Toxicity of Diquat (herbicide) to fingerlings of *clarias gariepinus*

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Abstract

Agrochemicals affect fish by either reducing their growth rate or interfering with their reproduction and behaviour with evidence of tissue damage or may lead to fish kill. Fish are particularly sensitive to a wide variety of herbicides and toxic conditions may arise not only from spillage or deliberate discharge of these chemicals into water ways but also from approved agricultural practices. Acute toxicity test of diquat (herbicide- a readily available and commonly used herbicide in Nigeria) on fingerlings of Clarias gariepinus were conducted employing the 96hours bioassay test. There were four different treatments and one control (absence of diquat) with each having three replicates. The treatment diquat (herbicide) was prepared by adding 1.8mg/l, 3.2mg/l, 5.6mg/l and 10.0mg/l. While there was no mortality in the control treatment, there were differences in the effect of concentration of diquat on total mortality percentage. The Lc 50 was found to be between 1.8mg/l to 2.0mg/l. The fish showed stress and erratic behaviours, body feature deformation, lesions and increased mucous layer on the body of the fish. Air bubbles were on the surface of the water in the treatment tanks indicating poor water quality as the experiment progressed. These effects increased with increased diquat concentration and duration of exposure. It was also observed that fish of the same size showed different levels of tolerance to the same concentrations of diquat. Histopathological examination of fish without diquat showed that there was no lesion, while fish in other treatments showed histopathological changes. Effect of the toxicity of diquat disappeared within a short period of time. It would be an ideal chemical for selective eradication of unwanted fish both from nursery and rearing ponds before stocking.

Introduction

Aquatic toxicity testing in water pollution control is very necessary to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between toxicant concentration and its effect on aquatic animals. One objective is often to ascertain the concentration which may be permitted in receiving waters without adversely affecting the fauna or prejudicing other uses (Hunter, 1978).

Herbicides can enter water courses intentionally or unintentionally. The most common examples of unintentional entry are from spray drift or run-off from terrestrial application on treated fields or from the irresponsible disposal of containers with concentrated chemicals. Intentional applications of aquatic herbicides are likely to be made in a wide variety of situations. Their most extensive use is in productive waters in low lying areas where weed growth seriously affects land drainage, irrigation canals and water transport (McEwen and Stephenson, (1979). Examples of herbicides are diquat, paraquat and dalapon. According to Holden, (1973) fish are particularly sensitive to a wide variety of herbicides and toxic condition may arise not only from spillage or deliberate discharge of these chemicals into water ways but also from approved agricultural practices if their use is excessive and thus can lead to detrimental influence on fish population. Since herbicides could enter a water course by accident or through ecological/geographical factors such as erosion and runoff of surface water, following its application in a field, knowledge of any potential aquatic effects must be ascertained.

Fish accumulates chemicals in their tissues and organs (Buhl and Hamilton 1996). Fish samples found around the dam site at Kainji, Nigeria were found to be contaminated predominantly by DDT complex and traces of polychlorinated biphenyls (PCB) (Ita, 1984). The use of pesticides in agriculture has sometimes resulted in the outbreak of diseases in fish (McEwen and Stephenson, 1979). In water

bodies, agrochemicals affect fish by either reducing their growth rate or interfering with their reproduction and behaviour with evidence of tissue damage or may even lead to fish kill (Buhl and Hamilton 1996). Toxicity testing of chemicals in experimental animals to detect potential hazard to human health and the environment has for decades been the cornerstone of natural and international programmes on chemical safety. Herbicides being one of such toxic chemicals may be ingested by man through aquatic organisms such as fish and other sources. Contamination of water with these recalcitrant chemicals results in bio- accumulation in fish and other biota, sometimes to biologically active levels. Hence, these chemicals have been suspected to be concern causing agents in fish and other aquatic organisms (GESAMP, 1991). The residues of these toxic chemicals found in water sediments, fish and other aquatic biota can pose a risk to aquatic organisms, to predators and to humans.

This work is therefore aimed at assessing the effect of herbicides (Diquat) - a readily available and commonly used herbicide in Nigeria - on fish adopting state acute bioassay technique using *Clarias gariepinus* fingerlings. The fingerlings are highly sensitive to pollutant at this stage and it is the stage at which fish mortality is high. It is a common practice to use herbicides in ponds during farming preparations after which fingerlings are stocked. Therefore the use of fingerlings in the bioassay test to ascertain their level of tolerance to the herbicide. The fish is hardy (tolerates high level of stress), highly priced and cultivated, and fast gaining prominence due to their aquacultural potentials in Nigeria.

Methodology

Fingerlings of *Clarias gariepinus* were purchased from a private fish farm in Ibadan. Mean weight and length of the fish were 15+-1g and 10+-1cm respectively. The fingerlings were acclimatized for three weeks in 20 litre capacity plastic aquaria at 10 fingerlings per aquarium and fed to satiation with 40% crude protein diets. After three weeks the fish were transferred into experimental aquaria containing 10l of water. The water was allowed to stay for 24 hours before using it. The fingerlings were weighed using a top -loading metteler balance (Ohaust - TM 160) and distributed randomly into fifteen aquaria. There were four different treatments and one control with each having three replicates. The treatment diquat (herbicide) was prepared by adding 1.8mg/l, 3.2mg/l, 5.6mg/l and 10.0mg/l which are standard concentrations (Donald and Oshida, 1987). The toxicant was diluted with deionized water by multiplying the percentage concentration by 1000 and subtracting the figure from 1000 to get the volume of diluting water required (Donald and Oshida, 1987).

Visual observations of the effect of the different concentrations of diquat on the fish were made every three hours for 96 hours. Physcio-chemical parameters of the water were determined after the introduction of toxicant and at the end of the experiment. Histopathological examination of tissues: gills, kidney and liver of the fish were carried out (Couch, 1975). Fish were randomly selected and dissected to extract the tissues after which they were preserved in 10% buffer formalin prior to the preparation of slides (Sutherland, 1996).

Results and discussion

Table 1: Preparation of toxicant

% Conc. (Mg/l) of Treatments A-E	Vol. Of diluting water (ml)	Vol. Of toxicant (ml)
0.0 (control) – A	1000	0
1.8 - B	982 = 98.2%	18
3.2 - C	968 = 96.8%	32
5.6 - D	944 = 94.4%	56
10.0 – E	900 = 90.0%	100

The 96hour Lc 50 of diquat to *Clarias gariepinus* fingerlings was 1.9mg/l. Hundred percent mortality was recorded in treatment E (10 mg/l of toxicant), while 83.4% mortality was recorded in treatment D with 5.6mg/l of toxicant. Treatment C with 3.2mg/l had 80% mortality. There was no mortality recorded in the control while 50% mortality was recorded in treatment B with 1.8mg/l of toxicant (Table 2). The fingerlings showed differences in tolerance to the same concentration of toxicant. They were observed swimming weakly and erratically and exhibited loss of balance, incessant gulping of air and the tendency of settling down at the bottom of the aquaria as time of exposure increased.

Table 2: Number of *Clarias gariepinus* survivors in 96 hours

Toxicant Conc. (mg/l)	Initial no of organism	Replicate 1	Replicate 2	Replicate 3
0 (control)	10	10	10	10
1.8	10	5	5	5
3.2	10	2	1	2
5.6	10	1	1	0
10	10	0	0	0

Physico-chemical parameters of experimental water showed a decrease in dissolved oxygen (DO) concentration after the toxicant was added and a further decrease at the end of the experiment. The other parameters investigated showed an increase in concentration except temperature which remained almost the same after introduction of toxicant and at the end of 96 hours (Table 3).

The tissues taken for histopathological examination were the gills, kidney and liver for all the treatments. There was discolouration in the gill, liver and kidney of the experimental fish but there was no discolouration in the organs of the control fish. Histophatological changes in the tissues of *Clarias gariepinus* fingerlings at different concentrations, and at different mortality time showed that lesions were essentially similar at different concentrations, there was mild lesion on the various organs, gill, liver and kidney. The intensity of cell damage increased with increasing period of exposure to diquat.

Table 3: Physico-chemical parameters of the water medium in the experimental tanks

Parameter	Before Toxicant addition	After addition of toxicant					End of experiment				
		A	B	C	D	E	A	B	C	D	E
Temp (°c)	26.8	27.5	27.4	27.5	28.0	28.0	27.5	27.5	27.6	27.8	8.3
PH	7.06	6.3	5.4	5.1	4.9	4.7	6.3	6.4	6.5	6.6	6.6
DO (mg/l)	6.83	6.4	5.4	5.1	4.9	4.7	6.4	5.2	4.4	4.1	3.9
NO3 (mg/l)	0.59	0.49	8.3	12.2	24.8	35.4	0.49	11.1	15.8	23.2	7.2
NO2 (mg/l)	0.29	0.33	3.5	4.1	4.3	5.3	0.33	1.2	1.8	2.1	3.3
NH3 (mg/l)	0.33	0.36	4.8	9.2	14.3	23.1	0.36	5.1	10.0	16.2	24.3

The gills of the control fish were composed of long slender branching projections lined by highly vascularised simple cuboids epithelium, with long slender rods based on cores of cartilage and with internal areola connective tissue. Whereas at different concentrations of diquat, there was destruction of villous and branching epithelial cells, squamous metaplasia from otherwise normal cuboids cell lining mononuclear cellular infiltration. Although at 1.8mg/l at 18 hours mortality, there was mild congestion of the submocosal capillaries, hyper pigmentation of the gill plate and moderate denudation of the gill epithelium. Deposition of dark brown granular material that resembles calcium was also observed which were absent in other concentrations. At 10mg/l at 27 hours mortality, there was severe congestion and also sloughing of the gill epithelium with presence of necrotic epithelial debris on the surface of the gill plate.

Kidney showed no significant lesion in all the concentrations when compared to the control fish's kidney. Also the liver of the control fish was without foci of degradation of hepatocytes with the parenchyma, necrosis and congestion. At 1.8mg/l at 18hours mortality, there were a few foci of degeneration of hepatocytes necrosis and congestion within the liver parenchyma, which was also observed at other concentrations except at 10mg/l at 27 hours mortality, where there was a severe and multiple foci of necrosis of hepatocytes in the liver parenchyma.

Conclusion

The stressful and erratic behaviour of C*larias gariepinus* fingerlings in the experiment indicates respiratory impairment, probably due to the effect of the toxicant diquat on the gills. The fishes became inactive at higher concentrations with increased time of exposure to toxicant. According to Kulakkattolickal (1997), this is a normal observation in acute and chronic toxicity test.

The high mortality rate of fish at 10mg/l of diquat (Treatment E) indicates that the higher the concentration of toxicant the higher the mortality, however, it was observed that the fingerlings of *C. gariepinus* showed variation in their tolerance of same concentrations of diquat (Table 4). This demonstrates the observation of Fryer (1977), that in all toxicants, a threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance. The time of toxicity disappearance and mortality were observed from the record of the relative mortality time in different concentrations of diquat for 96 hours. All fingerlings in the control experiment survived. The mortality rates at varying concentrations show that the time for 100% mortality was recorded at 30hrs at 10mg/l where all the fish were killed (Table 4), 48 hours at 6.5mg/l where only two of the thirty fingerlings survived. While the time for 50% mortality was also recorded at 72 hours at 1.8mg/l. The experiment shows that diquat had an Lc 50 of 1.8mg/l at which 50% of test organisms were killed. It was also observed that the higher the concentration of the toxicant, the higher the mortality rate. However, effect of the toxicity of diquat disappeared within a short period of time. It would be an ideal chemical for selective eradication of unwanted fish both from nursery and rearing ponds before stocking.

Conclusively, the present study shows that diquat had a 96 hour Lc 50 of 1.8mg/l; 100% mortality was recorded in 30 hours at 10mg/l. This shows that the higher the concentration the higher the mortality at a given time; Since *C. gariepinus* fingerlings only succumb to higher concentration of diquat in a short period of time, it is possible to use the herbicide as fish toxicant in stock assessment studies without any dangerous environmental consequences.

Table 4: Rate of mortality of *clarias gariepinus* fingerlings per treatment

Treat-ment	Hours of exposure (Death)																																Total Mortality
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	
A_1																																		0
A_2																																		
A_3																																		
B_1							1				1		1								1				1									
B_2							1				1			1		1							1											
B_3									1			1			1		1							1										15
C_1					1			1	1		1		1		1						1				1									
C_2					1			1			2		1		1					1		1		1										25
C_3				1			1			1		1		1		1	1						1											
D_1				1	1	1	1		2			1		1		1	1																	28
D_2				1		2		1		1		1	1		2																			
D_3			1	1		1	1		1		1		1	1		1	1																	
E_1			2	1	1	1	1	1	1	1	1																							
E_2			1	1	2	1	1	1	2	1																								
E_3			2	1	1	1	1	1	1	2																								

A = Control Treatment without toxicants, B= 1.8 mg/l of toxicant, C = 3.2 mg/l of toxicant, D = 5.6 mg/l of toxicant, E=10 mg/l of toxicant.

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