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ACUTE TOXICITY, PHYSICAL AND BEHAVIOURAL RESPONSES OF *Clarias gariepinus* JUVENILES EXPOSED TO ATRAZINE

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ABSTRACT
The impact of atrazine herbicide on juveniles of Clarias ganiepirus was evaluated using standard methods. The experiment was set up in a completely randomized design (CRD) using plastic aquarium tanks with five species of C. gariepinus in four treatments (including a control) and three replicates; a total of sixty (60). Laboratory conditions with a natural photoperiod (12 h light - 12 h dark) were set up at the College of Natural Resources and Environmental Management (CNREM), Michael Okpara University of Agriculture, Umudike, Nigeria. A four-day (24, 48, 72, and 96 hours) static renewal toxicity bioassay was conducted with atrazine concentrations of 7.5, 15.0, 22.5, 30.0, and 37.5 mg/l respectively. The results showed that the 24, 48, 72, and 96-hour LC_{50} of atrazine were 21.23, 19.57, 18.37 and 18.37 mg/l respectively. Physical and behavioural responses were observed and included dark pigmentation, haemorrhaging from gills, mucous secretion, and respiratory distress. These findings indicated that atrazine could be toxic to Clarias gariepirus and its use in agricultural systems close to aquatic bodies should be strictly monitored.

KEYWORDS: *Atrazine, Toxicity, Juvenile, LC_{50}, response, Behavioural*

INTRODUCTION

Fish is an affordable and critical source of animal protein and lipids for man and his animals (Banaee, 2013; Izah and Angaye, 2015). About 14 million people representing 10% of the population rely completely or partially on the fisheries sector for economic sustenance (FAO, 2006). Due to its readily digestible and immediate utilizability by the human body, fish is thus suitable and complementary for regions of the world with a high carbohydrate diet (FAO, 2006). The genus *Clarias* is the most acceptable and economically viable fish in Nigeria (Adewumi, 2015; Ogamba *et al.,* 2015).

The use of herbicides in weed control has been recognized as a part of agricultural practices worldwide (Meng *et al*., 2022; NPIC, 2024). Unregulated use of herbicides to boost agricultural productivity may negatively affect non-target organisms especially in the aquatic environment (Battaglin *et al.,* 2008; El-Nahhal and El-Nahhal, 2021). Herbicides are generally applied in the dry season or early rainy season, which often coincides with the breeding season of many fish species (Dinh *et al.,* 2022). Some of these fish breed in aquatic habitats receiving the runoff drained from the cultivation fields (Ladipo *et al.,* 2011).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is one of the most commonly used herbicides among rural farmers in Nigeria (Ekeleme *et al*., 2021; NAFDAC, 2023; Owagboriaye *et al*., 2023). Atrazine has relative mobility in soil and aquatic environments because of its low volatile nature and moderate solubility in water (Murphy *et al*., 2006). It tends to partition into the water column rather than sorb to the sediments (Giddings *et al.,* 2004). The persistence of atrazine in different environmental media is high. For example, higher half-life was recorded in soil (>100 days), 85 days in surface water and 14.30 days in sediments (Qu *et al*., 2017; Pérez *et al*., 2022). As a result of the long half-life duration and low absorption capacity, contamination of farmlands occurs (Meng *et al*., 2022). It is extensively used on corn, sorghum, sugarcane, pineapples, Irish potato, cassava and to some extent on landscape vegetation (Battaglin *et al.,* 2008; Fayinminnu *et al*., 2017; Obiazi *et al*., 2020; Ekeleme *et al*., 2021). Due to extensive and repeated use of atrazine herbicides to control weeds in agricultural fields, large quantities of the herbicide find their way into water bodies (Battaglin *et al.,* 2008; EEA, 2024). Atrazine concentrations of 0.01 - 0.08 mg/L have been recorded in drinking water sources in Southwest Nigeria (Owagboriaye *et al*., 2023). In some fish farms in Southwest Nigeria, Atrazine were also reported in fish feed (1.3–1.5 µg/kg) and fish (1.4–1.8 µg/kg) (Olatoye *et al*., 2021). Elsewhere, De Rosa *et al*. (2024) recorded Atrazine and its degraded products in the water dissolved phase (20.1-96.5 ng/L), in suspended particulate matter (5.4-60.2 ng/L) and sediment (4.7-19.8 ng/g); indicating the herbicide pollution within the watershed of Sele River estuary, Southern Europe. The use of Atrazine in Nigeria has been revisited, leading to its complete ban with effect from 1st January 2025 by National Agency for Food and Drug Administration and Control (NAFDAC) (NAFDAC, 2022; 2023). Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to the environment and humans (Saganuwan, 2017). Bioassay technique has been in the forefront of toxicological studies for chemical safety and ecosystem health (Rahnama *et al*., 2018). Aquatic bioassays are necessary in water pollution control to determine potential toxicants and their dangers to aquatic life (Azizullah and Häder, 2018).

However, behavioural ecotoxicology has been identified as an early warning sign for determining the quality of an environment (Hellou, 2011). It is slowly becoming more relevant because of its high level of sensitivity (10–1,000 times), when compared with LC_{50} associated with lethal or sub-lethal tests (Hellou *et al*., 2008; Robinson, 2009). Hellou (2011) also observed that apart from being very relevant ecologically, behavioural tests are faster, easy to carry out, noninvasive as well as cheap. Sharma (2019) also reported that very low concentrations of some chemicals can result in quick change of behaviour in some organisms. This study is aimed at determining the acute toxicity level, physical and behavioural responses of African catfish (*Clarias gariepinus*) juveniles exposed to atrazine.

MATERIALS AND METHODS

Experimental Animal

Clarias gariepinus juveniles of relatively uniform sizes were obtained from the MOUAU fish farm in February 2020 and transported in plastic containers to the laboratory. The fish was acclimated to laboratory conditions for two (2) weeks. During acclimation, the health status of fishes was checked for any disease condition. Water in the aquaria was changed every three days in order to prevent accumulation wastes and also to improve dissolved oxygen content. The fishes were fed to satiation with commercial feeds (2mm pelleted size) twice in day (morning and evening). The feeding was stopped 24 hours prior to the start of the experiment and throughout the exposure period (96 hours) and the body weight (g) was measured. This was necessary as feeding will increase the respiratory rate and excretory products, that could alter the toxicity of test solution (Popoola *et al*., 2018).

Preparation of Test Solution

Atrazine with trade name ATRAZ 50FW with manufacturing date (November 10, 2018) and expiration date (November 10, 2021) was obtained as a commercially available herbicide from an agro-based shop in Umuahia, Abia State at a concentration of 500g/L in a one-litre container. It was stored in a cool, dry place in the laboratory according to manufacturer's specification. From the 500g/L, a stock solution was prepared by adding 1ml of the herbicide to 99ml of water (Reish and Oshida, 1987). The stock solution was used to prepare different concentrations of the toxicant by diluting measured volumes of the toxicant with tap water.

Experimental Design and Setup

The study was carried out in the wet laboratory of the Department of Fisheries and Aquatic Resources, Michael Okpara University of Agriculture, Umudike, Nigeria. Completely randomized design (CRD) method was used in the study. A range-finding test was done to determine the concentrations of atrazine that were used in the definitive tests (Akin-Obasola, 2019). This was done by placing five concentrations of the test herbicide (5, 10, 15, 25, and 50 mg/l respectively) in separate plastic aquaria (using a pipette) containing 20 liters of dechlorinated tap water. The five concentrations used in the acute test were then selected, ranged between the highest and lowest concentrations, and made into duplicates. A total of sixty (60) healthy fish were selected, weighed, and randomly distributed into plastic aquarium tanks containing 20 liters of the test solution. Five species of *Clarias gariepinus* were randomly placed in four test aquarium tanks for the acute toxicity bioassay consisting of four treatments (including a control) in three replicates.

Bioassay Test

A four-day static renewal toxicity bioassay was conducted in the laboratory to determine the toxicity of atrazine to juveniles of *Clarias gariepinus* as described by (ASTM 1990). Ten fish specimens were selected randomly and stocked in each aquarium tank containing atrazine concentrations of 7.5, 15.0, 22.5, 30.0, and 37.5 mg/l respectively. These were replicated twice for each concentration. Fish were not fed during the experiment. The experiment was monitored for 24, 48, 72, and 96 hours, and lethal concentration (LCx) was used to determine toxicity as recommended by Boyd (2005) in a static experiment. The LCx was estimated using probit analysis developed by Finney (1971). The physical and behavioural responses of *Clarias gariepinus* exposed to different atrazine concentrations were also closely monitored.

RESULTS

The results of the mortality rate of *C. gariepinus* exposed to atrazine during the acute toxicity study showed the mortality pattern of exposed fish (Table 1). No mortality (0%) was recorded at concentration of 7.5 mg/L, 5% at 15 mg/L, 95% at 22.5 mg/L, and 100% at 30 and 37.5 mg/L atrazine concentrations after 96 hours of exposure.

The results of the 96hour LC_{50} are presented in Table 2. The 24hrs LC_{50} was 21.23 mg/L, 48hrs LC_{50} was 19.57 mg/L, 72hrs LC50 was 18.37 mg/L and 96hrs LC_{50} was 16.82 mg/L. The result showed that after 96 hours of exposure, lower concentrations caused more mortality than those required to cause mortality after 24, 48, and 72 hours.

Table 1: Mortality rate of *C. gariepinus* exposed to atrazine

Duration of exposure	Concentration of pesticide (mg/l)	Total exposed	Cumulative mortality count	Percentage mortality
24hr	7.5	20	0	0%
	15.0	20	1	5%
	22.5	20	11	55%
	30.0	20	20	100%
	37.5	20	20	100%
48hr	7.5	20	0	0%
	15.0	20	1	5%
	22.5	20	16	80%
	30.0	20	20	100%
	37.5	20	20	100%
72hr	7.5	20	0	0%
	15.0	20	1	5%
	22.5	20	19	95%
	30.0	20	20	100%
	37.5	20	20	100%
96hr	7.5	20	0	0%
	15.0	20	1	5%
	22.5	20	19	95%
	30.0	20	20	100%
	37.5	20	20	100%

Table 2: Lethal concentrations (LC_{50}) of atrazine after 96 hours

LC_X	Estimate (mg/L)	95% LCL(mg/L)	95% UCL(mg/L)
24hrs	21.229	19.270	23.117
48hrs	19.574	17.777	21.296
72hrs	18.370	16.823	20.055
96hrs	18.370	16.823	20.055

Legend: LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

The physical and behavioural responses of *Clarias gariepinus* exposed to different atrazine concentrations are presented Fig. 1. Juveniles were observed to avoid areas where atrazine was introduced into the aquarium. The number of physical and behavioural responses increased in number and frequency as the concentration and duration of exposure increased. At 7.5 mg/L atrazine concentration (control), no physical or behavioural changes were observed in the juveniles within 24 hours of exposure. However, dark pigmentation (DP) was observed from 48 hours while haemorrhages (H), secretion of mucous (MS) and sounding (S) were observed from 72 hours. All the responses were mild (1). At 15 mg/L atrazine concentration, dark pigmentation (DP), Haemorrhage (H) and mucous secretion from the gills (MS), and sounding (S) were observed from 48 hours. Respiratory distress (RD) in the form of frequency surfacing was observed after 72 hours while quiescent (Q) was observed from 96 hours. The frequency of dark pigmentation (DP), haemorrhages (MH) and mucous secretion (MS) increased to 2 (moderate).

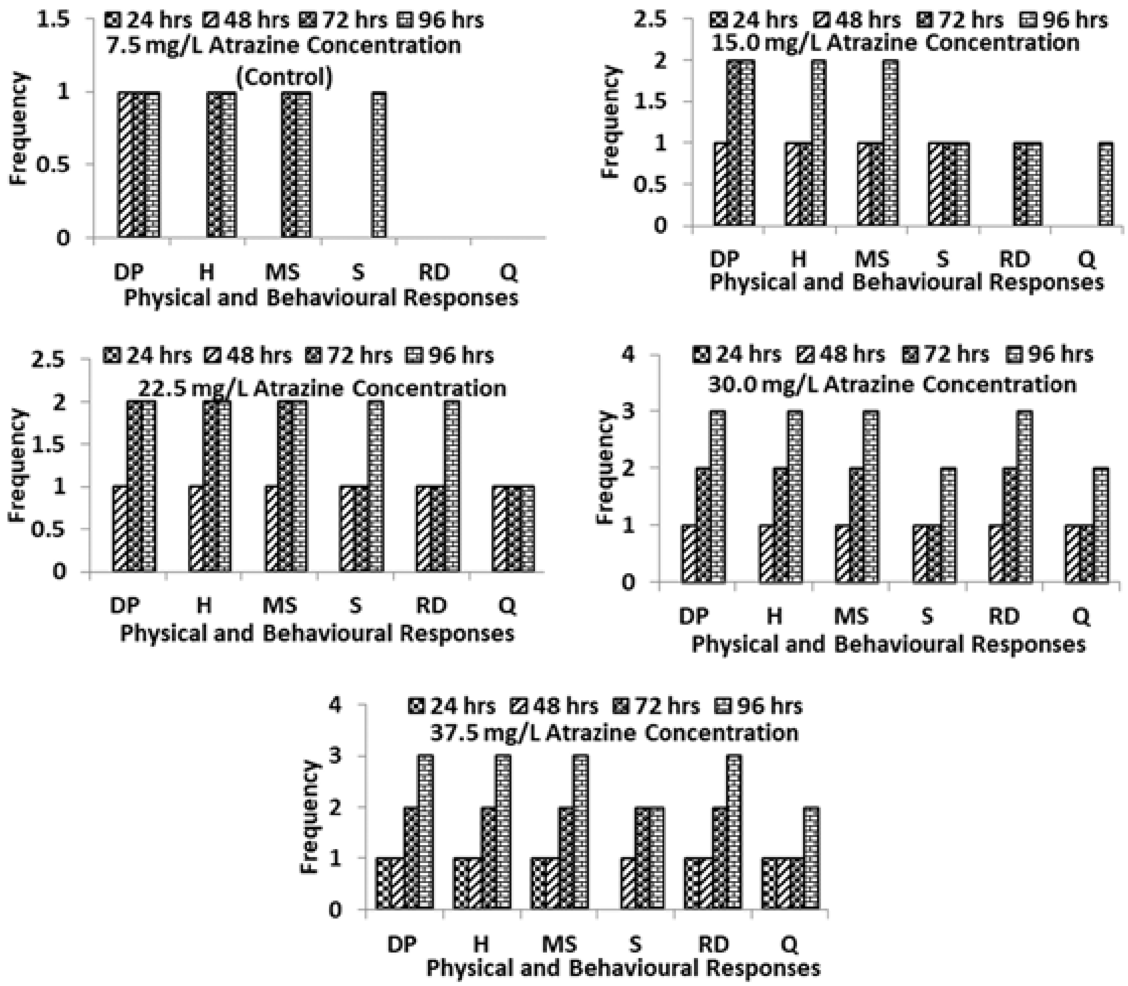

Fig. 1: Physical and behavioural responses of *Clarias gariepinus* exposed to different atrazine concentrations

Legend: DP = Dark pigmentation, H= Hemorrhaging from gill, MS = Mucous secretion, S = Sounding, RD = Respiratory distress, Q = Quiescent, 0 = none, 1 = mild; 2 = moderate, 3 = severe)

At 22.5 mg/L atrazine concentration, moderate dark pigmentation (DP), haemorrhage (H), and mucous secretion from the gills (MS) were observed after 72 hours. Moderate sounding (S) and respiratory distress (RD) were observed from 96 hours while mild quiescent (Q) occurred from 48 hours. At 30 mg/L atrazine concentration, severe (3) dark pigmentation (DP), haemorrhage (H), mucous secretion (MS), and respiratory distress (RD) occurred after 96 hours. Moderate dark pigmentation (DP), haemorrhage (H), and mucous secretion from the gills (MS) were observed after 72 hours as in 22.5 mg/l atrazine concentration while moderate sounding (S) and quiescent (Q) occurred after 96 hours. At 37.5 mg/L atrazine concentration, dark pigmentation (DP), haemorrhage (H), mucous secretion (MS), and respiratory distress (RD) started as mild after 24 hours and progressed to severe after 96 hours while sounding (S) and quiescent (Q) started after 24 hours as mild and progressed to moderate after 72 and 96 hours respectively.

DISCUSSION

The toxicity of Atrazine to *C. gariepinus* juveniles was evaluated as healthy fish specimens were exposed to varying Atrazine concentrations. Atrazine residues have been reported in fish feed and *Clarias gariepinus* fillets from farms in Southwestern Nigeria (Olatoye *et al*., 2021). Fish are increasingly used as sentinel organisms in eco-toxicological studies (Elías Sedeño-Díaz and Lopez-Lopez, 2012). They play important role in the transfer of energy and nutrient across the food web, bio-accumulate substances from the aquatic ecosystem, respond to low concentrations of xenobiotics and act as early warning detectors of aquatic pollution (Bae and Park, 2014; Barneche and Allen, 2018; Jonsson and de Queiroz, 2023). Acute toxicity data has been used to derive water quality guidelines for regulatory measures (Nwani *et al.,* 2010). Toxicants are classified based on the LC_{50} as highly toxic if the LC_{50} is between 0.1 and 1mg/L, moderately toxic if the LC_{50} is between 1 to 10 mg/L and slight toxicity if the LC_{50} is in the range of 10-100 mg/L. All the recorded LC_{50s} indicated that the formulated atrazine was slightly toxic to *C. gariepinus* juveniles. The LC_{50} (16.82 mg/l) after 96hr was higher than 0.18 ml/l (180 mg/l) recorded by Popoola *et al*. (2018) but lower than 0.55 mg/l by Doherty *et al*. (2019), 0.68 µg/L (0.00068 mg/l) by Opute *et al*. (2022) and 7.63 mg/l recorded by Akeredolu *et al*. (2022). The results showed that the toxicity of atrazine to *C. gariepinus* depends largely on both the chemical exposure duration and concentration because the mortality increased with both factors as observed by Popoola *et al*. (2018). In related studies, Akeredolu *et al*. (2022) recorded 24hr-11.40 mg/l and 96hr-LC_{50} of 7.63 mg/l for *C. gariepinus* juveniles and Agbohessi *et al.* (2022) recorded 96hr-LC_{50} of 3.17 mg/l also for *C. gariepinus* juveniles while Opute *et al*. (2022) reported LC_{50} of 0.68 µg/L (0.00068 mg/l) after 48hrs and 100 % mortality at 30 µg/L (0.03 mg/l). Apart from duration of exposure, differences in LC_{50} values of a toxicant generally depend on species type, life stage, size, health of the species and physico-chemical factors (Mahnaz and Sadegh, 2018). The toxicity of pesticide may also be influenced by other additives in the herbicide formulation (Pereira *et al.,* 2009) and testing protocols (Jones *et al.,* 2009).

In order for animals to survive, it is important for them to maintain stability in their internal conditions as their environment changes; therefore, animals have to adopt strategies for response and acclimatization to these changes (Fu *et al*., 2022). Behaviour is one of the acclimatization strategies of animals to environmental change and usually occurs when organisms are exposed to chemical concentrations that are low to cause death (Saaristo *et al*., 2018). Behaviors are integrated results arising from physiological regulation in response to changes in the environment; serving as a link between physiology and ecological outcomes (Sharma, 2019; Fu *et al*., 2022). Atrazine can also alter fish behaviour by altering neurophysiological responses (Khoshnood, 2024). Toxicant-induced behavioural impairment interferes with ecologically relevant behaviours of fish such as predator avoidance, reproductive, and social interactions which are essential to wellbeing and survival of fishes in natural ecosystems (Scott and Sloman, 2004). The behavioural changes observed in this study were consistent with previous related studies (Popoola *et al*., 2018; Opute *et al*., 2022). However, Agbohessi *et al*. (2022) reported other behavioral changes like hyperexcitation, disorientation, lethargy, surfacing activity, reduced swimming rate and vertical positioning. This could be attributed to the brand of Atrazine and concentrations used. Juveniles were observed to avoid areas where atrazine was introduced into the aquarium as observed by Agbohessi *et al.* (2022). Avoidance is the most common behavioural manifestation observed in many animals exposed to toxicants (Hellou, 2011). No physical or behavioural changes was observed in the initial 24 hours of exposure in the control treatment. However, changes like dark pigmentation, haemorrhages, secretion of mucous and sounding were observed from 48 hours. According to Sharma (2019), very low concentrations of some chemicals can result in quick change of behaviour in some organisms. The number of physical and behavioural responses increased in number and frequency as the concentration and duration of exposure increased as observed in related studies (Popoola *et al*., 2018; Agbohessi *et al*., 2022; Opute *et al*., 2022). The behavoural dysfunction elicited by atrazine was similar to effects elicited by other pesticides such as profenofos (Pandey *et al.,* 2011), diazinon (Ahmad, 2011), endosulfan (Shao *et al*., 2012) and malathion (Ahmad, 2012). Respiratory distress and quiescent were the last behavioural responses observed. Respiratory distress elicited by atrazine may be linked to increased ventilation caused by increased oxygen consumption from atrazine metabolism by CYP450 (Cedergreen, 2014). After the initial behavioural responses due to exposure to toxicant, fish becomes quiescent - stop swimming and remain in a fixed position (Khoshnood, 2014), leading eventually to death as the concentration of the toxicant increases.

CONCLUSION

This study demonstrated that the herbicide, atrazine was toxic to *Clarias gariepinus* juveniles, and the effect increased with increasing atrazine concentrations resulting in 96hrs LC_{50} of 16.82 mg/L. The study also showed that *Clarias gariepinus* exhibited a number of physical and behavioural responses as a result of the introduction of the herbicide. These responses were influenced by concentration of the herbicide and duration of exposure. Therefore, the use of atrazine should be monitored seriously because it is still extensively used despite the fact that it has been banned in Nigeria and globally.

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