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ACUTE TOXICITY OF GALEX TO *Oreochromis niloticus* (TREWAVAS) IN NIGERIA.

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Abstract.

The acute toxicity of galex (Metolachlor) to fingerlings of *Orechromis niloticus* (Trewavas) was determined in 96hour static bioassays. During the assays, opercular ventilation and tail fin movement rates of the fish were determined. Haematological parameters of the blood and histopathology of the gills were observed. The 96hours LC50 of galex to fingerlings of *O. niloticus* was observed to be 9.30mgL-¹± 1.04. Histopathology of gills of the fish exposed to 1100mgL¯1, 1200mgL and 13.00mg/L-1 showed a dose-dependent disruption of the architecture of the lamellae and atrophy which led to impairment in oxygen uptake. Other symptoms of toxicosis in exposed fish include haemorrhage of the gills and fins, blood shot eyes of the fish, copious production of mucus on the body and head, agitated swimming, eruption of the ventral side and spilling out of the digestive system of fish, air gulping and death.

Keywords: haematology, histopathology of gills, Nigeria, Orechromis niloticus, galex.

Introduction

Pests are organisms which man considers to be harmful and pesticides are chemicals used to control them. A pesticide is normally used against a particular organism. Ideally it should poison it, but be otherwise harmless. Although, there are many chemicals with a remarkable degree of selectively is virtually impossible. This means that there is always the risk that pesticides will cause damage to man or to other non-target organism (Mellanby, 1980).

In Nigeria in recent times, water-hyacinth has been a major problem to effective fishing in waters in Nigeria. Efforts made by the federal government to combat the water hyacinth problem through manual and mechanical methods have not yielded expected results, suggesting that herbicidal control of water hyacinth is possible. The question is the effects on fish and other non-target organisms. The extensive use of pesticides results in a large reduction in fish production, not only in capture fisheries sector but also in aquaculture. The indiscriminate use of pesticides has been identified as a cause of many previously unexplained fish kills in the fish farms in other parts in the world (Oloruntuyi *et al.*, 1992). Pesticides also get into the aquatic environment through accidents or through run off from surrounding farmlands resulting in fish mortality and morbidity.

Galex (Metachlor) CGA_247us, is an Organochlorine herbicide and chemically named 2-Chloro-N-(2-ethyl-6-methyl phenyl)-N-2- (2-methoxyl-1-methyl ethyl) acetamide. It is a pre –emergence selective herbicide of toxicity of LD50 about 2780mgL-1 from previous work (Amentid and Edwards, 1982). It is used in corn farm to destroy the weed at suitable concentration and it is toxic to fish (Pimemted and Edwards, 1982).

Inspite of obvious advantages in agriculture, indiscriminate use of pesticides has been identified as a cause for fish death (CIFA, 1981). It is therefore important to monitor the Levels in water bodies for the assessment of their impact on fish production and their fate. The objective of this study was to evaluate the effects of acute levels of paraquat on mortality, respiratory rate, tail fin, opercular movement rate, haematological parameters and histopathology in gills of *Oreochromis niloticus* and behaviour of test

organisms. The fish type was chosen because it is a local, hardy fish of economical importance and it is common in African Freshwater.

MATERIALS AND METHODS

TEST ORGANISM.

Fingerlings of *O. niloticus* of wet weight range and mean weight 6.7± 3.3g were obtained from the Ahmadu Bello University Zaria dam, from where they were transported in an ice-box containing sufficient water from the dam to the laboratory. The fish were acclimatized for two weeks in dechlorinated Zaria municipal water during which they were fed on pelleted diet made of corn (70%), fish meal (14%), groundnut cake (15%) and vitamin premix (1%). Municipal water was dechlorinated by allowing it to stand outside for 2 to 3 days in plastic containers prior to use. A daily photoperiod of 12/12hrs light and dark was maintained during the acclimatisation and assaying. The physico-chemical parameters of water during the assay are shown in Table 1.

TEST CHEMICAL

The test chemical was 20% solution of commercial galex. However, analysis of the chemical using gas liquid chromatography showed that it was 17% only and this was what was used for subsequent calculation of concentrations. Concentrated eluates were analysed on the GLC, varian model 3400 fitted with electron capture detector (Food and Drug Laboratory Kaduna). Measured quantities of galex were pipetted into each aquarium using the insulin syringes and mixed with appropriate volumes of dechlorinated tap water to achieve desired concentrations. The solution was stirred and the fish introduced.

EXPOSURE OF FISH TO GALEX

The static method of bioassay was used to determine the acute toxicity of galex on *O.niloticus*. Eight-glass aquaria tanks of size 30.5 x 30.5 x 92.5cm, each containing different concentration of galex and a control tank were used following methods of Sprague(1973) and APHA (1989). Appropriate volumes of the stock solution were takenand discharged into 50L of de chlorinated water in each test tank to give the following concentrations: 8.00mgl-^{1}, 9.00 mgl-^{1},9.80- mgl-^{1},11.00 mgl-^{1}, 12.00 mgl-^{1},13.00 mgl-^{1},14.00 mgl-^{1} and the last tank without the toxicant representing the control. The mixture was allowed to stand for 30minutes before introducing test organisms.

RANDOMISATION

Randomisation of fish of mean weight 6.7±3.3g was carried out as suggested by Sprague (1969). Eighty fingerlings were randomly distributed into the toxicant concentrations to give ten in each aquarium using the random table and to eliminate systematic error. In each series of test there was a control in which ten fish were exposed to chlorinated municipal water only. Each test was replicated. The test solutions were partially renewed once during each test by siphoning three-quarters of the test solution and faecal materials out of each aquarium. Physico-chemical parameters of test solution were determined daily. No feeding occurred during the ninety-six hour (96hr) bioassays.

Mortality was recorded at experimental exposure periods of 12, 24, 48, 72 and 96 hrs. LC_{50} was determined as a graphical summary of the mortality data using the method of Sprague (1973) (Figure 1). The 95% confidence limit value was determined by using probit method (Wardlaw, 1989). The measure of dispersion of the population was by estimate of standard error of all readings. Opercular ventilation and tail fin movement rates were determined for 4 fish sampled from each concentration once daily during the assay. Determination of haemoglobin (Hb) Haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC) and leucocytes differential counts (Klontz, 1972)

were carried out on all of the surviving/ all control fish survived, 2 survived in concentration 10.00 mgl-¹, 11.00 mgl-¹ and 12.00 mgl-¹, 4 survived in concentration 9.8 mgl-¹ and 6 survived in test concentration 9.00 mgl-¹. There was no survivor in concentration 13 and 14 mgl-¹. Gills of remaining fish at 96hour were excised and fixed in 10% neutral formalin for 24hours. Paraffin sections were stained in haematoxylin and eosin (HSE) following

TABLE 1: WATER QUALITY VALUES DURING TOXICITY ASSAYS WITH *O.niloticus* EXPOSED TO GALEX

Parameters	Range	Mean± S.D (n =) 4
TemperatureêC	25-26	25.5± 0.77
Dissolved Oxygen (mgll)	5.8-6.5	6.15± 0.42
Conductivity (µm/s)	5.0-5.5	5.25±0.83
Hardness(mgL-1 as caco3)	4.0-6.5 x 10^2	5.25± 0.83
Alkalinity (mgL-1)	13.0-16.0	14.5± 1.66
pH	7.0-8.0	7.5± 0.77

RESULTS

The physico-chemical parameters of the test solution did not differ significantly ($p<0.05$) From those for the control during the exposure period (Table 1). The dose-response Relationship between galex and probit kill of O. *niloticus* is presented in Figure 1. The 96hr LC_{50} was calculated as 9.30mgL-1 (95% C.L, 9.3 0±1.06mgL-1). Signs of toxicosis observed in the exposed fish included loss of balance, air gulping, copious production of mucus on head and body, bloodshot eyes, erratic movement to avoid the toxicant at immediate exposure to test solutions and haemorrhaging of gills and fins.

These signs were pronounced in the aquaria containing higher concentrations of galex.

Opercular ventilation rates (Figure 2) show an almost constancy in control fish; however a dose dependent increases was observed in other test water. At 96hr increase in opercular ventilation rate was highest. Opercular ve ntilation rates in surviving fish at 96hr were significantly higher ($p> 0.05$) than in 0-72hrs. The effect of the different concentrations of galex on the opercular rate was highly significant for 96hr period.

The gills of control fish (Plate 1) has normal structure, consisting of finger-like filament attached to a cartilagenous gill bar with delicate lamellae. However, the gills of fish exposed to toxicant 8.0, 9.0, 9.8,10.0,11.0,12.0 and 13.0mg/L showed some histological damages (Plate 2-5). With increasing concentration of the toxicant, there was an increase in the disruption of the gill architecture. There was a mild hyperplasia at lower concentrations of 8-10mgL-1 but atrophy was observed only at high concentration of 130mg/L. It was observed that the higher the concentration the higher the opercular movement throughout the 96-h test period. The control fish showed a high and relatively constant ventilation rate and maintained this throughout the test period. At 96-h an increase in opercular movement was observed in all the test solutions showing higher ventilation (Table 3). Statistical analysis showed a significant difference ($P< 0.05$) for the 4 days of exposure. Also a highly significant difference was observed in the effect of concentrations on the opercular movement for the 96-h periods ($P<0.01$).

It was observed that the higher the concentration, the higher the tail fin movement for the first 24h in the fish exposed to different galex concentrations however by 72h and 96h the tail fin/min movement slowed down.

Table 2: Opercular Ventilation Rates of *O. niloticus* Exposed to Various Acute Concentrations of Galex 96

Concentration (mg/l)	0	1	2	3	4
Control	74.50	74.50	97.75	93.25	113.0
9.0	55.00	70.25	24.50	59.75	86.00
10.0	52.50	56.50	31.50	37.33	125.00
11.0	44.50	63.33	43.33	42.00	95.50
12.0	34.50	69.00	58.00	67.00	95.67
13.0	38.00	71.00	49.75	53.00	0.00

Table 3: Tail-fin Movement of *O. niloticus* exposed to Galex for 96

Concentration (mg/l)	0	1	2	3	4
Control	32.50	19.00	12.50	10.00	27.25
9.0	37.33	80.67	52.67	20.00	15.33
10.0	62.50	39.00	16.00	12.00	31.00
11.0	55.67	41.33	21.67	42.00	65.00
12.0	64.67	26.00	50.67	35.00	52.50
13.0	82.00	27.67	41.67	45.50	0.00

3.0 DISCUSSION

The calculated 96g LC_{50} of galex to *O. niloticus* in this study was 9.30mgL-1 (95% C.L 1.04) (FIGURE 1). The calculated safe concentration for galex was 0.93mg/L. This was derived by multiplying the 96hr LC50 with an application factor of between 0.01 to 0.1 (Koesoemadinate, 1980) depending on the persistence of the pollutant. As discovered by Babatunde (1997), Paraquat seems to be less toxic compared to galex from the safe concentration values in which paraquat's is 1.18mg/l while galex is 0.93± 0.13mg/l. Paraquat and galex are both highly toxic to *O.niloticus* in the tropics and thus the application (Babatunde, 2001). Nehring (1966) reported that Diquat and Paraquat have a low level of toxicity to perch (*Perca fluviatilis)* and roach (*Rutilis rutilis*). The median ventilation at 24-hours for the high toxicant concentration as observed by Babatunde *et al.* (2001) in case of paraquat than a subsequent increase from 48th to the 96-hour of exposure period. This indicates hyperventilation. Indices of opercular ventilation have been reported to be a strong indicator of stress when fish are in unfavourable environments (Sprague, 1973 and Butler, 1974, Babatunde et al., 2001).

The photomicrograph of the gill at high toxicant concentration of 12.00 and 13.00mg/l show remarkable structural disruption of the gills in which case architectural disruption of the gill filaments were observed (Auta *et al.(2005)*, Babatunde *et al.* (2001) in acute toxicity test of Paraquat to *O. niloticus* reported that the opercular ventilation rate and tail fin beat followed the same pattern that in there was an initial sharp increase being directly proportional to the toxicant concentrations, then a drop from 48hrs to the end, suggesting avoidance syndrome in the test fish. Galex also has the same effects on the test fish in this research.

Haemorrhage gills and under fins and blood shot eyes were observed in the *O. niloticus* exposed to high concentration of galex Auta *et al.(2005)* reported similar reaction in acute toxicity of *O. niloticus* to Endosulfan. Behavioural pattern observed in the fish included, Vertical movement, sporadic jumping out of water, fish laying on their dorsal part for few Seconds and rapid death in test concentrations of 12mg/l and 13mg/l. These differ in Some ways observed in reaction of *O. niloticus* to paraquat as observed by Babatunde (1997) in which reaction included agitated swimming, air

gulping period of quiescence and death. Annun *et. al.* (1991) reported a similar reaction in acute toxicity of zinc to fingerlings of *C.lacera* and *O. niloticus*. Sublethal concentration did not show much visible difference in behaviour those of the control. Anees (1975) is of the opinion that behavioural reaction of *C. punctatus* against acute concentration of organophosphate insecticides, which showed hypersensitivities were not detected.

Fish tended to increase in opercular ventilation as was observed in 24-h for the high toxicant concentration, a subsequent decrease at 48-h and a final decrease in 72 and 96-h. This indicates hyperventilation. The same pattern was observed in acute toxicity of Endosulfan to *O. niloticus* (Auta, 2005) and Paraquat (Babatunde, 1997 and 2001). Opercular ventilation has been reported to be a strong indicator of stress when fish are in unfavourable environment (Sprague, 1973 and Butler, 1974). This indicates the damage being caused by galex on the gills, the fish, thus increased its ventilation rates in an attempt to take oxygen. The photomicrograph of the gills at high toxicant concentrations of 11, 12 and 13mg/l show structural disruption of the gills. This corroborates the result obtained in the opercular movement showing that damage to the gills actually might have induced the hyperventilation which led to fatigue and finally death of the fish. This finding agrees with the results of Omoregie *et. al.* (1991) in the acute toxicity of actellic 25EC on *O. niloticus,* Babatunde et. al. (2001) in acute toxicity test of Gramoxone on *O. niloticus* and Ogundele et. al.(2005) in effect of linear Alkyl Benzene Sulphonate (LAS) on the weight gain of *Clarias gariepinus*.

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