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Haematology in Juveniles of African Catfish (*Clarias gariepinus*) Exposed to Primextra Gold® (Atrazine) Herbicide

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Research Article

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Abstract

The widespread increase of pesticides application in crops frequently leads to the contamination of the fresh water ecosystem. One of the most commonly used pesticides in Nigeria is Primextra Gold® (atrazine) herbicide. The aim of this study is to evaluate the toxicity of Primextra Gold® (atrazine) on the juveniles of the African catfish, *Clarias gariepinus*. The toxicity was carried out using 120 healthy fish that were divided into six treatments (control inclusive) with each treatment having ten fish and the setup was replicated. The fish were exposed to 0.00 (control), 0.15, 0.29, 0.44, 0.59 and 0.74mg/L concentrations of Primextra Gold® (atrazine) for two weeks. During the exposure period, physicochemical parameters were determined. After two weeks of the experiment, blood samples were collected for heamatology using standard methods. Data obtained were subjected to ANOVA at $P<0.05$using Minitab 17. Results of the physico-chemical parameters of water exposed to concentrations showed that there was a significant decrease ($P<0.05$) in the amount of dissolved oxygen with increase in concentrations of the herbicide while pH, total dissolved solids (TDS) and electrical conductivity increased. Temperature showed no significant difference ($P>0.05$). Haematological parameters reduced significantly ($P<0.05$) in amount of PCV, Hb and RBC with increase in concentrations. Meanwhile WBC increased significantly ($P<0.05$) with increase in concentration. A haematological change observed during the exposure is an indication of anaemia, leukocytosis, and haemoglobinaemia. The study revealed that Primextra Gold® (atrazine) was moderately toxic to *Clarias gariepinus* juveniles and affected the haematology as well as Physicochemical parameters of the exposed water. These results show that the contamination of water by pesticides either directly or indirectly could be lethal to fish or may reduce fish productivity. Elevated concentrations of undesirable chemicals in edible fish tissue can affect the health of humans eating these fishes.

Keywords: *Clarias gariepinus*; Heamatology; Herbicides; Primextra Gold® (Atrazine)

Abbreviations: PCV: Packed Cell Volume; RBC: Red Blood Count; WBC: White Blood Cell; MCHC: Mean Corpuscular Haemoglobin Concentration; MCH: Mean Corpuscular Haemoglobin; MCV: Mean Corpuscular Volume; EDTA: Ethylene Diamine Tetra Acetic Acid; TDS: Total Dissolved Solids; EC: Electrical Conductivity; DO: Dissolved Oxygen.

Introduction

The ever-increasing world population and the attendant increase in food demand necessitated that new ways of increasing agricultural output be sought. In an attempt to increase agricultural output, man relies heavily on the use

of chemicals to protect crops from pests, right from the time of dressing of seeds before planting, through fighting weeds and other pests on the farm, to the preservation of already harvested products. On one side, benefits derived from the use of pesticides in agriculture are immense, but on the other side, environmental pollution and/or degradation is one major problem that is linked to their application [1].

The presence of pesticides in the environment has caused significant social and scientific development anxiety worldwide, as all-over-the-world their extensive use can create potential risks to the environment and human health, and easily pollute bodies of water thereby resulting in extensive damage to non-target species, including fish [2]. Be it intentional or unintentional application, water is contaminated through direct application into the aquatic system, drifts during spray, atmospheric fallout as rain and dust, soil erosion, sewage, industrial effluent and occasionally by spillage Moreno MA, et al. [2].

The aquatic environment is particularly one vulnerable area as it is the ultimate recipient of pollutants due to basin drainage. The aquatic ecosystems have been known to receive a wide spectrum of pollutants, which may be introduced to them directly or indirectly. The indiscriminate use of chemicals has resulted in large scale reduction in aquatic productivity. Pesticides have different diverse impacts on aquatic animals especially fishes which are of economic importance and high value from the point of biological conservation [3]. Environmental pollution by pesticides has become a serious problem in terms of global conservation and animal and human health [4,5]. Besides overexploitation and habitat loss, pollution from herbicides is one of the main causes of fish species and other aquatic organisms' loss [6].

Atrazine (2-Chloro-4-ethylamino-6-Isopropylamino-s-triazine) is one of the most currently and widely used herbicides in this part of the world and several studies have detected its presence in water bodies at levels above the limits determined by local and international authorities (for example the Maximum Contaminant Level of 3µg/L by USEPA) [5]. Due to its potential to bio accumulate in organisms and its proposed negative effects, the application of atrazine has been banned in many countries (in the EU region by European Commission Directive No. 2004/248/EC) [7].

Fish is highly nutritious, easily digestible and a much sought after food. Nutritional value of fish depends on their biochemical composition, which is affected by water pollution. The African catfish, *Clarias gariepinus* is one of the richest sources of animal protein to man [8]. The aim of this study is to evaluate the toxicity of Primextra Gold® (atrazine) to the juveniles of the African catfish, *Clarias gariepinus*.

Materials and Methods

Study Area

The study was carried out in the Department of Fisheries and Aquaculture, Joseph SawuanTarka University Makurdi, Benue State. Makurdi is located Latitude 7° 44' 1.50" N and Longitude: 8° 31' 17.00" E and it is situated at elevation 104 meters above sea level.

Experimental Fish

A total number of 250 (two hundred and fifty) healthy and active juveniles of African catfish *Clarias gariepinus* with standard length 17.91±0.21 cm and mean weight 36.40±0.01 g were collected from Joseph SarwuanTarka University, Makurdi experimental Fish farm in for the study The fish were acclimatized for seven days in the fish hatchery, Department of Fisheries and Aquaculture, Joseph SarwuanTarka University, Makurdi. The fish were fed twice daily at 3% of their body weight during acclimatization. The complete randomized design was used.

Exposure of *Clarias gariepinus* to Primextra Gold® (Atrazine) Herbicide

The herbicide Primextra Gold® (atrazine) was obtained from Franken technologies Agrochemical chemical shop in Makurdi, Benue State. At the end of the acclimatization period, the tanks (Aquaria) were filled with water to about 20litres. A pilot test was carried out to determine the range of concentrations for the actual tests; from the findings, five concentrations of atrazine from the LC50 of 8.84 mg/L (0.15 mg/L, 0.29 mg/L, 0.44 mg/L, 0.59 mg/L, 0.74 mg/L and 0.00 mg/L as control) were used. The fish were randomly selected, weighed and stocked ten (10) per tank. The concentrations used were calculated using the formula C1V1=C2V2 [6]. The test herbicide Primextra Gold® (atrazine) was measured using a micro-pipette and was introduced into the tanks containing 20litres of water. The fish were fed with Coppens twice daily at 5% of their body weight. The water was changed weekly with the renewal of the herbicide. The exposure lasted for a period of 28 days.

Procedures for Haematological Studies of *Clarias gariepinus* Exposed to Primextra Gold® (Atrazine) Herbicide

At the end of the two (2) weeks, blood samples were collected from the heart of the fish in the various treatments using a 2mm needle and syringe. The blood samples collected were poured into ethylene-diamine-tetra-acetic acid (EDTA) bottles to prevent coagulation. The blood were

analysed at the University of Agriculture Makurdi Veterinary Teaching Hospital, North bank, for the following parameters: haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Count (RBC), White Blood Cell (WBC), Mean corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and leucocytes blood differentials using an automated haematology analyser mindrey vet 2800 model by inserting the needle of the analyser into the sample. The read button was pushed, the samples were read and displayed on the LCD and further printed.

Physicochemical Parameters of the Test Solution

The water quality parameters were determined using HANNA® dissolved oxygen (DO) meter, Model HI 93246 for dissolved oxygen and HANNA® multi parameter water tester, model HI 98129 for pH, TDS, EC and temperature following the manufactures guide as described by Odo O, et al. [6].

Data Analysis

Data of the physico-chemical parameter and haematology of fish collected were analyzed for descriptive statistics (mean and standard error of means) using Minitab 17. The data were further subjected to analysis of variance (ANOVA). Results are presented in tables.

Results

Bioassay Test of *Clarias gariepinus* Juveniles Exposed to Concentrations of Primextra Gold® (Atrazine) Herbicide

Significant variations (P<0.05) were observed in blood parameters determined with increase in concentration of the toxicant as presented in Table 1. PCV, Hb and RBC decreased with increase in concentration. WBC increased significantly (P<0.05) with increase in concentration. On the other hand, derived erythrocyte indices MCH, MCV and MCHC although differ significantly (P < 0.05), did not follow any trend.

Concentration (mg/L)	Parameters						
	Hb (g/dL)	RBC (fL)	WBC (fL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC(g/dL)
0.00(Control)	10.05±0.05e	3.06±0.10d	41.60±0.40a	30.00±0.10f	107.15±0.05d	35.75±0.05e	33.35±0.05b
0.15	9.00±0.00d	2.55±0.05c	48.45±0.05b	28.00±0.01e	112.55±0.05e	37.55±0.05f	33.35±0.05b
0.29	8.57±0.05c	2.45±0.05c	49.55±0.45b	26.00±0.01d	100.01±0.05c	33.25±0.05c	33.25±0.05b
0.44	8.41±0.01b	2.15±0.05b	53.64±0.04c	24.00±0.01c	104.20±0.01c	35.25±0.05d	33.70±0.10c
0.59	8.20±0.01b	2.06±0.06a	55.90±0.10d	23.00±0.01ab	83.35±0.05a	27.35±0.05a	32.85±0.05a
0.74	7.41±0.01a	2.01±0.01a	57.75±0.25e	21.00±0.01ab	96.25±0.05b	32.35±0.05b	33.70±0.10c
p-value	<0.01	<0.01	<0.01	0.01	0.01	0.02	0.03

Means in the same column with different superscripts differ significantly (P<0.05). KEY: Hb = Haemoglobin, RBC = Red Blood Cells, WBC = White Blood Cells, PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

Table 1: Mean Hematological Parameters of Clarias gariepinus juveniles exposed to different concentrations of Primextra Gold® Atrazine herbicide.

Results of juveniles of *Clarias gariepinus* exposed to varied concentrations of Primextra Gold® (Atrazine) herbicide showed that the control (0.00 mg/L) had the highest haemoglobin (Hb) mean value of 10.05±0.05 g/dL while the last concentration 0.74 mg/L had the lowest haemoglobin of 7.41±0.01 g/dL. Highest mean Red Blood Cells (RBC) value of 3.06±0.10 fL was recorded from the control (0.00) concentration and lowest Red Blood Cells of 2.01±0.05 fL was recorded from 0.74 mg/L. white blood cells (WBC) had the highest value recorded for 0.74 mg/L concentration with 57.75±0.25fLwhereas the lowest white blood cells value of 41.60±0.40fL was obtained in the control concentration. The control (0.00 mg/L) recorded the highest packed cell volume (PCV) value of 30.00±0.10 % while 0.74 mg/L had the lowest packed cell volume of 21.00±0.01%. The value of mean corpuscular volume (MCV) in concentration 0.15 mg/L was recorded highest with 112.55±0.05 fL while the lowest mean corpuscular volume value of 83.35±0.05 fL was obtained in concentration 0.59 mg/L. Highest mean corpuscular haemoglobin (MCH) of 37.55±0.05 pg

was recorded from the concentration 0.15 mg/L while the lowest mean corpuscular haemoglobin of 27.35±0.05 pg was recorded from 0.59 mg/L concentration. Mean corpuscular haemoglobin concentration (MCHC) varied slightly from the highest mean value of 33.70±0.10 g/dL recorded in both concentrations 0.44 mg/L and 0.74 mg/L to the lowest mean value of 32.85±0.05 g/dL obtained in concentration 0.59 mg/L.

The results for the physicochemical parameters of water during the exposure of Primextra Gold® (Atrazine) herbicide on C. gariepinus juveniles are presented in Table 2. Dissolved oxygen (DO), pH, total dissolved solids (TDS) and electrical conductivity (EC) varied significantly ($p<0.05$) from the control. Highest mean temperature of 26.98±0.26°C was recorded from the control (0.00 mg/L) and lowest temperature of 26.51±0.39°C was recorded from 0.59 mg/L solution. Although there was no statistical difference ($p>0.05$). All parameters determined increased with increase in concentration except for dissolved oxygen which decreased with increase in concentration. The Highest mean pH value of 7.72±0.03 was recorded from the concentration 0.74 mg/L while the lowest pH of 7.26±0.20 was recorded from the control (0.00 mg/L) solution. Mean value of dissolved oxygen in the control 6.51±0.22 mg/L was the highest recorded and it varied significantly from the lowest dissolved oxygen value of 2.72±0.21 mg/L obtained from concentration 0.74 mg/L. The highest mean value of total dissolved solids 832.42±9.82 mg/L was recorded from the concentration 0.74 mg/L whereas the lowest total dissolved solids mean value of 607.75±27.37 mg/L was recorded in the control test solution. Highest mean electrical conductivity (EC) of 1653.80±23.37 µS/cm was recorded from the concentration 0.74 mg/L test solution while the lowest electrical conductivity of 956.58±19.00 µS/cm was recorded from the control solution.

Concentration (mg/L)	Parameters				
	Temp(oC)	pH	DO(mg/L)	TDS (mg/L)	EC (µS/cm)
0.00 (Control)	26.98±0.26	7.26±0.20a	6.51±0.22d	607.75±27.37a	956.58±19.00a
0.15	26.53±0.40	7.52±0.11ab	4.91±0.34c	695.08±12.47b	1292.10±55.07b
0.29	26.55±0.41	7.57±0.05b	4.67±0.32bc	701.75±1.58b	1403.80±3.11c
0.44	26.58±0.41	7.62±0.05b	3.96±0.31b	706.75±1.66b	1401.40±7.42c
0.59	26.51±0.39	7.66±0.06b	3.14±0.18a	724.17±1.76b	1428.40±16.40c
0.74	26.57±0.40	7.72±0.03b	2.72±0.21a	832.42±9.82c	1653.80±23.37d
p-value	0.95ns	0.04	0	<0.01	<0.01

Means in the same column with no ranking are not statistically different ($p<0.05$),ns = not significant. KEY: Temp = Temperature, DO = Dissolved Oxygen, TDS = Total Dissolved Solids, EC = Electrical Conductivity.

Table 2: Mean Physico-chemical parameters of the water during exposure of *Clarias gariepinus* Juveniles to different Concentrations of Primextra Gold® (Atrazine) herbicide.

Discussion

The health status of blood in living organisms is imperative because of its numerous roles including Oxygen and nutrient transportation. Blood plays a major function in the regulation of internal environment of a system (homeostasis) as stated by Ada FB, et al. [9]. After exposure to the concentrations of Primextra Gold® (Atrazine) herbicide, the results for haematological indices showed significant differences in Hb (haemoglobin), RBC (Red Blood Cells), WBC (White Blood Cells), PCV (packed cell volume), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) between the control and the various treatments. Usually any intense or severe attack on it can out rightly lead to death of an organism. Reduction in haemoglobin concentration with increased concentration of atrazine observed in this study conforms to the report of Edori OS, et al. [10] in which *Clarias gariepinus* was exposed to sublethal concentrations of paraquat. The decrease in haemoglobin may have led to a condition in which the capacity of the blood to transport oxygen to tissues is reduced; consequently, decline in red blood cells may depict a reduction in cellular iron which supports Oxygen carrying potential of blood. Ada FB, et al. [9] opined that haemoglobin reduction invariably contributes to the stress and anaemic state of organisms which further alters respiration, metabolism and induces morbidity and mortality. Hence, haemoglobin reduction in treated fish might have impaired Oxygen supply to the various tissues. The red blood cells decreased with increase in concentration of atrazine which is similar to findings of Odo JI, et al. [11] who revealed that reduced red blood cell count implies a reduction in the level of Oxygen carried to the tissues. According to Adedeji OB, et al. [12], high concentration of pesticides or long term exposure of fish to different concentrations of pesticides usually decreases red blood cells indices and

this is evident in the present study that showed reduction in all the concentrations compared to the control. Adedeji OB, et al. [12] revealed that red blood cells break down at an early stage in the incidence of pernicious anaemia. White blood cells showed significant difference, it increased with increase in concentration of atrazine and this is contrary to the findings of Ajani F, et al. [13] who reported a continuous decrease with increase in concentration of malathion. The increase in white blood cells from this investigation with increase in concentration could be due to attempts made by the fish to fight against the effects of the pollutant which could have led to production of more white blood cells to improve the health status of the fish. Increase in white blood cell count is seen as an adaptation of organisms and their efforts to combat invaders from body cells [9]. The population of exposed white blood cells which have been swiftly multiplied by previously surviving ones according to Popoola OM [14] is usually comprised of new or younger cells (because the old ones are killed) that would fight the invaders. They increase in the number of younger cells may be responsible for the differences in the white blood cell volume observed between exposed and control groups. Therefore, sharp increase in white blood cells (WBC) count above that of the control group could be resistance to prevalent unwanted change and adaptability to the environment. Packed cell volume decreased in this study and that implied poor transportation of oxygen and absorbed nutrients which could possibly have resulted to a decreased status of fish condition. An anaemic response in the test fish was perhaps also due to the destruction of intestinal cells [11,15] reported similar trends in fish exposed to pedimentiline. The fluctuations in the MCV, MCH and MCHC in this study indicates that the concentration of haemoglobin in the red blood cells were lower in the exposed fish than in the control over the exposure period. Mean corpuscular haemoglobin, which indicates blood level conditions, fluctuated, thus indicating anaemic condition in the test fish exposed to atrazine. Ada FB, et al. [9] reported that herbicides may trigger the multiplication of the blood cells to compensate for the low load of haemoglobin per cell whereas Adeyemo OK [16] explained that decrease in MCV, MCH and MCHC imply the malfunctioning of the organs responsible for blood production in fish. However, MCV fluctuations in values recorded in fish exposed to concentrations of atrazine did not concur with the findings of Ada FB, et al. [9] who reported that MCV did not change significantly in the exposed groups compared with the control. Moreover, the reduction in these blood metabolites is an indication of anaemia caused by exposure to the toxicant.

Water physicochemical parameters usually serve as indicators of the quality of water bodies, revealing the toxic levels and effects of chemicals present in aquatic media and on aquatic organisms. During the test period, only temperature showed no significant difference between the mean values of the treated fish and that of the control. Significant changes were however recorded in the mean values of pH, dissolved Oxygen (DO), total dissolved solids (TDS) and electrical conductivity (EC) between the control and various treatments. Generally, means of temperature and pH fluctuated slightly between the control and the treated solutions; this is line with studies of Ayoola SO [17] who reported slight changes in test water temperature and pH on exposure of *O. niloticus* juveniles to glyphosate herbicide. There was a significant relationship ($p<0.05$) between the rates of dissolved Oxygen with atrazine concentration, as the mean values decreased with increase in concentration of the toxicant in the test solutions. The significant negative correlation between dissolved Oxygen (DO) values and toxicant concentration as dissolved oxygen decreased considerably with increasing concentrations of atrazine is in consonance with earlier reports of Ajani and Awogbade [18] and Ada FB, et al. [9] that reported same decline in dissolved Oxygen (DO) in exposed fish studied under laboratory conditions. This may be due to stress induced by atrazine which resulted in agitation and abnormal behaviours of the test fish, thereby, leading to reduced dissolved Oxygen (DO) level in the test solutions. Ada FB, et al. [9] suggests that it may be possible that some herbicides make use of oxygen in decomposition process; this may probably be one of the reasons for decline in oxygen in treated test solutions in the present study. Total dissolved solids (TDS) and electrical conductivity (EC) increased with increasing concentrations of atrazine indicating organic matter content and electrolyte concentrations. The changes might have been as a result of the active ingredient and also the level of concentration of the toxicant in test solutions. These are in agreement with the findings of Akaahan TJ, et al. [19] on the effects of zinc on water quality when *C. gariepinus* juveniles were examined and on the toxicity of paraquat dichloride on water quality during exposure of adult *C. gariepinus* Popoola OM [14,20,21].

Conclusion

Atrazine alter the pristine state of aquatic environment and kill non-target organisms like fish. The effects of atrazine on haematology and water physicochemical parameters were examined under laboratory conditions. An indication of subtle but rapid deterioration of life due to the effects of atrazine exerted on fish was evident in the blood parameters because small concentrations of toxicants in water bodies are harmful. Concentrations of atrazine on juveniles of *Clarias gariepinus* caused alterations in haematological parameters (Haemoglobin, Red Blood Cell, White Blood Cell, Packed Cell Volume, Mean Corpuscular Volume, mean corpuscular haemoglobin and Mean Corpuscular Haemoglobin Concentration) of exposed fish and caused a range of defects and health problems like anaemia as a result of increased white blood cell of the test fish. It is however imperative

to note that the life of an organism is in the blood, so the enormous impact of atrazine on the blood characteristics posed an adverse damage to the treated fish. At exposure to concentrations of atrazine, fish showed abnormal behaviours such as restlessness, gasped for air, and vertical movements. Dissolved Oxygen level in the test solutions reduced drastically and this can induce death in fish. Atrazine application to enhance crop yield; although produces an immediate satisfactory result, but it consequently carries an accompanying side effect, moreover, when we act locally, the impact may be felt globally so let us be environmentally conscious and seek to achieve environmental sustainability for future generations.

Recommendations

1. Based on the findings of this work that atrazine affects physicochemical parameters of water and fish haematology, Biological methods of controlling weeds and organic farming should be encouraged.
2. Atrazine should be applied at a minimum effective concentration so that non-target organisms will not be affected.
3. Researches on the rate of atrazine in Nigeria water bodies should be conducted and the effects of atrazine to other biochemical parameters on *Clarias gariepinus* should be examined due to the fact that atrazine affects physicochemical parameters of water and fish haematology.

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