 ± 0.20^{c}	0.30 ± 0.00^{a}
	T5	31.10 ± 0.00^{a}	7.87 ± 0.00^{d}	170.83 ± 0.12^{e}	1.57 ±0.12^{d}	85.37 ± 0.07^{e}	0.30 ± 0.00^{a}
	T6	31.60 ± 0.17^{a}	7.86 ± 0.00^{e}	181.33 ± 0.12^{b}	3.33 ±0.12^{b}	90.49 ± 0.01^{b}	0.40 ± 0.00^{a}
4 hr	**T1**	29.77 ± 0.06^{e}	7.98 ± 0.00^{e}	180.53 ± 0.12^{c}	2.83 ±0.06^{b}	89.99 ± 0.00^{c}	0.40 ± 0.00^{a}
	T2	30.20 ± 0.00^{cd}	7.97 ± 0.00^{f}	183.03 ± 0.12^{b}	2.33 ±0.06^{e}	91.59 ± 0.12^{a}	0.40 ± 0.00^{a}
	T3	30.30 ± 0.00^{bc}	8.40 ± 0.00^{a}	174.03 ± 0.12^{e}	2.57 ±0.06^{d}	87.06 ± 0.06^{e}	0.30 ± 0.00^{a}
	T4	30.37 ± 0.12^{b}	8.03 ± 0.00^{d}	178.20 ± 0.17^{d}	2.63 ±0.06^{cd}	88.95 ± 0.01^{d}	0.30 ± 0.00^{a}
	T5	30.13 ± 0.06^{d}	8.39 ± 0.00^{b}	183.47 ± 0.35^{a}	3.63±0.06^{a}	91.40 ± 0.01^{b}	0.40 ± 0.00^{a}
	T6	31.97 ± 0.06^{a}	8.14 ± 0.00^{c}	96.07 ± 0.06^{f}	2.73 ± 0.12^{bc}	48.05 ± 0.04^{f}	0.20 ± 0.00^{a}
8 hr	**T1**	30.47 ± 0.12^{a}	8.68 ± 0.01^{a}	780.63 ± 0.12^{a}	2.60 ± 0.00^{c}	390.30 ± 0.17^{a}	1.50 ± 0.00^{a}
	T2	30.37 ± 0.12^{a}	8.08 ± 0.00^{c}	188.97 ± 0.29^{e}	1.17 ± 0.12^{e}	94.40 ± 0.02^{d}	0.40 ± 0.00^{a}
	T3	30.63 ± 0.06^{a}	8.03 ± 0.00^{e}	180.70 ± 0.69^{f}	2.80 ± 0.17^{b}	90.48 ± 0.31^{e}	0.40 ± 0.00^{a}
	T4	29.30 ± 0.69^{b}	8.12 ± 0.00^{b}	194.57 ± 0.92^{d}	2.43 ± 0.12^{c}	96.98 ± 0.23^{c}	0.40 ± 0.00^{a}
	T5	30.30 ± 0.00^{a}	8.08 ± 0.00^{d}	195.60 ± 0.17^{c}	3.27 ± 0.06^{a}	97.60 ± 0.05^{b}	0.40 ± 0.00^{a}
	T6	30.53 ± 0.12^{a}	8.01 ± 0.00^{f}	197.47 ± 0.23^{b}	2.13 ± 0.06^{d}	97.50 ± 0.01^{b}	0.40 ± 0.00^{a}

Table 3 continue

12 hr	**T1**	**31.73 ± 0.12^{a}**	**8.38 ± 0.00^{b}**	**179.77 ± 0.23^{e}**	**4.17 ± 0.12^{c}**	**89.79 ± 0.04^{e}**	**0.4 ± 0.00^{a}**
	T2	29.97 ± 0.06^{c}	7.99 ± 0.00^{f}	188.80 ± 0.17^{d}	4.50 ± 0.17^{b}	94.55 ± 0.11^{d}	0.40 ± 0.00^{a}
	T3	30.37 ± 0.06^{b}	8.40 ± 0.00^{a}	353.43 ± 0.29^{a}	4.67 ± 0.12^{ab}	126.70 ± 0.17^{a}	0.50 ± 0.00^{a}
	T4	30.33 ± 0.12^{b}	8.03 ± 0.00^{d}	193.83 ± 0.12^{c}	4.83 ± 0.06^{a}	96.90 ± 0.02^{c}	0.40 ± 0.00^{a}
	T5	30.43 ± 0.06^{b}	8.32 ± 0.00^{c}	114.23 ± 0.29^{f}	3.67 ± 0.12^{a}	57.06 ± 0.06^{f}	0.20 ± 0.00^{a}
	T6	30.43 ± 0.06^{b}	8.01 ± 0.00^{e}	195.67 ± 0.40^{b}	4.07 ± 0.12^{c}	97.69 ± 0.07^{b}	0.40 ± 0.00^{a}
16 hr	**T1**	29.67 ± 0.12^{e}	7.79 ± 0.00^{f}	178.07 ± 2.23^{e}	2.633 ±0.06^{d}	89.70 ± 0.06^{f}	0.40 ± 0.00^{a}
	T2	30.67 ± 0.12^{b}	7.97 ± 0.00^{d}	157.97 ± 1.27^{f}	3.367 ±0.12^{c}	93.65 ± 0.38^{e}	0.40 ± 0.00^{a}
	T3	32.27 ± 0.12^{a}	8.24 ± 0.00^{b}	248.47 ± 0.23^{b}	2.367 ±0.06^{e}	154.90 ± 0.35^{b}	0.50 ± 0.00^{a}
	T4	29.97 ± 0.12^{d}	8.33 ± 0.00^{a}	830.7 ± 5.72^{a}	4.033 ±0.06^{b}	417.00 ± 0.17^{a}	1.60 ± 0.00^{a}
	T5	30.27 ± 0.12^{c}	8.01 ± 0.00^{c}	199.77 ± 0.23^{d}	3.467 ±0.12^{c}	99.54 ± 0.12^{d}	0.40 ± 0.00^{a}
	T6	30.57 ± 0.12^{b}	7.88 ± 0.00^{e}	207.47 ± 0.23^{c}	4.233 ±0.06^{a}	103.83 ± 0.12^{c}	0.40 ± 0.00^{a}
20 hr	**T1**	31.53 ± 0.06^{a}	7.86 ± 0.10^{de}	200.73 ± 0.29^{e}	4.83 ± 0.06^{a}	100.73 ± 0.12^{e}	0.40 ± 0.00^{a}
	T2	31.27 ± 0.06^{b}	8.12 ± 0.00^{b}	195.37 ± 0.01^{f}	3.33 ± 0.06^{d}	97.59 ±0 .12^{f}	0.40 ± 0.00^{a}
	T3	31.23 ± 0.06^{b}	8.20 ± 0.00^{a}	203.53 ± 0.12^{c}	4.03 ± 0.12^{b}	102.20 ± 0.17^{c}	0.40 ± 0.00^{a}
	T4	30.87 ± 0.06^{c}	7.98 ± 0.00^{c}	317.67 ± 0.40^{a}	3.83 ± 0.12^{c}	159.47 ± 0.23^{a}	0.60 ± 0.00^{a}
	T5	31.23 ± 0.06^{b}	7.82 ± 0.00^{e}	202.27 ± 0.58^{d}	4.07 ± 0.06^{b}	101.17 ± 0.23^{d}	0.40 ± 0.0 0^{a}
	T6	30.67 ± 0.06^{d}	7.90 ± 0.00^{d}	210.50 ± 0.17^{b}	3.17 ± 0.12^{e}	105.13 ± 0.12^{b}	0.40 ± 0.00^{a}
24 hr	**T1**	30.63 ± 0.06^{ab}	8.21 ± 0.00^{c}	318.77 ± 0.23^{b}	5.13 ± 0.06^{b}	159.87 ± 0.58^{b}	0.60 ± 0.00^{a}
	T2	30.57 ± 0.12^{b}	8.03 ± 0.00^{e}	201.83 ± 0.12^{e}	3.67 ± 0.12^{f}	100.97±0.06^{e}	0.40 ± 0.00^{a}
	T3	30.73 ± 0.06^{a}	8.13 ± 0.00^{d}	202.83 ± 0.12^{d}	4.03 ± 0.06^{e}	101.53±0.12^{d}	0.40 ± 0.00^{a}
	T4	30.27 ± 0.12^{c}	7.94 ± 0.00^{f}	197.77 ± 0.23^{f}	5.03 ± 0.42^{a}	98.83±0.12^{f}	0.40 ± 0.00^{a}
	T5	30.77 ± 0.12^{a}	8.40 ± 0.00^{a}	204.43 ± 0.29^{c}	4.67 ± 0.12^{c}	102.27±0.23^{c}	0.40 ± 0.00^{a}
	T6	30.80 ± 0.00^{a}	8.28 ± 0.00^{b}	684.83 ± 0.12^{a}	4.07 ± 0.12^{d}	342.97±0.23^{a}	1.30 ± 0.00^{a}
48 hr	**T1**	27.63 ± 0.12^{c}	7.65 ± 0.01^{a}	380.10 ± 0.87^{f}	4.07 ± 0.06^{a}	189.67±1.10^{f}	0.70 ±0.00^{e}
	T2	27.87 ± 0.06^{b}	6.31 ± 0.00^{f}	733.83 ± 0.12^{a}	2.67 ± 0.06^{c}	366.73±0.29^{a}	1.40 ± 0.00^{a}
	T3	27.87 ± 0.06^{b}	7.28 ± 0.00^{d}	494.80 ± 0.17^{b}	2.57 ± 0.06^{c}	248.47±0.23^{b}	1.00 ± 0.00^{b}
	T4	27.93 ± 0.06^{b}	7.33 ± 0.00^{c}	400.80 ± 1.04^{d}	3.97 ± 0.06^{a}	200.17±0.40^{d}	0.80 ± 0.00^{d}
	T5	27.87 ± 0.06^{b}	7.53 ± 0.00^{b}	435.37 ± 0.40^{c}	3.97 ± 0.06^{a}	218.00±0.17^{c}	0.87 ± 0.06^{c}
	T6	28.27 ± 0.06^{a}	7.20 ± 0.00^{e}	389.30 ± 0.35^{e}	3.53 ±0.058^{b}	194.93±0.4619^{e}	0.83 ± 0.06^{cd}
72 hr	**T1**	27.27 ± 0.06^{a}	6.47 ± 0.28^{e}	474.90 ± 0.17^{b}	3.37 ± 0.06^{a}	270.93±28.96^{d}	0.90 ± 0.00^{a}
	T2	28.27 ± 0.06^{a}	7.35 ± 0.00^{c}	773.37 ± 118.1^{ab}	3.27 ± 0.12^{a}	420.83±0.12^{b}	1.60 ± 0.00^{a}
	T3	27.43 ± 1.68^{a}	7.75 ± 0.00^{a}	853.4 ± 373.78^{ab}	3.27 ± 0.06^{a}	320.23±0.64^{c}	1.30 ± 0.00^{a}
	T4	28.67 ± 0.06^{a}	6.47 ± 0.00^{e}	1046.70 ± 413.4^{a}	3.03 ± 0.06^{b}	642.80±0.17^{a}	2.50 ± 0.00^{a}
	T5	28.23 ± 0.12^{a}	6.50 ± 0.00^{d}	555.20 ± 25.29^{b}	3.07 ± 0.06^{b}	285.43 ± 0.29^{d}	1.10 ± 0.00^{a}
	T6	23.37 ± 0.06^{a}	7.72 ± 0.00^{b}	526.27 ± 0.58^{b}	3.33 ± 0.06^{a}	263.63 ± 0.46^{d}	1.00 ± 0.00^{a}
96 hr	**T1**	27.17 ± 0.06^{d}	6.60 ± 0.00^{d}	563.83 ± 0.12^{c}	4.20 ± 0.00^{ab}	282.43 ± 0.29^{c}	1.00 ± 0.17^{c}
	T2	27.87 ± 0.06^{a}	6.87 ± 0.00^{c}	480.17 ± 0.23^{d}	3.97 ± 0.06^{c}	240.27 ± 0.23^{e}	0.97 ± 0.16^{c}
	T3	27.33 ± 0.06^{c}	6.61 ± 0.00^{d}	572.33 ± 29.04^{c}	4.27 ± 0.06^{a}	252.73 ± 0.12^{d}	1.00 ± 0.00^{c}
	T4	27.73 ± 0.12^{b}	6.86 ± 0.00^{c}	312.27 ± 0.23^{e}	4.03 ± 0.06^{c}	155.77 ± 0.23^{f}	0.60 ± 0.00^{d}
	T5	27.77 ± 0.06^{ab}	7.40 ± 0.00^{a}	949.70 ± 0.17^{b}	3.57 ± 0.06^{d}	475.67 ± 0.12^{b}	1.30 ± 0.17^{b}
	T6	27.80 ± 0.00^{ab}	7.07 ± 0.02^{b}	1270.60 ± 0.00^{a}	4.17 ± 0.06^{b}	635.00 ± 0.17^{a}	2.50 ± 0.00^{a}
After	**T1**	27.37 ± 0.46^{b}	6.47 ± 0.00^{f}	245.23 ±0.29^{f}	3.80 ± 0.00^{b}	121.97 ± 0.23^{f}	0.50 ± 0.00^{e}
	T2	27.83 ± 0.16^{a}	6.95 ± 0.00^{e}	264.80 ±0.17^{e}	2.57 ± 0.06^{c}	132.57 ± 0.23^{e}	0.50 ± 0.00^{e}
	T3	27.7 ± 0.12^{ab}	7.04 ± 0.00^{c}	496.30 ±0.17^{d}	4.57 ± 0.06^{a}	248.20 ± 0.17^{d}	0.57 ± 0.06^{d}
	T4	27.73 ± 0.12^{ab}	7.23 ± 0.00^{b}	606.20 ±0.17^{b}	3.73 ± 0.06^{b}	303.50 ± 0.35^{b}	1.20 ± 0.00^{b}
	T5	27.73 ± 0.12^{ab}	6.96 ± 0.00^{d}	877.27 ±0.23^{a}	4.27 ± 0.40^{a}	439.53 ± 0.98^{a}	1.70 ± 0.00^{a}
	T6	27.83 ± 0.12^{a}	8.25 ± 0.00^{a}	580.77 ±0.23^{c}	4.23 ± 0.15^{a}	290.47 ± 0.23^{c}	1.10 ± 0.00^{c}

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

CONCLUSION

Hence, it can be concluded from the present study that Nicosulfuron herbicide is moderately toxic to *C. gariepinus* and even low doses of it could modify the hematological profile of fish. The toxicity could be of low impact but the bioaccumulation effect can be hazardous. The result of this work can serve as baseline information to develop models on effects of Nicosulfuron herbicides on ecological system of aquatic environment.

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