

Haematological Responses of *Clarias Gariepinus* and *Oreochromis niloticus* Juveniles Exposed To Sub-Lethal Concentrations of Glyphader 480[®] Herbicide

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ABSTRACT

This study was designed to determine the haematological responses of juveniles of *Clarias gariepinus* and *Oreochromis niloticus* exposed to different sub-lethal concentrations of Glyphader 480 herbicide during a period of 8 weeks. The toxicant led to significant ($P < 0.05$) changes in haematological parameters as the toxicant concentration increased. Haemoglobin content (Hb), Mean Red Blood Cells (RBC), Packed Cell Volume (PCV), Haematocrit (HCT) reduced as the concentration of toxicant increased while other parameters (WBC, MCV, MCH, PLT and MCHC) increased proportional with the toxicant concentration. Replacement of Glyphader 480 with less harmful and more biodegradable herbicides is recommended.

INTRODUCTION

Glyphader 480, contains 360g/L glyphosate in the form of 480g/L isopropylamine salt, it is used by farmers in the tropics to control weeds prior to or after planting. It is used to control weeds like *Pennisetumsp*, *Panicum maximum*, *Cynodondactylon* etc. It is one of the widely used herbicide that is persistent and mobile in soil and water, and it is known to be one of the most common terrestrial and aquatic contaminants.

Haematological analyses has been used in determining the physiological state of animals which can be affected by different environmental factors, it is used in the diagnosis of many diseases and in evaluating the responses to therapy in both animals and human (Solomon and Okomoda 2012). Its usage in fish is growing and becoming very important for toxicological research. Shah and Altindag (2004) noted

that studies on fish blood gives the possibility of knowing physiological conditions within the fish long before there is an outward manifestation of diseases because under stressful condition as well as environmental imbalances some parameters in the fish blood changes in response to reflect the change.

The present study seeks to determine changes in hematological parameters of *C.gariepinus* and *O. niloticus* exposed to various concentrations of Glyphader 480.

MATERIALS AND METHODS

Juveniles of *Clarias gariepinus* and *Oreochromis niloticus* with mean weight of $35.85 \pm 0.5g$ and $16.85 \pm 0.5g$ respectively were collected from the University of Agriculture Fish Farm, Makurdi. The fish were acclimatized for 14 days (2 weeks) in glass aquaria tanks containing de-chlorinated and aerated tap water at room temperature of $27.87 \pm 0.19^{\circ}C$. During the period of acclimation, fish were fed twice daily with Coppens at 3% body weight. Water was changed every day to prevent the build-up of metabolic wastes and was aerated to increase oxygen supply. During the period of acclimation, fish were examined for pathogens and diseases. There was no mortality during the acclimation period.

Blood samples were obtained by randomly selecting the fish in each treatment baths and injecting a 2mm needle and syringe through the dorsal aorta to collect blood. The blood was kept in specimen bottles treated with EDTA to prevent coagulation and analysed at Federal Medical Centre Makurdi, for the following haematological parameters; Haemoglobin (Hb), Packed cell volume (PCV), Red blood cell (RBC), White blood cell (WBC), using a automated haemoglobin analyzer (Cobas U411 Model), while Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular haemoglobin (MCH) and

Mean corpuscular volume (MCV) were determined by calculation using the equations below;

$$MCV = Hct / RBCs \times 10$$

$$MCH = Hct / Hb \times 100$$

(Feldman *et al.*, 2000).

The statistical analysis was performed through Analysis of variance (ANOVA) and correlation test of 0.05 was chosen as the level for accepted significance.

RESULTS

Haematological parameters are potential biomarkers of exposure to agrochemicals due to their sensitivity to certain toxic agents (Heath, 1995). Exposure of *Clarias gariepinus* and *Oreochromis niloticus* to sub-lethal concentrations of Glyphader 480 for 8 weeks caused a significant ($P < 0.05$) decrease in Packed cell volume (PCV), Haemoglobin, white blood cell and Haematocrit as the concentration increases while Red blood cell, Mean corpuscular volume, Mean corpuscular haemoglobin increases as the concentration increase for the two fish species (Table 1 and 2).

Table 1: Effects of Sub-lethal concentration Of Glyhader 480 on Heamatology of *Clarias gariepinus* for 8 weeks

Conc. (Mg/L)	Haematological Parameters								
	PCV	Hb	WBC	RBC	MCV	MCH	PLT	MCHC	HCT
0 (ctrl)	24.50±0.50 ^a	8.20±0.10 ^a	148.95±0.25 ^d	2.29±0.01 ^a	100.70±0.10 ^f	38.30±0.20 ^d	44.50±0.50 ^f	27.80±2.00 ^d	59.75±0.05 ^a
2.00	21.50±0.50 ^b	7.35±0.15 ^b	166.60±3.20 ^c	2.58±0.00 ^b	126.20±0.05 ^e	39.60±0.00 ^d	52.00±0.00 ^e	26.60±0.10 ^d	53.65±0.05 ^b
4.00	16.50±0.50 ^c	5.85±0.05 ^c	184.90±1.60 ^b	2.28±0.05 ^c	129.20±0.30 ^d	41.30±0.20 ^c	57.50±1.50 ^d	29.20±0.60 ^c	48.45±1.25 ^c
6.00	13.50±0.50 ^d	4.65±0.05 ^d	217.30±0.90 ^a	2.1±0.01 ^{cd}	133.85±0.65 ^c	44.35±0.85 ^b	63.50±1.50 ^c	32.00±0.50 ^c	35.05±0.55 ^d
8.00	12.00±0.00 ^d	4.20±0.10 ^e	220.40±0.90 ^a	2.08±0.06 ^d	147.40±1.30 ^b	48.20±0.70 ^a	73.50±0.50 ^b	36.7±0.20 ^b	28.80±0.90 ^e
10.00	9.50±0.50 ^e	3.40±0.10 ^f	224.05±3.25 ^a	1.69±0.01 ^e	151.35±0.75 ^a	48.80±0.10 ^a	92.00±1.00 ^a	41.45±0.65 ^a	23.92±0.52 ^f
<i>PValue</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means on the same column with different superscript are statistically significant (p<0.05)

KEYS: PCV = Packed cell volume, Hb=Haeoglobin, WBC=White blood cell, RBC=Red blood cell, MCV=Mean Corpuscular Volume, MCHC=Mean Corpuscular Haemoglobin concentration, HCT=Haemotocrit,

Table 2: Effects of Sub-lethal concentration Of Glyhader 480 on Heamatology of *Oreochromis niloticus* for 8 weeks

Conc. (Mg/ L)	Haematological Parameters								
	PCV	Hb	WBC	RBC	MCV	MCH	PLT	MCHC	HCT
0 (ctrl)	24.80±0.50 ^a	8.80±0.10 ^a	127.95±0.25 ^d	2.52±0.01 ^a	99.40±0.10 ^f	36.80±0.20 ^d	43.40±0.50 ^f	27.30±2.00 ^d	58.55±0.05 ^a
2.50	21.80±0.50 ^b	7.95±0.15 ^b	145.60±3.20 ^c	2.18±0.0 ^b	124.90±0.50 ^e	38.10±0.00 ^d	50.90±0.00 ^e	26.10±0.10 ^d	52.45±0.05 ^b
3.00	16.80±0.50 ^c	6.45±0.05 ^c	163.90±1.60 ^b	1.88±0.05 ^c	127.90±0.30 ^d	39.80±0.20 ^c	56.40±1.50 ^d	28.70±0.60 ^{cd}	47.25±1.25 ^c
3.75	13.80±0.50 ^d	5.25±0.05 ^d	196.30±0.90 ^a	1.7±0.01 ^{cd}	132.55±0.65 ^c	42.85±0.85 ^b	62.40±1.50 ^c	31.50±0.50 ^c	33.80±0.55 ^d
5.00	12.30±0.00 ^d	4.80±0.10 ^e	199.40±0.90 ^a	1.6±0.06 ^d	146.10±1.30 ^b	46.70±0.70 ^a	72.40±0.50 ^b	36.20±0.20 ^b	27.60±0.90 ^e
7.50	9.80±.0.50 ^e	4.00±0.10 ^f	203.05±3.25 ^a	1.29±0.01 ^e	150.05±0.75 ^a	47.30±0.10 ^a	90.90±1.00 ^a	40.95±0.65 ^a	22.72±0.52 ^f
<i>P</i> - <i>Value</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means on the same column with different superscript are statistically significant (p<0.05)

KEYS: PCV = Packed cell volume, Hb=Haeoglobin, WBC=White blood cell, RBC=Red blood cell, MCV=Mean Corpuscular Volume, MCHC=Mean Corpuscular Haemoglobin concentration, HCT=Haemotocrit.

DISCUSSION

The significant reduction in some of the hematological parameters (PVC, Hb, RBC, MCHC and HCT) is an indication of severe anaemia caused by destruction of erythrocytes (Kori-Siakpere *et al.*, 2009), Haemodilution (Adeyemo, 2005 and Ayuba 2008) resulting from impaired osmoregulation across the gill epithelium and according to Okomoda *et al.*, (2010) could be as a result of the destruction of intestinal cells. Gaafar *et al.*, (2010) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants. The present study reveals that mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in control fish depicting anaemic condition (Table 1 and 2). This is similar to the observations of Bhagwart and Bhikajee (2002). According to Dzenda *et al.*, (2004), Mean Corpuscular Haemoglobin concentration is a good indicator of red blood cell swelling hence it reduces as concentration of the toxicant increases. White Blood Cells (WBC) count increase with increasing level of the toxicant in both fish species, this increase is due to heightened immune mechanism of the experimental fish species stimulated to fight against the toxic pollutant. This is in line with Ayuba and Ofojekwu, (2002). Solomon and Okomoda (2012) however, reported reduced WBC with increase stress from photoperiod manipulation, the difference between this finding and that of the present study is likely due to differences in the stressor involved as well as time of exposure to the stressor, as the present study use a toxicant for 8 weeks while Solomon and Okomoda, (2012) exposed fish to different photoperiod for six weeks.

From the study carried out, the result showed that *Clarias gariepinus* was more resistant to the test chemical than *Oreochromis niloticus*, this could be as a result of higher white blood cell count in the *Clarias gariepinus* than the *Oreochromis niloticus*.

CONCLUSION

The present study showed that Glyphader 480 is harmful to *Clarias gariepinus* and *Oreochromis niloticus*. The exposure of fish to this herbicide resulted in significant reduction in the studied haematological parameters such as PVC, Hb, RBC, MCHC and HCT and an increase in WBC, MCV, MCH and PLT of the studied species. The increase and decrease in the studied parameters were more severe in *O. niloticus* than *C. gariepinus*. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment as well as culture conditions. This should be considered when farmers use Glyphader 480 to control weeds in their fields. Replacing of Glyphader 480 with less harmful and more biodegradable herbicides is recommended.

REFERENCES

- Aderolu, A.Z., Ayoola S.O and Otitolaju A.A. (2010). Effects of Acute and sub-lethal concentrations of Actellic on Weight changes and Haematology parameters of *Clarias gariepinus*. *World Journal of Biological Research*. 3: 30-39
- Adeyemo, O.K. (2005). Haematological and histopathological effects of cassava mill Effluent in *Clarias gariepinus*. *African Journal of Biomedical Research*. 8: 179-183.
- Ayuba, V.O and Ofojekwu, P.C. (2002). Acute toxicity of the root extract of Jimson's Weed: *Datura innoxia* to the African catfish (*Clarias gariephinus*) fingerlings. *Journal of Aquatic Science* 17(2): 131 – 133.
- Bhagwant, S. and Bhikajee, M. (2000). Introduction of hypochromic macrocylic anaemia in *Oreochromis hybrid (Cichlidae)* exposed to 100mg/l (sublethal dose) of Aluminium. *Science and Technology Reserve Journal*. 5: 9-21

- Dzenda, T., Ayo, J.O., Adelaiye A.B. and Adaudi A. O. (2004a). Effect of crude methanolic leaf extract of *Tephrosia vogelii* on contraction of isolated rabbit jejunum. *XXIV Ann. sci. conf. physiology sos.* Nigeria Delta State University.
- Feldman, B.F. Zinkl, J.G. and Jain, N.C. (2000). Schalm's Veterinary Hematology. 5th ed. Lippincott Williams & Wilkins, pp. 1120-1124.
- Gaafar, A.Y., El-Manakhly, E.M., Soliman, M.K., Soufy H., Mona, S., Z., Mohamed, S.G. and Hassan, S.M. (2010). Some Pathological, Biochemical and Haematological Investigations on Nile Tilapia (*Oreochromis niloticus*) following Chronic Exposure to Edifenphos Pesticide. *Journal of American Science.* 6(10): 542-551
- Henry, C.J., Higgins K. F., and Buhl K. J. (1994). Acute toxicity and hazard assessment of RodeoR, X-77 SpreaderR, and Chem-TrolR to aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* 27: 392-399.
- Houston, A.H. (1990). Blood and circulation. In: C.B. Schreck, and P.B. Moyle (eds) *Methods in fish biology.* American Fisheries Society. Bethesda: Maryland., pp. 273-335.
- Kolo, R.); Yisa, T.A. and Esogban, S.A. (2009). Acute toxicity of Round up (Glyphosate) on Juvenile Tilapia zilli. *Journal of Applied Agricultural Research* 1(1): 105-109
- Kori-Siakpere, O., Ogbe, M.G. and Ikomi, R.B. (2009). Haematological response of the African cat fish, *Clarias gariepinus* (Burchell, 1822) to sublethal concentrations of potassium permanganate. *Scientific Research and Essay* 4(5):457-466

- Okomoda, V.T. and Ataguba G.A. (2011): Blood glucose response of *Clarias gariepinus* exposed to acute concentrations of glyphosate-isopropylammonium (Sunsate®). *Journal of Agricultural and Veterinary Sciences*. 3(6): 69-75
- Oloruntuyi O.O., Mulero O., Odukale B., (1993). The Effects of two Pesticides on *Clarias gariepinus*. *Proceeding of the 10th Annual Conference of the Fisheries Society of Nigeria held in Abeokuta, 16 – 20th November, 1992*. 173-177
- Servizi, J.A., Gordon R.W., Martens D.W. (1989): Acute toxicity of Garlon 4 and round up herbicides to salmon, daphnia and trout. *Bull. Environ. Contam. Toxicol.* 39: 15-22
- Shah, S.L., Altindag A. (2004). Haematological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. *Bull. Environ. Contam. Toxicol.* 73: 911 – 918
- Solomon, S.G. and Okomoda V.T. (2012). Effects of photoperiod on the haematological parameters of *Clarias gariepinus* fingerlings reared in water re-circulatory system. *Journal of Stress Physiology & Biochemistry*, 8(3): 24