Journal of Stress Physiology & Biochemistry, Vol. 9 No. 2 2013, pp. 271-278 ISSN 1997-0838

ORIGINAL ARTICLE

Hematological Response of *Clarias gariepinus* Fingerlings Exposed to Acute Concentrations of Sunsate®

Okomoda V.T.*, Ataguba G.A. and Ayuba V.O.

Department of Fisheries and Aquaculture, University of Agriculture Makurdi, Nigeria

Tel: +2348033319959

*E-Mail: okomodavictor@yahoo.com

Received January 4, 2013

The present study was designed to evaluate the 96 hours LC_{50} of Sunsate® and the hematological variation of fingerlings of African catfish exposed to different concentrations of the herbicides. Using static bioassays with continuous aeration under laboratory conditions acute toxicity of Sunsate® was determine to be 18.33 mg l^{-1} with upper and lower limit of 20.93 and 16.05 respectively. Also 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), Platelet count (PLT), Packed Cell Volume (PCV), reduced as the concentration of toxicant increased while other parameters increased proportional with the toxicant concentration. Precautious use of Sunsate® for the control of Aquatic weeds is recommended.

Key words: African catfish, acute toxicity, LC50, Herbicide

ORIGINAL ARTICLE

Hematological Response of *Clarias gariepinus* Fingerlings Exposed to Acute Concentrations of Sunsate®

Okomoda V.T.*, Ataguba G.A. and Ayuba V.O.

Department of Fisheries and Aquaculture, University of Agriculture Makurdi, Nigeria

Tel: +2348033319959

*E-Mail: okomodavictor@yahoo.com

Received January 4, 2013

The present study was designed to evaluate the 96 hours LC_{50} of Sunsate® and the hematological variation of fingerlings of African catfish exposed to different concentrations of the herbicides. Using static bioassays with continuous aeration under laboratory conditions acute toxicity of Sunsate® was determine to be 18.33 mg l^{-1} with upper and lower limit of 20.93 and 16.05 respectively. Also 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), Platelet count (PLT), Packed Cell Volume (PCV), reduced as the concentration of toxicant increased while other parameters increased proportional with the toxicant concentration. Precautious use of Sunsate® for the control of Aquatic weeds is recommended.

Key words: African catfish, acute toxicity, LC50, Herbicide

Glyphosate based herbicide are widely used for the control of Aquatic and terrestrial plants, they are sold with different trade name and formulation (Okomoda and Ataguba 2011). Like other herbicides, Glyphosate based herbicide have harmful effect on aquatic life beyond their save limit of usage, of all formulations having this active ingredient; MONO818®, Rodeo®, Round up®, Gramoxone® basically have been studied and their toxic effect well documented by several authors (Servizi *et al*., 1989; Henry *et al*., 1994; Oloruntuyi *et al*., 1993; Kolo *et al*. 2008), while research in other combination are very much scarce. Depending on the formulation involved in a particular herbicide; toxic effect could differ in herbicide even containing same active ingredient and with different life stages of the fish.

Sunsate® particularly, contains 360g/L glyphosate in the form of 480g/L isopropylamine salt (Manufacturers label), it is widely used by farmers in the tropics to control weeds prior to or after planting is done. It is used particularly to

control weeds like Pennisetumsp, Panicum maximum, Cynodondactylon, etc. (Manufacturers label). Glyphosate based herbicides are one of the widely used herbicides that could be persistent and mobile in soil and water, and it is known to be one of the most common terrestrial and aquatic contaminants, Hence the present study seeks to determine the active toxicity of this herbicide to a commonly cultured fish in Nigeria.

Haematological analyses has been routinely used in determining the physiological state of animals and known to be affected by different environmental factors, it is used as a guide in the diagnosis of many diseases and in evaluating the responses to therapy in both animals and human (Solomon and Okomoda 2012). Hence it use in fish culture 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 therefore examine changes in hematological parameters with varying concentration of Sunsate®.

MATERIALS AND METHODS

The experiment was conducted at the R. and D. Department of Felicity Foods and Beverages Nigeria Limited Lokoja, Kogi state, Nigeria. Fingerlings of *Clarias gariepinus* were gotten through induced breading and raised in plastic bowls prior to the commencement of the experiment. Feeding was based on 5% of the body weight of the fish but was suspended 24 hours prior to the commencement and during the period of the experiment. The concentration of Sunsate® (10.0 mgL^{-1}, 12.5 mgL^{-1}, 15 mgL^{-1}, 17.5 mgL^{-1} and 20mgL^{-1}) used for the acute toxicity test were determined by a preliminary test as described by Solbe (1995), Ten Fingerlings of *Clarias gariepinus* with average weight 4.58g $\pm$ 1.43 were randomly selected and transferred from the holding plastic bowls into 5 replicate test plastic bowls of 40Litters capacity, this was filled to 20Litters mark for the purpose of the study, the toxicant concentrations decided were measured into each plastic bowl using a calibrated measuring cylinder and marked accordingly. The Control tanks also had ten fish with 0% of the herbicide. Mortality of the fish was recorded for 1 hr, 6hrs, 12hrs and subsequently every 24 hours up to 96 hours of exposure. Toxicosis symptoms were also observed for the different concentration. Fish were considered dead when gill movement ceased and no response were observed upon gentle prodding. The physico-chemical parameters (Temperature, pH, Dissolved Oxygen, Total alkalinity and Free Carbondioxide) were analyzed according to standard method by APHA (1985). Descriptive Statistical Analysis, as well as Analysis of variance of results was done with Gen stat discovery edition statistical package.

At the end of the experiment, blood was collected from anaesthetized fish by cutting the caudal peduncle. Blood from two to three fish were pooled to obtain enough samples for hematological analysis. The collected blood were placed in coded 1.5mL heparinized plastic tubes, stored on ice according to the procedures established by Campbell and Murru (1990), standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (HGB) concentration was estimated as

cyanmethemoglobin (Brown, 1980), Packed Cell Volume (PCV) was determined using microhaematocrit method of Snieszko (1960). The Red Blood Cell (RBC) were counted using haemocytometer (Improved Neubauer Weber Scientific Ltd), according to Wintrobe (1978). Also the total White Blood Cell Counts (WBC) was enumerated with an improved Neubauer

Haemocytometer using Shaw's diluting fluid (Miale, 1982). Platelet (PLT) count was performed according to Rees and Ecker method (Seivered, 1983). The Red Blood Cell indices that include Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated using the formula mentioned by Dacie and Lewis (2001).

RESULTS AND DISCUSSION

Physicochemical parameters measured in the present study were observed not to differ significantly from one another (Table 1) and were within tolerance range recommended for cultured fish by Mackereth (1963). Similar findings were also reported by Adigun, (2005), Kolo, *et al.,* (2008), Kolo *et al*. (2009) and Okomoda and Ataguba (2011) for other toxicant. Hence, outcome of the studies on water quality were not thought to be the course of fish mortality.

Observations by Kolo *et al.,* 2009 reported initial reaction of fish to include swim actively due to the effect on the nervous system; the rapidity of swimming was directly proportional to the concentration of the chemical this toxicosis symptoms were also observed in the present study. The stressful and erratic behaviour of the test organism also indicate respiratory impairment probably due to the effect of the chemical on the gills (Okomoda and Ataguba 2011). Experiment conducted on acute toxicity of glyphosate on *C. gariepinus* fingerlings by Auta and Ogueji (2007) reveals several abnormal behaviors such as restlessness, uncoordinated movement, loss of equilibrium, air gulping and staying motionless, This is similar to the result of the present study and the findings of Okomoda and Ataguba (2011), Ayuba and Ofojekwu, (2002), Onusiriuka (2002), and Aderolu *et al.,* (2010) who observed similar response on fish subjected to varying toxicants.

The present study shows that the 96 h LC_{50} value of Sunsate® herbicide was 18.33mg/l with upper and lower limit of 20.93 and 16.05 respectively. According to WHO (1994) LC_{50} values of glyphosate vary widely from 2 to 5 ppm depending on fish species and the test conditions as well as glyphosate formulations, experiment by Servizi *et al*., (1989) on MONO818® a glyphosate formulation reveals LC_{50} of 2-3 mg/L for sockeye, rainbow, and coho fry while LC_{50} of Roundup® for bluegill sunfish and rainbow trout is only slightly higher at 6-14 mg/L and 8-26 mg/L, respectively However Okomoda and Ataguba (2011) reported LC_{50} of 17.5 mg/L for African catfish exposed to acute concentrations of Sunsate®. 96 hour LC_{50} of formalin was reported by Okomoda *et al.,* (2010) to be 114.83µl/l for the African catfish, Oronsaye and Ogbebo (1997) also reported LC_{50} of 0.4mg/l for *Clarias gariepinus* exposed to 96 hour of copper sulphate, Ayuba and Ofojekwu, (2002) also reported 204.17 mg/L for *Datura innoxia* root extracts. Acute concentration of different toxicant differs with formulation and with environmental conditions.

Exposure of *Clarias gariepinus* to acute concentrations of Sunsate® caused a significant ($P<0.05$) decrease in Packed cell volume (PCV), Haemoglobin, and erythrocytes of the fish. Similar results had been reported by Adeyemo, (2005),

Aderolu *et al.,* (2010), and Okomoda *et al,.* (2010), with freshwater fishes exposed to different toxicants under laboratory conditions. The significant reduction in these parameters is an indication of severe anaemia caused by destruction of erythrocytes (Kori-Siakpere *et al.,*2009), Heamodilution (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. This is similar to the observations of Bhagwart and Bhikajee (2002). According to Dzenda *et al* (2004), MCHC is a good indicator of red blood cell swelling hence it reduction as concentration of toxicant increases. White Blood Cells (WBC) count increase with increasing level of the toxicant, this increase is likely due to heightened immune mechanism of the experimental fish species stimulated to fight against the toxicant pollutant. This assumption is also in line with Ayuba and Ofojekwu, (2002) vedict. Solomon and Okomoda (2012) however, reported reduced WBC with increase stress from photoperiod manipulation, the different 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 96hrs while Solomon and Okomoda (2012) exposed fish to different photoperiod for six weeks.

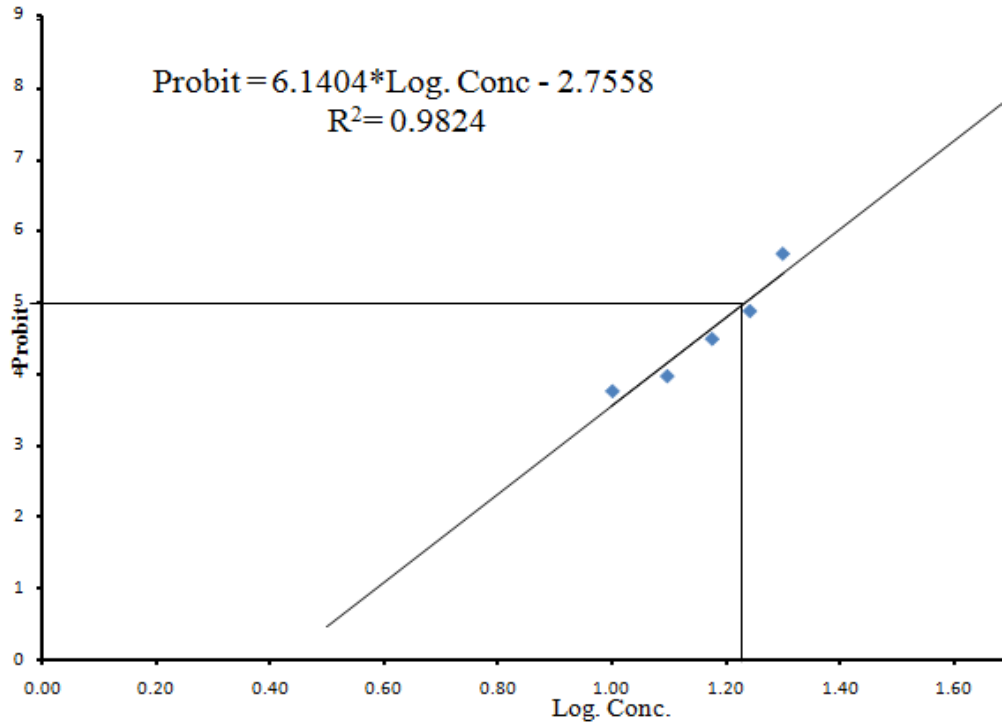

Figure 1. Linear relationship between mean probit mortality and log concentration of *Clarias gariepinus* fingerlings exposed to acute concentrations of Sunsate® for 96 hours.

Table 1: Physico-chemical parameters of test solution

Parameters	**Sunsate (ml/L)**				
	Controls	10.00	12.50	15.00	17.50
Temperature (^{0}C)	26.05±0.02	26.12±0.01	26.28±0.01	26.00±0.01	26.30±0.1
D.O (Mgl$^{-1)}$	7.33±0.02	7.30±0.03	7.20±0.4	7.16±0.02	6.49±0.06
pH	5.62±0.01	6.02±0.01	6.05±0.01	6.05±0.02	6.03±0.02
Alkalinity (Mgl$^{-1)}$	25.63±0.02	26.63±0.01	25.60±0.36	25.90±0.01	26.10±0.01
Free CO_2 (Mgl^{-1})	2.38±0.01	2.38±0.01	4.94±0.01	5.10±0.01	5.30±0.01

Table 2: Haematological parameters of *Clarias gariepinus* exposed to Lethal concentrations of Sunsate® for 96 hours.

Parameters	**0mg/l**	**15 mg/l**	**17.5mg/l**	**20mg/l**	**P**
WBC (10^8 /L)	2.5±0.25^c	57±2.1^b	90±0.2^b	189±1.3^a	**0.01**
Hb (g/dL)	2.25±0.5^a	1.22±0.12^b	0.75±0.05^c	0.33±0.15^c	**0.002**
RBC (10^{10} /L)	29.5±0.045^a	11.5±0.21^b	7±0.01^b	1.5±0.015^c	**0.011**
PCV (%)	3.5±0.9^a	1.23±1.1^b	0.6±0.2^c	0.15±0.15^c	**0.05**
PLT (10^9 /L)	55.5±42.5^a	18.9±0.39^b	9.50±8.5^c	1.00±1.00^d	**0.04**
MCV(10^{-12}fl)	1±0.11^d	7.2±0.67^c	12.9±0.2^b	83.6±0.23^a	**0.001**
MCHC(g/dL)	64.28±5.2^c	103±2.3^b	125±4.1^b	220±8.6^a	**0.003**
MCH(10^{-12}pg)	1.1±1.5^c	15.2±2.1^b	22.5±2.11^b	76.3±2.1^a	**0.03**

Means in the same roll with different superscripts differ significantly (P<0.05)
NS- Not significant.

REFERENCES

Aderolu, A.Z., Ayoola S.O and Otitoloju 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.

Adigun, B.A. (2005). Water Quality Management in Aquaculture and Freshwater Zooplankton Production for use in Fish Hatchery. *NIFFR annual report*, New Bussa, Niger State. 2-11.

APHA/AWWA/WPCF (American Public Health Association, American Water Works Association and Water Pollution Control Federation) (1985). Standard methods of examination of water and waste water. 16th edition. APHA, Washington, DC, USA, 1268 pp.

Auta, J. and Ogueji, E.O. (2007).Acute Toxicity and behavioural effects of chlorpyrifos-Ethyl pesticide to juvienile of *Clarias gariepinus* Teugels. *Procceeding, 22nd Annual Comference of Fisheries Society of Nigeria (FISON) held at School of Nursing, BirninKebbi, 12th -16th November, 2007.* 264-272.

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.

Ayuba, V.O., Ofojekwu, P.C. and Francis, A. (2008). Growth and Heamatological Changes in *Clarias gariepinus* fingerlins exposed to *Datura imoxia* seed extract for 12 weeks leaf on weight gain in the African catfish. *Annals of research in Nigeria* **6***:*46-55.

Bhagwant, S. and Bhikajee, M. (2000). Introduction of hypochromic macrocylia anaemia in *Oreochromis* hybrid (Cichlidae) exposed to 100mg/l (sublethal dose) of Aluminium. *Science and Technology Reserve Journal.* **5**: 9-21

Blaxhall, P.C. and Diasley, K. W. (1973). Routine haemotological methods for use with fish blood. *Journal of fish Biology* **5**: 771 – 781

Campbell T.W. and Murru F. (1990) An introduction to fish hematology. *Compendium of Continuing Education in Veterinary Science,* **12**, 525-533.

Dacie, J.V and Lewis, S.M. (2001). *Practical Haematology* (9th edition). Churchill Livingstone, London. Brown, B.A. (1980). *Haematology. Principle and Procedure* (3rd) Lea and Fabinger, Philadelphia.

Dzenda, T. Ayo, J.O., Adelaiye A.B. and Adaudi A. O. (2004a). Effect of crude mathanolic leaf extract of *Tephrosia vogelii* on contraction of isolated rabbit jejunum. XXIV Ann. sci. conf. physiology sos. Nigeria Delta State University.

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 haematolocal investigations on Nile Tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pestiside. *Journal of American Science*. **6(10)**: 542-551

Henry, C.J., HigginsK. F., and BuhlK. 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.

Kolo, R.J., Lamai, S.L. and Ojutiku, R.O. (2008). Sub-Acute toxicity of gramoxone to *Clarias gariepinus*. *Annual Conference of Fisheries Society of Nigeria (FISON) held on 27th – 30th October, 2008 at Arewa House, Kaduna State.*

Kolo, R.J.; 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

Mackereth, F.J.H. (1963). Some Methods of water Analysis for Limnologists. Publication No. **21**. *Freshwater Biological Association* 70 pp.

Miale, J.B. (1982). *Laboratory medicine haematology* 6th edition. The CV Mosby Publishing London.

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

Okomoda, J., Ayuba, V.O. and Omeji S. (2010), Heamatological Changes of *Clarias gariepinus* (Burchell, 1822) Fingerlings Exposed To Acute Toxicity of Formalin. *Publication of Nasarawa State University, Keffi PAT June, Online copy available at www.patnsukjournal.net/currentissue*.

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.

Onusiriuka, B.C. (2002). Effect of sub-lethal concentration of formalin on weight gain in the African cat fish, *Clarias garipinus* (tougals) *Journal of Aquatic Sciences*, **17**: 66 – 68.

Oronsaye, J.A.O. and Ogbebo, P.E . (1997). Acute toxicity of copper to *Clarias gariepinus* in soft water. *Journal of Aquatic Sciences* **10**: 19-23.

Seiverd, C.E. (1983). Haematology for medical Technologists. Lea and Febiger, Philadelphia, USA. 946 pp.

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

Snieszko, S.F. (1960). Microhaematocrit as a tool in fisheries management. Special scientific report Wintrobe, M.M. (1978). Clinical Haematology. H. Kimpton, London, UK.

Solbe J.F (1995). Fresh water in: Handbook of Ecotoxicology (Edited byPeter Collins) Black Well Science Ltd. Osneymeed OX 20EL. 683pp.

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)**: 247-253

World Health Organization (WHO) (1994): Glyphosate. Environmental Health Criteria, Publication NO 159, Geneva, Switzerland.