Acute Toxicity of Fingerlings of *Clarias gariepinus* exposed to Glyphosate and 2, 4 dichlorophenoxyacetic acid

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***Abstract*— The acute toxicity effect of the exposure of glyphosate and 2, 4-dichlorophenoxyacetic acid on catfish fingerlings was evaluated using Lorke's method. The acute lethal study of glyphosate and 2, 4-D on catfish fingerlings showed that no animal died within 24 hours. But for the mixture of glyphosate and 2, 4-D, at higher concentrations, death occurred. So, the LD_{50} was less than 2500mg/kg (2154.07mg/kg). The physicochemical parameters of water recorded were within the permissive limits fixed by WHO which are 6.5 and 6.5- 8.0 for dissolved oxygen and pH respectively. Although the presence of herbicides caused some changes in the quality of water in and around sprayed areas and decrease the dissolved oxygen in water, along with an increase in temperature, which may pose a threat to the survival of fish species, the results of the present study indicates that application does not result in significant changes in the physicochemical parameters to a point that is capable of causing visually observable deleterious effects in fish. This is probably because the concentrations studied here are of lower magnitude compared to those applied in agricultural practices. Also, several abnormal behaviours such as restlessness, erratic swimming, air gulping, respiratory distress, loss of equilibrium, and resting motionless at the bottom of the bowl (for those exposed to the mixture of glyphosate and 2, 4-D) were observed at higher concentrations of the toxicants. These results show that glyphosate and 2, 4-D may not have lethal effects on the fingerlings of *Clariasgariepinus*, but the mixture, at higher concentrations (≥2900mg/kg) may cause death. Hence, the use of glyphosate and 2, 4-D herbicides in agricultural farms should be monitored to avoid continuous leaching into water bodies.**

***Keywords*— Clarias gariepinus, acute toxicity, herbicides, glyphosate, 2,4-D.**

I. INTRODUCTION

Appreciation of fisheries and aquatic systems has been accompanied by increasing concern about effects of growing human population and human activities on aquatic life and water quality. The environmental impact of pesticides is often more than the target intended by those who use them. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species including non-target species, air, water, bottom sediments, and food (Miller and Miller, 2004).

Pesticide-contaminated water may have undesirable effects on fish and other aquatic life biota. Pesticide runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream (Toughill, 1999). Herbicides can accumulate in water bodies at levels that kill zooplankton, the main source of food for young fish. Accidental spills and dumpsites also account for a part of the environmental pesticides input (Doherty *et al.*, 2011). The toxicity of some chemicals can also be enhanced or mitigated in the presence of other chemicals (Wilson, 2006).

In addition to killing the organisms, some pesticides can have negative but nonlethal effects on individual organisms and populations, such as reduced reproduction, reduced mobility to escape predation, or alteration in behavior (Wilson, 2006). Besides the direct health effects, the subtle danger of pollutants lies in the fact that they may be mutagenic (or toxic) and lead to several human diseases like cancer, atherosclerosis, cardiovascular diseases and premature ageing (Grover and Kaur, 1999).

Glyphosate-based herbicides are among the most widely used broad-spectrum herbicides in the world because they are highly efficacious, cost effective, and relatively non-toxic and degrade readily in the environment (Giesy*et al*., 2000; Williams *et al.*, 2000). ForceUp® and Cotamine® 2, 4-D Amine herbicides were used. ForceUp® is used to control the emerged weeds. ForceUp® is a non-selective systemic foliar-applied, total agricultural herbicide with rapid translocation throughout the plant for the control of most stubborn annual and perennial grasses, broad-leaved weeds and sedges in arable and plantation crops, under pre-plant, post-emergence directed conditions, reduced or zero tillage systems. 2, 4,-Dichlorophenoxylacetic acid is a herbicide, and secondarily a plant growth regulator (Tomlin, 2006). Cotamine® 2, 4-D Amine herbicide is an herbicide used for the control of broadleaf weeds in crops such as maize, rice, sugarcane, sorghum, groundnut, palm, banana, plantations and for control of some aquatic weeds. Toxicity to fish and aquatic invertebrates varies widely depending on chemical form, with esters being the most toxic (Tomlin, 2006; WHO, 2011).

Acid and amine salt LC_{50} range from greater than 80 to 2244mg acid equivalents per litre whereas the ester range from less than 1.0 to 14.5mg acid equivalents per litre. The greater toxicity generally of the ester in fish is likely due to the greater absorption rates of the esters through the gills where they are hydrolyzed to the acid form (WHO, 1989).

II. MATERIALS AND METHODS

Test samples: ForceUp® glyphosate and Cotamine® 2, 4-D Amine herbicides were used. These were purchased from a chemical shop at Aba, Abia State, and taken to the lab for stock preparations.

2.1 Acute toxicity of glyphosate and 2, 4-D herbicides on Clarias gariepinus fingerlings: Fifty fingerlings of *Clarias gariepinus*, of mean weight 5.8 ± 0.2g and mean length 4.2 ± 0.3cm, aged 10 weeks, collected from Decfiro Standard Link farm, 7 Wogu street, Oyigbo city, PH, Rivers State, were used for the investigation. The fingerlings were acclimatized for seven days in plastic black bowls containing de-chlorinated and aerated tap water, at room temperature of 28.43 ± 0^oC, following the methods of Hoque *et al*. (1993). During the acclimation period, fingerlings were examined for pathogens and diseases. There was no mortality during the acclimation period. Water was changed at three days interval to prevent the build-up of metabolic wastes, and was aerated to increase oxygen supply. Fingerlings were fed twice daily with 2mm feed pellets at 3% body weight. Feeding was stopped 24 hours prior to and the during exposure period, that lasted for 48 hours. This was necessary because feeding increases the rate of respiration and excretory products, which may influence the toxicity of test solution.

The method used to determine acute toxicity was as described by Lorke (1983). The study was conducted in two phases.

Phase 1: Nine fingerlings were used during this phase. Nine fingerlings were divided into three groups of three fingerlings each. These were put in three different 30-litre capacity black bowls, labelled A, B and C, based on the weight of the fingerlings. Each group of fingerlings were administered different doses (10, 100 and 1000mg/kg of body weight) of the glyphosate, 2,4-D and a mixture of both herbicides, according to Lorke, 1983, and then topped up with tap water to the 20-litre mark. The fingerlings were placed under close observation for 1hr, 12hrs, 18hrs and 24hours to monitor their behaviour as well as if mortality will occur. Observations were noted.

Phase 2: During this phase, three fingerlings were used. Three fingerlings were distributed into three groups of one fingerling each, based on the weight of the fingerlings. The fingerlings were administered higher doses (1600, 2900, and 5000mg/kg) of the glyphosate, 2,4-D and a mixture of both herbicides, according to Lorke, 1983, and then observed for another 24hours for signs of toxicity, abnormal behaviour as well as mortality. From the data obtained, LD_{50} was determined. The control sample was prepared without adding the herbicides.

The LD_{50} was calculated using the formula:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$.

Where D_0 = Higher dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Temperature condition was kept at room temperature, and all bowls were exposed to equal amount of natural light. Fish were examined for abnormal behaviours and mortality for 1 hour, 12 hours, 18hours, and 24 hours during the period. The 24 hour LD_{50} toxicity for each glyphosate, 2, 4-D, and glyphosate/2, 4-D mixture concentrations were determined as a summary of percentage mortality data following the methods of Lorke (1983). Which were immediately removed and counted in every bowl at each observation time during the exposure periods.

Physico-chemical parameters of water from the acute toxicity tests: Some amount of water were collected, using sterile plastic white containers, from the tanks housing the catfish fingerlings, after treatment. These were kept in a freezer maintained at 4^oC until they were taken to the lab for physico-chemical assessments. The parameters assessed included: Alkalinity, Ammonia, Carbon dioxide, Chloride, Electrical conductivity, Dissolved Oxygen (DO), Nitrate, pH and Hardness. Standard methods were used to assess these

III. RESULTS AND DISCUSSION

Physico-chemical parameters of water from the acute toxicity tests: Water quality attributes are prime factors that influence fish survival, reproduction, growth performance, and overall biological production (King, 1998; King and Jonathan, 2003). They affect aquatic biotic integrity by directly causing mortality and/or shifting the equilibrium among species due to subtle influences such as reduced reproductive rates or alternations in competitive ability.

Physico-chemical parameters measured (Table 1) seemed to be within optimum range for fish culture as reported by Omitoyin *et al.*, (2006) and Olaifa *et al*. (2003). There was a significant change in water quality resulting from application of the toxicants compared to the control. This observation was in line with Okoli-Anunobi *et al*., (2002) who investigated the lethal effect of the ElephantBlue® detergent on the Nile Tilapia *Oreochronisniloticus*. In the case of dissolved oxygen, there was a slight decrease in the range for glyphosate- (4.7mg/l) and 2, 4- D-treated water (4.5mg/l) (Table 1), while there was much decrease in the dissolved oxygen for the mixture of glyphosate/2, 4-D water (3.2mg/l) (Table 1), compared with the control (5.0mg/l). This decline can be attributed to a situation referred to as oxygen sag, which is characterized by high mortality within short time. Death recorded in the phase two of the acute toxicity test of the mixture of glyphosate and 2, 4-D (Table 4) could therefore have occurred because of this. Warren, 1977 had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration, which will impair respiration, leading to asphyxiation. This was probably why the fishes exposed to higher concentrations of a mixture of glyphosate and 2, 4-D were stressed progressively with time before death.

TABLE 1. Physico-chemical parameters of the experimental water and control for phase-1 and phase-2 water for acute toxicity tests

Parameters	Control	Glyphosate	2, 4-D	Mixture of Gly. and 2, 4-D
Temperature (^{0}C)	25	28	29.8	31
Alkalinity (m/L)	12	10	12	14
Ammonia	0.5	0.4	0.5	0.7
Carbon dioxide	3.0	3.5	4.0	4.4
Chloride	4.5	4.5	4.8	5.1
Dissolved Oxygen (mg/L)	5.0	4.7	4.5	3.2
Ph	7.0	6.04	6.08	6.30
Hardness (mg/L CaCo3)	35.0	30	35	38

The pH fluctuated slightly from the treatment water compared with the control water, but was not upto the alkaline death point as recorded by Okoli-Anunobi *et al*. (2002). The water hardness for glyphosate decreased slightly (30mg/l) compared with the control (35mg/l) and increased slightly for 2, 4-D (35mg/l) and the mixture of glyphosate and 2, 4-D (38mg/l). This could be as a result of the high toxicity of these toxicants. This will invariably affect the optimum growth and development of the cultured fish. Bacteria and other germs will thrive with bad water quality. But the temperature, ammonia, chloride and carbon dioxide values were almost the same with the value of the control.

The physico-chemical parameters recorded were within the permissive limits fixed by WHO which are 6.5 and 6.5- 8.0 for dissolved oxygen and pH respectively. Although the presence of herbicides cause some changes in the quality of water in and around sprayed areas and decrease the dissolved oxygen in water, along with an increase in temperature, which may pose a threat to the survival of fish species, the results of the present study indicates that application does not result in significant changes in the physicochemical parameter to a point that is capable of causing visually observable deleterious effects in fish. This is probably because the concentrations studied here are of lower magnitude compared to those applied in agricultural practices. Also, the water quality parameters were within the recommended range for the culture of tropical fishes (Olaifa, *et al*., 2003; Omitoyin *et al*., 2006). Boyd (1979) recommended a pH range of 6.5 - 9 and Davis and Parker (1990) recommended a temperature range of 25^{0}C-32^{0}C. Similar findings were also reported by Adigun (2005) and Kori-Saikpere *et al*. (2010).

The acute lethal study of glyphosate and 2, 4-D (Tables 2 and 3) on catfish fingerlings showed no animal died within 24 hrs. The LD_{50} was therefore greater than 5000mg/kg b.w. But for the mixture of glyphosate and 2, 4-D, at higher concentrations (2900mg/kg and 5000mg/kg), during the phase-2 of the herbicide mixtures (glyphosate and 2, 4-D) exposed to the fingerlings, death occurred (Table 4). So, the LD_{50}, calculated from the formula stated above, was less than 2900mg/kg (2154.07mg/kg). Several abnormal behaviours such as restlessness, erratic swimming, air gulping, respiratory distress, loss of equilibrium, and resting motionless at the bottom of the bowl (for those exposed to the mixture of glyphosate and 2, 4-D) were observed at higher concentrations of the toxicants, similar to Lovely (1998) observations.

The no toxic effects (no mortality) seen in the results of the acute toxicity tests of the glyphosate-treated and 2, 4-D-treated fingerlings showed the LD_{50} was greater than 5000mg/kg. This could be because the acid forms of these herbicides were used in this study instead of the ester forms. This was in line with the findings of Tomlin (2006). He asserted that the toxicity to fish and aquatic invertebrates varies widely depending on chemical form, with esters being the most toxic (Tomlin, 2006; WHO, 2011).

Acid and amine salts LC_{50} range from greater than 80 to 2244mg acid equivalents per litre, whereas the ester forms range from less than 1.0 to 14.5mg acid equivalents per litre. The greater toxicity generally of the ester in fish is likely due to the greater absorption rates of the esters through the gills where they are hydrolysed to the acid form (WHO, 1989),

TABLE 2**.** Acute lethal effect of Glyphosate on fingerlings of *Clarias gariepinus*

Experiment	Dose (mg/kg b.w.)	No. of dead fingerlings after 12 hrs.	No. of dead fingerlings after 24 hrs.
Phase-1	*10*	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	0	0/3	0/3
Phase-2	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1*

*LD_{50}>5000mg/kg b.w.

TABLE 3 Acute lethal effect of 2, 4-D on fingerlings of *Clarias gariepinus*

Experiment	Dose (mg/kg b.w.)	No. of dead fingerlings after 12 hrs.	No. of dead fingerlings after 24 hrs.
Phase-1	*10*	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	0	0/3	0/3
Phase-2	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1*

*LD_{50}>5000mg/kg b.w.

As with fish, esters are more toxic than acid or amine salt forms to fresh water aquatic invertebrates, with LC_{50} values ranging from 25 to 643 mg ac/L (for esters). The relative toxicities for acids and salts are slightly toxic to practically non-toxic, whereas the esters are moderately to slightly toxic. The acute lethal concentrations of a mixture of glyphosate and 2, 4-D on the catfish fingerlings showed that the herbicides have significant effects on the physiological parameters of the fingerlings.

Several abnormal behaviours such as restlessness, erratic swimming, air gulping, respiratory distress, loss of equilibrium, and the fingerlings resting motionless at the bottom of the bowl (dead) were observed. The erratic swimming, restlessness, gulping of air, and eventual resting motionless at the bottom of bowl (for higher concentrations of the mixture of glyphosate/2, 4-D-treatments) observed in this investigation are indications that mortality of the exposed fish is not only due to impaired metabolism, but could in addition be due to nervous disorder. This is similar to the findings of Oti (2000) and Annune, *et al* (1994), who reported these abnormal behavioural responses in fingerlings of the hybrid, *Hetero-clarias* exposed to toxicants at 96 hours period.

The impairment of respiration due to the toxic effects of glyphosate herbicide on the gills has been reported by Omitoyin *et al*. (2006). At higher concentrations of the mixture (2900mg/kg and 5000mg/kg), there was an increase in the opercular movements within the first 12 hours of exposure. This might be suggestive of a physiological response to dissolved oxygen stress. Opercular movements reduced by the 24^{th} hour. This is an indication that the fingerlings were gradually assuming a new homeostatic balance to a polluted environment, before death occurred. A similar explanation may probably be made for the increase in tail movement observed by the 12^{th} hour and reduction by the 24^{th} hour. Ogueji *et al*., (2013) reported that surviving fish were maximally intoxicated at this period due to maximum bioconcentration and bioaccumulation of toxicants. Also, there was marked increase in opercular ventilation and tail fin beats per minute. This may be because the exposed fish needed more oxygen for increased metabolic rate, especially within 12 hours of exposure. This behaviour suggests respiratory impairment, due to changes in gill pathology resulting in reduced oxygen exchange at the gills, thereby leading to a hypoxic condition within the fish internal environment.

TABLE 4. Acute lethal effect of a mixture of Glyphosate and 2, 4-D on fingerlings of *Clarias gariepinus*

Experiment	Dose (mg/kg b.w.)	No. of dead fingerlings after 12 hrs.	No. of dead fingerlings after 24 hrs.
Phase-1	*10*	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	0	0/3	0/3
Phase-2	1600	0/1	0/1
	2900	0/1	1/1*
	5000	1/1	1/1

*LD_{50}<2900mg/kg b.w.

Lloyd (1992) reported that an increase in oxygen consumption may be associated with additional energy requirements for detoxification or it may be caused by the extra activity necessary for an avoidance reaction to the toxicant and also, an attempt to escape from the toxicant environment. This suggests a decrease in oxygen consumption and reduction of respiratory and metabolic rates. The reduction in respiratory and metabolic rates are pointers to the onset of fatigue due to several attempts to escape from the toxic medium or frequent surfacing to facilitate more oxygen intake. Similar findings were observed by Auta (2002) after exposing Dimethoate to juveniles of *Oreochromisniloticus* and *Clarias gariepinus*.

Air gulping was lower in the control fishes in comparison with exposed fishes at higher concentrations (2900mg/kg and 5000mg/kg) at the phase-2 of the mixture of glyphosate/2, 4-D. This is an indication that the fish required increased supply of oxygen and had to swim to the surface to gulp air. This activity was observed to be at its highest within 12 to 24 hours after exposure. This period coincided with a period of reduction in opercular ventilation, stressed cellular respiration and hence the need for an alternative oxygen source. Air gulping significantly reduced after 24 hours, which suggests physical fatigue due to swimming and other cumulative physiological effects of the toxicants on fish. Ogueji *et al*. (2013) also reported increased air gulping activity within 24 hours of exposure to lambda cyhalothrin.

Also, although the physicochemical parameters of water fluctuated slightly during the bioassay, this fluctuation was not enough to have caused the mortality. Death of test fish exposed to the mixture of glyphosate and 2, 4-D herbicides at higher concentrations may be attributed to the destruction of such organs as the gills, liver, kidney, brain, blood system and the pancreas. Annune *et al*., (1994) also reported that gill tissues are the most sensitive to water pollutants, since gills are the primary sites for osmoregulation and respiration. They are highly vulnerable to lesions due to their immediate contact with aquatic pollutants. While some pollutants enter the body, there is evidence that some of them exert their effects on the external surface of the fish especially the gills.

The very high toxicity of the herbicides (mixture of glyphosate and 2, 4-D) could probably be attributed to some possible synergistic effects likely to be produced by the active ingredients in the herbicides, of which is the compound that exist separately as herbicide and likely to be equally toxic to fish. The herbicide mixture is acutely toxic to catfish (*Clarias gariepinus)*.

IV. CONCLUSION

Glyphosate and 2, 4-dichlorophenoxyacetic acid, were found to be nontoxic to fingerlings of *Clarias gariepinus.* The LD_{50} of the herbicides to fingerlings of *C. gariepinus* was determined, although the herbicides (glyphosate and 2, 4-D) did not affect the catfish fingerlings, even at higher doses, but when they were mixed, there were deleterious effects, especially at higher doses. So, the mixture of glyphosate and

2, 4-D was found to have an LD_{50} less than 2,900mg/kg, and so was more toxic. Hence, the use of glyphosate and 2, 4-D herbicides in agricultural farms should be monitored to avoid continuous leaching into water bodies.

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