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Toxicity of Paraquat-Based Herbicide on Survival of African Catfish *Clarias Gariepinus*Fingerlings

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ABSTRACT: Paraquat is a toxic chemical used as a broad spectrum, contact herbicide for aquatic weed control in many countries including Nigeria that has been implicated in many non-target effects in the environment. *Clarias gariepinus*, an important fish in aquaculture in Nigeria is also used as a bio-indicator species, as it plays an increasing important role in the monitoring of water pollution because it responds with great sensitivity to the changes in the aquatic environment. Therefore, this study investigated the effect of paraquat-based herbicide on the survival *C. gariepinus* fingerlings. Fingerlings were exposed to three concentrations of paraquat viz 3.44ml, 6.88ml and 13.75ml in tanks of 5 replicates. Percentage mortality was recorded after 24, 48 and 72-hour exposure. Paraquat exhibited a high degree of mortality against fingerlings, but the observed mortality was observed to be concentration and exposure time dependent. All concentrations used were below the manufacturer's recommended concentration for aquatic weed control and they caused between 85 to 100% mortality after 72-hour exposure. Results from this study suggest that paraquat is highly toxic to *C. gariepinus* fingerlings and can therefore serve as reliable indicators of toxicity in environmental impact assessment programmes. Studies focusing on the effects of paraquat on aspects of the behaviour and physiology of juvenile stages of *C. gariepinus* are needed to fully understand the effects of this herbicide as results like this study show that paraquat can affect food supply (protein) and income to fish farmers.

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The use of pesticides (herbicides) in water has always been regarded with suspicion because of the peculiar possibilities of dangers to man, domestic animals, irrigated crops, wildlife, and aquatic organisms (Way et al., 1971). In addition to possible direct toxicity, secondary hazards may arise from the disappearance of the bulk of the primary producers from the aquatic ecosystem. The continual use of these herbicides has prompted some concern regarding the effects of these chemicals on the early life stages of fish (Skea et al., 1987). Paraquat is among the leading herbicides used in Nigeria (Udensi, 2020) to control both aquatic and terrestrial weeds (e.g., Chromolaena odorata, Eichornia crassipies) although it is banned in the country (Mokwunye et al., 2010) and worldwide (over 50 countries) (Fortenberry et al., 2016; Konthonbut et al., 2018) and its presence has been reported in many water sources in the world (Brown et al., 2004; Gao et al., 2010; Ismail et al., 2011). Paraquat is thought to be the most highly acutely toxic herbicide to be marketed over the last 60 years (Watts 2011). "The only highly toxic herbicide of the post-war years" is how the

World Health Organisation has described paraquat (WHO, 1991). Paraquat is on PAN International's Dirty Dozen (1985) (PANNA, 2002) and Highly Hazardous Pesticides (2009) lists for global phase-out by public interest organisations (Dinham, 2004). Paraquat has been reported to have both short- and long-term effects when used for controlling aquatic weeds in ponds (Newbold, 1975). This herbicide has been proven to be either fatal or elicit further physiological and behavioural effects clearly suggesting that it poses a danger to extant life forms present on earth (Rosic et al., 2020). Paraquat affects different non-target organisms such as bees (Moffet et al., 1972), Collembola (Choi et al., 2008), fishes including fingerlings (Eisler, 1990; Nkeeh and Jamabo, 2019), birds (Bauer, 1985), gastropoda (Bacchetta et al., 2002), periwinkle snails (Ogeleka et al., 2017) and mice (Dial and Dial, 1987). The high toxicity of paraquat to fish, and other aquatic species, including teratogenic effects in birds and amphibians and waterways has benefitted from reviews (Sartori and Vidrio, 2018; Rosic et al., 2020). Bortolotto et al.

(2014) reported decreased locomotion and distance travelled in adult Zebrafish injected with paraquat after 24 hours. A study in India on controlling water hyacinth (Eichhornia crassipes) found that paraquat caused increased mortality to the common carp (Kathiresan and Deivasigamani, 2015). A negative effect that can trigger death in fish has been reported in Nigeria for Nile Tilapia (Fidelis et al., 2012) and catfish (Kori-Siakpere et al., 2007). Other cases of paraquat concerns in Nigeria have been the effect on food chain (Shallangwa and Auta, 2008) in the aquatic ecosystem. Kori-siakpere et al. (2007) reported that 96 h of exposure of C. gariepinus to paraquat dichloride revealed low values of plasma glucose, triglyceride and plasma protein levels compared to the control. Ladipo et al. (2011) reported that paraquat dichloride caused mortality and histopathological effects in C. gariepinus exposed for 96h. The authors also reported respiratory stress, erratic swimming, and instant death of fish in exposed fishes. Akinsorotan et al. (2019) also reported acute toxicity in Oreochromis niloticus fingerlings exposed to paraquat herbicide. In general, paraquat is more toxic to aquatic fauna in soft water than in hard water, more toxic to early developmental stages than to juveniles or adults, and more toxic in formulations containing wetting agents than in formulations without them (Summers, 1980). Fish are among the organisms mostly used in ecotoxicological studies probably due to their sensitivity to the aquatic pollutants (Akinrosotan et al., 2019). Aquatic bioassays are important in control of water pollution to determine whether a potential toxicant is dangerous to aquatic life and to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa et al., 2003). To this effect, catfish especially C. gariepinus (Ladipo, 2011, Odo et al., 2017) are the most common group of fish use in toxicological studies in Nigeria. Clarias gariepinus are found in inland waters throughout much of the old world and is one of the most widely spread catfish genera in the world (Kori-siakpere et al., 2007). It is widely cultivated and found in water bodies in Nigeria hence used as biological indicators in ecotoxicological studies (Welker, 2000). Clarias gariepinus, as a bioindicator species, plays an increasing important role in the monitoring of water pollution because it responds with great sensitivity to the changes in the aquatic environment (Adeolu et al., 2009). The use of fish as a test organism in eco-toxicological studies is essential because of the link to human via food chain. Here, we test the hypothesis that paraquat herbicide has a toxic effect on C. gariepinus fingerlings. This study was aimed at investigating the lethal effects of Paraquatbased herbicide, on Clarias gariepinus fingerlings.

MATERIALS AND METHODS

Study Area: This study was conducted at the Ecotoxicology Research unit at the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin.

Test Chemicals: Weedcrusher® (Paraquat) a commonly used herbicide in Edo State, Nigeria, was chosen for this study. The herbicide was purchased from the local market near Ring Road, Benin City, Edo State. Weedcrusher® contains 200g/l paraquat ion (276g/l as paraquat dichloride), manufactured by Alderelm limited, United Kingdom. A range of concentrations were tested from below to far above manufacturers recommended values for usage in The recommended aquatic weed control. concentration is 27.5ml of Weedcrusher® (Paraguat) aquatic weed control in water bodies. Concentrations used were 2x below the recommended value, 4x below the recommended value and 8x below the recommended value recommended concentration.

Test organism: Five hundred (500) healthy fingerlings of C. gariepinus of the same age class (3 weeks old) were purchased from the Aquaculture and Fisheries Management Department of the Faculty of Agriculture, University of Benin, Benin City, Edo state, Nigeria, on May 17th, 2021. The fishes were transported in a well aerated container containing water from the fish farm to the laboratory. They were acclimatized for one week in a plastic drum with capacity of 160L but filled to the 130L mark with dechlorinated tap water. The de-chlorination of the water was done by allowing the water to stand for 24hours. The temperature of the water was 27°C and pH was 8.3. The fishes were fed three times per day with fish pellets (0.7mm) during which the water was renewed daily to prevent build-up of metabolic waste.

Preparation of dosages: For the bioassay, the different concentrations (dosages) of the herbicide were measured using a measuring cylinder. The herbicide was prepared by dissolving each concentration in 5 litres of water in a 10-litre tank then shaken to ensure an even solution before introduction of the fishes. The concentrations used were 3.44ml containing 6.88ml and 13.75ml.

Experimental design: Clarias gariepinus fingerlings were exposed to varying concentrations of atrazine this was carried using the static bioassay procedure. For the bioassay, five fingerlings of similar sizes were introduced into each concentration including control. The fishes were fed with 0.5g of 0.7mm pellets of vital fish feed containing 40% protein contents. Test water used for the bioassay was de-chlorinated tap water. A chemical free control was also set up using only water from the unit. The weight of each fish was between 2.2g and 1.9g the weight was measured using Atom Electronic Compact Scale before exposure. The experiment is to determine the toxicity on fingerlings, fish mortality was recorded every 12 hours till 72hours. The fishes were considered dead when they did not respond to prodding with a glass rod after being observed for almost 15min. The experimental setup was replicated 5 times. Percentage mortality of fish fingerlings was calculated thus:

Mortality
$$\% = \frac{NDF}{TNF} \times 100$$

Where NDF = number of dead fishes; TNF = total number of fishes exposed to the toxicant

Statistical analysis: The effects of different concentrations of the herbicide and exposure time on *C. gariepinus* mortality was analysed using a Generalized Linear Model (GLZ) (assuming normal distribution with an identity link function). When the overall results were significant in the GLZ analysis, the difference among the treatments was compared using the sequential Bonferroni test. Probit regression was used to estimate the concentrations of the herbicide estimated to cause 50 and 90% mortality (LC₅₀ and LC₉₀), the concentrations causing 50% and 90% of tested individuals to die in each period (i.e., 24, 48 and 72 hours). All analyses were performed using SPSS Statistical software, version 20.0 (IBM SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Mortality in *Clarias gariepinus* fingerlings was significantly influenced herbicide concentration and duration of exposure. Following a 24-hour exposure of *C. gariepinus* fingerlings to different concentrations of the herbicide, percentage mortality varied significantly (GLZ: Wald $\chi^2_3 = 66.429$; P=0.0001) with 13.75ml exhibiting the highest percentage mortality (100%) (Figure 1). Paraquat also caused high percentage mortalities in *C. gariepinus* fingerlings following a 48-hour exposure. Percentage mortality differed (Wald $\chi^2_3 = 34.205$; P=0.0001) with the lowest concentration 3.437ml causing the lowest mortality (36.7%) (Figure 2).

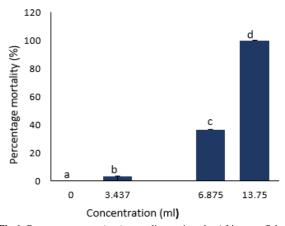


Fig 1: Percentage mean $(\pm se)$ mortality against the African catfish *Clarias gariepinus* fingerlings following exposure to different concentrations of Weedcrusher® for 24 hours. Means capped with different letters are significantly different (Sequential Bonferroni test: P< 0.05).

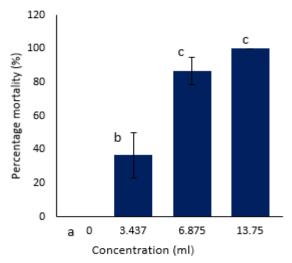


Fig 2: Percentage mean (\pm se) mortality against the African catfish *Clarias gariepinus* fingerlings following exposure to different concentrations of Weedcrusher® for 48 hours. Means capped with different letters are significantly different (Sequential Bonferroni test: P< 0.05).

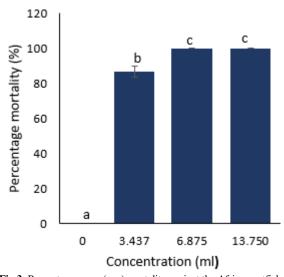


Fig 3: Percentage mean $(\pm se)$ mortality against the African catfish *Clarias gariepinus* fingerlings following exposure to different concentrations of Weedcrusher® for 72 hours. Means capped with different letters are significantly different (Sequential bonferroni test: P< 0.05).

Fish percentage mortality did not differ when exposed to 6.88ml and 13.75ml both causing 86.7% and 100% respectively. Percentage mortality caused by paraquat following 72 hours exposure was significantly influenced by concentrations (Wald $\chi^2_3 = 40.000$; P=0.001) (Figure 3). There was no difference in percentage mortality when C. gariepinus was exposed to 6.88ml and 13.75ml of paraquat as both resulted in 100% respectively. There was no mortality recorded in the control irrespective of exposure time. The acute toxicity profile of paraquat was estimated based on the mortality tests and concentrations estimated to cause 50% (LC₅₀) and 90% (LC₉₀) were calculated at different exposure times. LC₅₀ and LC₉₀ decreased with increase in exposure time (Table 1). When C. gariepinus fingerlings were exposed for 24 hours, LC_{50} was 7.225ml while LC_{90} was 10.972ml. LC_{50} was 4.046ml and LC_{90} was 7.248ml when fingerlings were exposed for 48 hours. Following a 72-hour exposure of fingerlings to different concentrations of paraquat LC_{50} was 1.910ml and LC_{90} was 3.658ml.

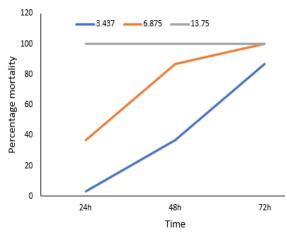


Fig 4: Percentage mortality of the African catfish *Clarias gariepinus* fingerlings following exposure to different concentration of Weedcrusher® at different time periods.

Table 1: Index of toxicity (LC_{50} and LC_{90}) of the African catfish *Clarias gariepinus* fingerlings when to different concentrations of Weedcrusher® for various durations.

Exposure	Index of toxicity		Probit line
time (hours)	$LC_{50}(g)$	$LC_{90}(g)$	equation
24	7.225	10.972	Y = 5x + -4.5
48	4.046	7.248	Y = 5x + -3
72	1.910	3.658	

This study investigated the effect of paraquat based herbicide on the survival of *C. gariepinus* fingerlings. This study was done to further elucidate the effects of paraquat on *C. gariepinus* as the herbicide has been associated with death and various mental and physical dysfunctions in several animals (e.g., soil fungi and bacteria, earthworms, fish, and dogs) (Watts, 2011). Studies have indicated that many herbicides are toxic to biota at varying concentrations. This study showed that exposure to paraquat, a broad-spectrum contact herbicide can result in significant mortality in *C. gariepinus* fingerlings. Aquatic organisms with fatigue and stress symptoms resulting from exposure to herbicide may not be productive to meet the increasing demand for aquatic food.

In this study, concentrations below the manufacturer's recommended concentration for Weed crusher® were applied to *C. gariepinus* fingerlings. This study showed that exposure of catfish to concentrations of herbicide below the manufacturer's recommended concentration caused high mortality (80-100%), but the results were dependent on duration of exposure. This corresponds with report by Aghoghovwia and Izah (2018) and Akinrosotan *et al* (2019) who reported that paraquat caused increased mortality in *Heterobranchus bidorsalis* fingerlings and *Oreochromis niloticus* fingerlings upon increased exposure time and concentrations. Rosica *et al.* (2020)

also reported that at realistic field concentrations, paraquat increased fish kills of common carp three times more than the weed (water hyacinth) that it was employed to control.

The LC₅₀ values reported in this study were higher than the findings of Ladipo (2011) that reported LC₅₀ value of 1.75 mg/l after 96 hours of exposing juvenile C. gariepinus with mean weight of 47.97g to paraquat dichloride. The variation could be associated to the weight, length and and biochemical characteristics of C. gariepinus used for the study as well as the chemical nature of the herbicides (Aghoghovwia and Izah, 2018). The decrease in LC₅₀ and increased mortality as the exposure period and concentration increased could be associated to stress and/ or alteration on the various organs/systems. Typically, when fishes encounter paraquat it could lead to disorders in the activity level of several enzymes involved in cellular and biochemical activities, change of blood biochemical factors, damage tissue and cause oxidative stress (Banaee et al., 2013). The LC₅₀ values depend on fish species and the test conditions as well as herbicide formulations (WHO, 1994). Neibor and Richardson (1980) reported that the level of toxicity of any pesticide depends on its bioaccumulation, the different chemistries of the compound forming the pesticide and the reactions of the organisms receiving the toxicant.

Conclusion: Our study further reiterates that the continued use of paraquat based herbicides threatens the survival of all extant forms of life on earth despite futile efforts to reduce its use. Therefore, governing authorities should strongly control every aspect of the supply chain and disposal of paraquat-based herbicides especially in aquatic weed control. Studies focusing on the effects of paraquat on aspects of the behaviour and physiology of juvenile stages of *C. gariepinus* are needed to fully understand the effects of this herbicide as results from this study show that paraquat can affect food supply (protein) and income to fish farmers.

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