BLOOD GLUCOSE RESPONSE OF CLARIAS GARIEPINUSEXPOSED TO ACUTE CONCENTRATIONS OF GLYPHOSATE-ISOPROPYLAMMONIUM (SUNSATE®)

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ABSTRACT
Clari**a**s gariepinus with mean weight 6.86g ±0.34 obtained from a homogenous source through induced breeding were exposed to different concentrations of Sunsate® for 96hours using static bioassays with continuous aeration under laboratory conditions to determine the acute toxicity of glyphosate in Sunsate® on the fish. LC_{50} of the exposed fingerlings were found to be 17.5 mg l^{-1}. The fish showed toxicosis symptoms such as loss of balance, respiratory distress, vertical and erratic movement, accumulation of mucus on the body surface and death. Result on blood glucose response shows that as the concentration increases so the blood glucose, therefor precautious use of Sunsate®in the control of aquatic weed is advised.

INTRODUCTION
The use of pesticide for agricultural purpose is a wide phenomenon. This has caused aquatic pollution through indiscriminate use and washing into water bodies through surface runoff during the raining season (Mbagwu and Ita, 1994). These indiscriminate use of herbicides, coupled with careless handling, accidental spillage as well as discharge of untreated effluents into natural waterways have harmful effects on fish population and other forms of aquatic life and may contribute long term effects in the environment (Ramah, 2011). Fish species however, are most sensitive to aquatic pollutants during their early life stages (Jiraungkoorskul*et al*., 2002). Pesticides at high concentrations are known to reduce the survival, growthand reproduction of fish and produce many visible effects on fish, more so, due to the residual effects of chemical, important organs in fish are damaged (Rahman*et al.*, 2002). Glyphosate based herbicide are widely used in Nigeria for the control of Aquatic and terestial plants, they are sold with different trade name and formulation,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 among the most widely used broad-spectrum herbicides in the world because they are highly efficacious, cost effective, practically non-toxic, and degrade readily in the environment (Williams *et al.,* 2000). The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding while in the aquatic system, fish have become the indicator species for the evaluation of the effects of obnoxious compounds in the aquatic ecosystem (Ervnest, 2004).*Clarias gariepinus* also called North African catfish has been widely introduced around the world, and found as far south of Africa as South African Catfish. It has been introduced in

Europe, the Middle East and part of Asia; they are potamodramous, which means they migrate within streams and rivers. *Clarias gariepinus* is a common tropical freshwater fish and is widely used in aquaculture in Africa; Cat fishes which are the most commercially culture species of fish in Nigeria are greatly affected by aquatic pollutionhence the emphasis on this species. Blood glucose levels have long been used as indicators of stress in fish (Wedemeyer and McLeay,1981). Yet, in many studies under stressful condition, blood glucose either remained unchanged or take a longer duration of stress to show the change (Pottinger *et al*., 2002), studies on blood glucose response of fish to acute concentration of herbicides are scares and few result gotten are highly varied, hence this study is designed to determine the acute toxicity of glyphosate in Sunsate® on *Clarias gariepinus* and the variation of blood glucose to different concentration of the herbicide.

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 inducedbreading and acclimatized for 14 days in plastic bowls prior to the commencement of the experiment.The water was changed at three days interval to prevent the buildup of metabolic wastes and ensure aeration of the medium throughout the period of acclimatization.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 concentrationof Sunsate®used for the acute toxicity test were determined by a preliminary test as described by Solbe (1995), Ten Fingerlings of *Clarias gariepinus* of average weight6.86g $\pm$0.34 were randomly selected and transferred from the holding plastic bowls into 5 replicate test plastic bowlsof 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 recoded for 1 hr, 6hrs, 12hrs and subsequently every 24 hours up to 96 hours of exposure.Fish were considered dead when gill movement ceased and no response observed upon gentle prodding. Toxicosis symptoms were also observed for the different concentration. Blood glucose was determined using a blood glucose kit. Blood samples were taken from two fish randomly selected from each treatment, a drop of the blood sampled was placed on the strips connected to the Accu-chek active kit model CE00HH and results were obtained in situ. The physico-chemical parameters (Temperature, Hydrogen ion concentration (pH), Dissolved Oxygen (DO), Total alkalinity and Free Carbondioxide) were analyzed according to standard method by APHA (1985). Descriptive Statistical Analysis, as well asAnalysis of variance of results was done with a computer Programme Gen stat discovery edition.

RESULTS

Table 1, shows physico-chemical parameters of the test solutions obtained were not significantly different from each other and were within standard range for optimum fish growth therefore slight fluctuation in this parameters were not thought to be the cause of fish mortality.Symptoms of toxicosis observed included loss of balance, respiratory distress,

vertical and erraticmovement, accumulation of mucus on the body surface and gill filament and death. The degree of death occurrence increased with increase in concentration of the toxicant as shown in Table 2. Probit transformation as well as probit curve of Sunsate®in

Figure 1 reveals acute toxicity of the chemical to be 17.5 mg l^{-1}.The result on blood glucose level as revealed in Table 2 shows that blood glucose increased as the concentration of concentration increased.Blood glucose was highest in 20mg l-1 concentration (6.9± 1.1) and least in the control (2.9± 1.2)

DISCUSSION

The physicochemical parameters measured were not significantly different among the concentration as shown in Table 1,so were not thought to be the course of fish mortality as they were found to be within tolerance range as suggested by Mackereth, (1963). Though Oloruntuyi*et al*., (2000) reported that herbicides cause changes in the quality of water in and near sprayed areasas decrease in dissolved oxygen in the water, along with an increase in temperature, may pose threat to the survival of fish species after herbicide applications,contrary to this, the result of the present study have shown that Sunsate® application those not result in significant changes in the physicochemical parameter to a point that is capable of causing deleterious effects on fish. Similar findings were also made by Adigun, (2005) and Kolo, *et al.,*2009. Also the water quality parameters were within the recommended range for the culture of tropical fishes. Boyd, (1979) recommended a pH range of 6.5-9 and Parker and Kenneth (1981) recommended a temperature range of 25^0C-32^0C which were in range with the present experiment.. However, itshould be noted that herbicide effect on physicochemical parameter of water is specific to the individual kind of herbicide used and may differ from one region to another. Sunsate® toxicity increased with increased concentration. The observation is in consonance with earlier reports on other glyphosate based herbicides (Kolo*et al.,* 2009). The initial reaction of the fish was to swim actively due to the effect on the nervous system; the rapidity of swimming was directly proportional to the concentration of the chemical. The stressful and erratic behaviour of the *Clarias gariepinus*fingerlings also tend to indicate respiratory impairment probably due to the effect of the chemical on the gills. Fish breathe by movement of water, dissolved oxygen and any water contaminants present, in and out through their gills, so the gills are usually site of first contact of the internal organ. This agrees with the finding of Onusiriuka&Ufodike (1994). Similarly, 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. The fish became inactive at higher concentration for a much longer time which is been described as a normal observation in acute and chronic toxicity tests by Kulakkattolikal, (1987) which shows that the higher the concentrations of the chemical, the resistance time of the fish to the chemical become shorter. Mortality increased sharply and then decreased gradually as the number of hours increased, this finding is in agreement with the work carried out by Edet, and Ikpi, (2008).

The Nature Conservancy (2001) declares that Glyphosate itself is of moderate toxicity to fish. The present study shows that the 96 h LC_{50} value of glyphosate in Sunsate® herbicide was 17.5mg/l. This value is higher than the concentration range reported by WHO (1994) as they stated that the LC_{50} values of glyphosate vary widely from 2 to 5 ppm. These LC_{50} values however depend on fish species and the test conditions as well as glyphosate formulations (WHO, 1994). BCPC (2005) stated the following as eco-toxicity of aquatic animals when glyphosate-isopropylammonium based herbicides are used; Fish LC_{50} (96 h) for trout and bluegill sunfish >1000, fathead minnows 97, channel catfish 130 mg/l. Touchdown 4-LC® and Bronco®Two glyphosate formulation have low LC_{50}s of about <13 mg/L, compared to Rodeo®which has a relatively high $LC_{50}$of about >900 mg/L (Henry *et al*. 1994).According to Servizi*et al,*. (1987) the LC_{50} of MONO818® a glyphosate formulation is 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. The present study has shown that acute concentration of herbicide lead to significant changes in the blood glucose level of the fish and suggest that as the concentration increased the blood glucose also increased in a roughly linear dimension, increased glucose level have been linked to stress in many literature. Report by Almazan Rueda *et al.,* (2005) shows that *Clarias gariepinus* exposed to different photoperiod had increase in plasma glucose levels and were attributed to increased swimming as a result of stress, therefore the increase in the rapidity of swimming, uncoordinated movement, as well as air gulping could be the reason for increased glucose level and this increases with increasing levels of concentration. In line with this assumption, Lactate level is been reported to be a function of anaerobic glycolysis (break-down of glucose) in white muscle due to frequent burst activity i.e. where fish spent more time swimming and more aggressive or stressed as reported by Kieffer*et al*., (1994).

Mortality of fish exposed to different levels of Sunsate®Concentration.

Treatment	Conc. (Mg/l)	Per. Mortality (%)
1	20.0	80
2	17.5	50
3	15.0	40
4	12.5	20
5	10.0	10
6	Control	0

Physico-Chemical Parameters and blood glucose level of the fish exposed to acute concentrations of Sunsate herbicide

Concentration(mg/l)	*DO(mg/l)*	*pH*	*Temp °C*	*Bloodglucose(mmol)*
0	5.8± 0.1	6.0 ± 0.1	27.0 ± 0.8	2.9 ± 1.2^{d}
10	5.2± 0.1	6.9 ± 0.3	27.0 ± 0.1	3.2± 1.6^{cd}
12.5	5.3 ± 0.6	6.7 ± 0.1	27.2 ± 0.1	3.8± 2.3^{c}
15	5.2 ± 0.1	6.6 ± 0.3	27.4 ± 0.3	4.8± 2.1^{b}
17.5	4.3± 0.3	6.3 ± 0.1	27.5 ± 0.1	5.3 ± 1.3^{b}
20	4.2± 0.1	6.3 ± 0.1	28.0 ± 0.3	6.9 ± 1.1^{a}

Means in the same column with different superscripts differ significantly (P<0.05)

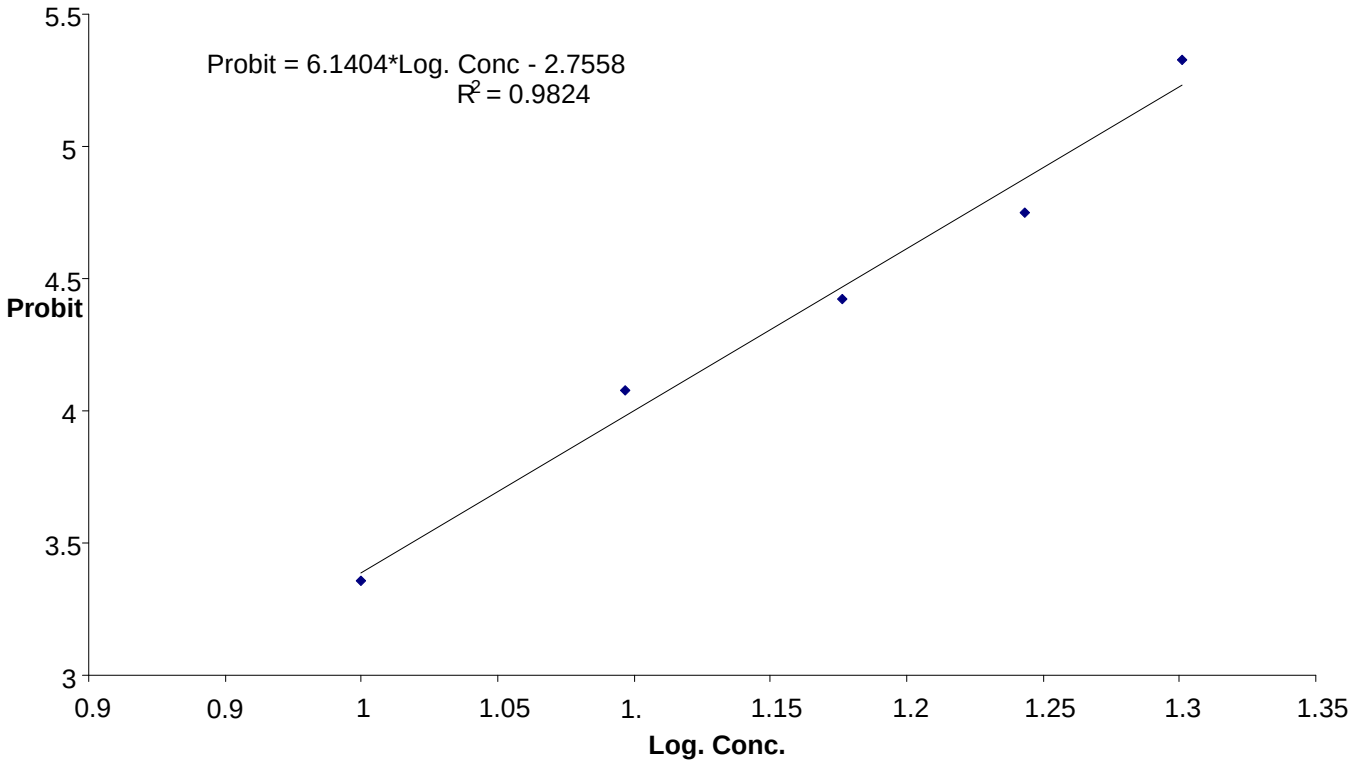

Fig. 1 Linear relationship between mean probit mortality and log concentration of Sarosate on *C. gariepinus* Juveniles for 96 hours

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