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Atrazine-Induced Impairment of Fecundity and Fertilization in Exposed Adult *Clarias gariepinus*: Consequences for Hatchability and Larval Survival

Opute, P.A. and Uwaifo, F.O.

Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

*Corresponding author Email: ashibudike.opute@uniben.edu, Tel: +234 (0) 803 364 4134

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ABSTRACT: This research investigates the impact of atrazine, a widely used herbicide, on the reproductive capabilities of adult *Clarias gariepinus*, a freshwater catfish of ecological and economic significance. Twelve months old specimens of *C. gariepinus* comprising of twenty (20) males and twenty (20) females with mean average weight of 1024 ± 88.4g and 1100 ± 97.4g respectively and mean total length of 52.8 ± 2.3cm and 52.7 ± 1.1cm respectively, were exposed to exposed to four different sub-lethal concentrations of atrazine (40µg/L, 60µg/L, 80µg/L, and 100µg/L) in a semi-static renewal assay. Atrazine exposure was found to significantly diminish fecundity in exposed individuals, resulting in a marked reduction in the number of viable eggs produced. Furthermore, the herbicide exhibited a detrimental influence on hatchability, leading to a decreased percentage of successfully hatched embryos. Subsequent examination of larval survival revealed that atrazine exposure hindered the normal development and viability of the offspring. The findings of this study underscore the reproductive hazards associated with atrazine exposure in *C. gariepinus*, highlighting the potential ecological consequences for the species and the ecosystems they inhabit. Understanding the adverse effects of atrazine on key reproductive parameters is crucial for informing conservation and management strategies, as well as for developing policies aimed at mitigating the environmental impact of widespread herbicide use.

Keywords: *Clarias gariepinus*, Atrazine, Fecundity, Hatching, Survival

Introduction

In Nigeria, the imperative for agricultural intensification stems from the country's burgeoning population, resulting in both land scarcity and heightened food demand (Opute and Isibor, 2024). Agriculture, a major contributor to the degradation of surface and groundwater resources through erosion and chemical runoff, has sparked global concerns regarding water quality (Issaka and Ashraf, 2017). Herbicides have become indispensable in modern agricultural systems to enhance food production efficiency (Sarkar *et al.,* 2021). However, the management of herbicides in developing countries often falls short. Across the globe, natural water bodies are rapidly deteriorating due to run-offs and underground water leachates resulting from the application of synthetic fertilizers, pesticides, and herbicides to bolster agricultural production (Fiorino *et al.*, 2018; Stara *et al.,* 2019). This concerning trend in water quality, accompanied by its adverse effects on biological systems and human health, has evolved into a significant public and environmental issue (Opute *et al.*, 2021).

Atrazine holds the designation of a priority substance according to the United States Environmental Protection Agency, Agriculture Canada, and the European Commission (Lazarko-Connon and Achari, 2009). The potential health risks associated with triazine chemicals have garnered ongoing global attention from scientists. Many herbicides are under suspicion for potentially acting as endocrine disruptors, meaning substances that interfere with the body's endocrine system, leading to adverse developmental, reproductive, neurological, and immunological effects in both humans and wildlife (Olatoye *et al.*, 2021).

Atrazine, identified as a prevalent endocrine-disrupting chemical (Sanchez *et al.,* 2020), can have detrimental effects on the development, behaviour, and reproduction of freshwater fishes. This occurs through the disruption of physiological activities and endocrine systems (Wirbisky *et al.,* 2016; Opute and Oboh, 2021; Opute *et al.,* 2021). Atrazine, classified as a Class III herbicide in the triazine family, stands out as one of the most used pesticides among farmers in many developing countries, Nigeria included (Uddin *et al.,* 2015). Notably, a significant portion of Nigeria's farmland, approximately eighty percent, is under the management of smallholders cultivating up to 10 hectares each (Mgbenka *et al.,* 2015). Despite its widespread use, there is a notable lack of data concerning the fate and effects of these chemicals once applied to crop fields in regions where farmers may not fully grasp safe practices and the associated health risks (Opute and Oboh, 2021).
Fecundity in fish refers to the reproductive capacity or potential of a fish species, specifically the ability to produce offspring. It is a key aspect of the reproductive biology of fish and is often measured in terms of the number of eggs produced by a female fish during a single reproductive event (Hossain *et al.,* 2012). Fecundity can vary widely among fish species, and it is influenced by various factors such as the size and age of the fish, environmental conditions, and the availability of resources. Some fish species exhibit high fecundity, producing a large number of small-sized eggs in each reproductive cycle, while others may have lower fecundity with larger-sized eggs. Understanding fecundity is important in fisheries management and conservation efforts, as it provides insights into the reproductive potential of fish populations. It helps fisheries scientists and managers make informed decisions about sustainable harvest levels, population dynamics, and conservation measures to ensure the long-term health of fish populations (Hasan *et al.,* 2020). While numerous studies have explored the reproductive effects of atrazine on commonly studied model organisms, the existing literature indicates a significant gap in our understanding of the impact of Endocrine-Disrupting Chemicals (EDCs) on well-cultivated food fish, such as *Clarias gariepinus* (Opute *et al.,* 2021). This study aims to clarify the potential impairment of fecundity and fertilization in adult *C. gariepinus* exposed to atrazine, with subsequent implications for hatchability and larval survival.

Material and methods

Procurement and acclimatization of experimental fish: Forty (40) 12months old specimens of *C. gariepinus* comprising of twenty (20) males and twenty (20) females with mean average weight of 1024 ± 88.4g and 1100 ± 97.4g respectively, and mean total length of 52.8 ± 2.3cm and 52.7 ± 1.1cm respectively, and mean total length of 52.8 ± 2.3cm and 52.7 ± 1.1cm respectively, were purchased live from the Fisheries and Aquaculture department of the Faculty of Agriculture, University of Benin, Benin City and transported to the Animal House, Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin. The fish were carefully examined for physical deformities and injuries before purchase. They were kept in tanks filled with 30L of dechlorinated tap water for 7 days (acclimatization period) and were not fed during this period.
Test chemical: The test chemical was atrazine (CAS Number 1912-24-9, technical grade, 98.9% purity). The brand used was AltraForce, produced by Zhejiang Zhongshan Chemical Industry Group, China. The solution was packaged in a 1litre bottle containing 50% suspension concentrate of atrazine. The concentration of atrazine in the stock solution was 39.685 g/L. Atrazine (2-Chloro-4-ethylamino-6-Isopropylamino-s-triazine) is a commonly used herbicide by farmers in Nigeria (Uddin *et al.,* 2015).
Test Water: Test water used for the bioassay was dechlorinated tap water. The dechlorination of the test water was done by allowing it to stand exposed for 36 hours (Ezemonye and Enuneku, 2005). The dechlorinated water was used for the acclimatization period, the control tanks and the experimental tanks with contaminants.
Water quality: The water quality was monitored during the acclimatization, acute and sublethal bioassay procedures. Physico-chemical parameters such as Turbidity, Total alkalinity, Water hardness and Conductivity were measured using standard methods (APHA, 1998). The pH, temperature and DO was measured insitu using the pH meter, thermometer and DO meter, respectively.
Artificial reproduction of the African catfish, C. gariepinus: Gonadal stimulation was conducted using OvaprimTM at a rate of 0.5 ml/kg of body weight. Prior to injection, brood stock individuals were anesthetized using five to ten drops of Guinaldine to facilitate the process. Ovaprim was administered intramuscularly at an angle of 30-45°, approximately 2-3 cm behind the dorsal fin in the direction of the tail. After injection, each fish was placed in separate tanks, and a latency period of eleven hours was allowed for the stimulation process.
Stripping, fertilisation and incubation: Injected female broodstocks were removed from their tanks after 11 hours (latency period), weighed and then stripped by gently pressing the abdomen with a thumb from the pectoral fin towards the genital papilla into well-labelled dry bowls. Milt of each male broodstock was removed after dissecting it and the sperm collected by laceration of the testis with a clean razor blade into 25ml of normal saline in a container. The sperm of each male was mixed with the eggs of the corresponding female and spread

on a plastic netting substrate placed in a 50L bowl, containing about 20L of water. The temperature of the water used was 27°C and the pH was 6.6 (regulated using soda ash and measured using a portable pH-meter). Thirty (30) eggs each were counted and removed from the original mixture and spread on a netting substrate in smaller bowls containing 2L of water to use for further studies such as fertilization rate, hatchability, and survival rate. This was done for each of the egg-sperm mixtures of the four (4) concentrations of atrazine as well as the control. The large and small bowls were all labelled appropriately with the codes AT_1, AT_2, AT_3, AT_4 and C.

Estimation of number of eggs stripped per fish, percentage fertilization, percentage hatchability, survival rate, mortality rate, and percentage deformity of larvae: The number of eggs released was determined by subtracting the weight of the broodstock after spawning (W_{ii}) from the weight before spawning (W_i) in grams and multiplying the difference by 700 (1gm = 700 eggs) (Viveen *et al*., 1985).

Fertilization rate was determined when the eggs reached the 4-8 celled stage of embryonic development. For calculating percentage fertilization, a sample of about 30 eggs from each replicate of each treatment were carefully taken on a petri dish containing water and the number of fertilized and unfertilized eggs were counted under a microscope (×40) (Adebayo, 2006). The fertilization rate was then calculated by the following equation according to Adebayo (2006):

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{Number of eggs counted}} \times 100$$

The eggs were then transferred to their original lot for hatching. After hatching, the numbers of hatchlings within each batch were carefully counted and the hatching rate was calculated using the following equation (Adebayo, 2006):

$$\text{Hatching rate} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs in a batch}} \times 100$$

$$\text{Percentage deformed larvae} = \frac{\text{Number of deformed larvae}}{\text{Total number of larvae}} \times 100$$

$$\text{Survival rate} = \frac{\text{Number of hatchlings alive up to larvae stage}}{\text{Total number of hatchlings}} \times 100$$

Adult reproductive toxicity: Forty (40) 12months old African catfish (*Clarias gariepinus*), twenty (20) males and twenty (20) females with mean average weight of 1024 ± 88.4 g and 1100 ± 97.4 g respectively and mean total length of 52.8 ± 2.3 cm and 52.7 ± 1.1 cm respectively were exposed to four different sub-lethal concentrations of the pesticides (40 µg/L, 60 µg/L, 80 µg/L, and 100 µg/L). Each set of experiment was accompanied by a control. After 28 days of continuous stress of the pesticides, the gonads of the male and female fish from control and treatment tanks were extracted. Gonadal examination and histological studies were carried out.

Estimation of the fecundity: Fecundity was estimated gravimetrically. It was determined as the product of gonad weight and oocyte density. Oocyte desity was taken as the number of oocytes per gram of ovarian tissue, and it was determined by counting the number of oocytes in the weighed sample of ovarian tissue. After weighing the ovaries, three (3) subsamples of known weight were extracted from different parts of the ovary lobe. The number of oocytes (eggs) was estimated using the following equation:

$$\text{Fecundity } (F_1) = \frac{\text{No. of eggs in sub-sample} \times \text{Gonad}}{\text{Weight of sub-sample}}$$

F_1 represents the fecundity for one sub-sample of the ovarian tissue. The mean number of the three sub-sample fecundities (F_1, F_2 and F_3), was then calculated for each female fish as follows:

$$\text{Fecundity (Fe)} = \frac{F_1 + F_2 + F_3}{3}$$

The gonadosomatic index (GSI) was calculated for each of the male and female fish using the following formula:

$$\text{GSI} = \frac{\text{Weight of ovary}}{\text{Weight of fish}} \times 100$$

Data analysis: Data across exposure concentrations were analyzed using computer software, Statistical Package for Social Scientists (SPSS, 21), Microsoft Excel, 2010 and Graphpad Software (2018). Data were expressed as mean ± standard error (SEM). One-way analysis of variance (ANOVA) was used to test significance among exposure chemical and concentrations followed by a post-hoc Tukey's multiple tests, for statistical comparisons among the groups (differences between means was considered significant at $p<0.05$.

Results and Discussion

Physic-chemical properties of the test media: The results of the physico-chemical parameters were almost the same across treatment groups. The parameters showed no significant differences (P>0.05) from the control. This result is consistent with the report of Papoulias *et al.* (2014) on atrazine. They reported that atrazine exposure to Japanese medaka (*Oryzias latipes*) measured with ELISA remained constant and near nominal concentrations for the entire duration of the experiment.

Reproductive performance of control and exposed adult C. gariepinus fecundity: The control group recorded the highest average number of eggs (87,500 eggs) while (43,350 eggs), (38,971 eggs), (35,500 eggs) and (31,660 eggs) were recorded in AT_1, AT_2, AT_3, and AT4 respectively (Figure 1). Significant ($p < 0.05$) reduction in fecundity was observed in all the treatment groups, with 59.43% and 63.82% reduction recorded in fish treated with 80µg/l and 100µg/l atrazine respectively. While not constituting adult exposure, Cleary *et al.* (2019) documented the exposure of the parent (F0) generation of medaka to atrazine (ATZ) at concentrations of 5 or 50 µg/L during the initial twelve days of development, with no subsequent exposure observed over three generations. Their findings revealed that fecundity remained unaffected by atrazine across the F0 through F2 generations. However, a reduction in fertilization rates was observed in both low atrazine and treatment lineages within the F2 generation of Medaka (*Oryzias latipes*). Elias *et al.* (2020) reported a significant decrease ($p < 0.01$) in the relative fecundity indices (FBW, FBL, and FGW) in fish from all three groups exposed to thiobencarb for varying periods, compared to those of the control. The absolute fecundity values of females exposed to thiobencarb for different durations exhibited a highly significant decrease ($p < 0.01$). The lowest value (181.8) was observed after a 15-day treatment, followed by treatments lasting 3 and 9 days (532.5 and 1090.9, respectively), in contrast to the control group (33135.0).

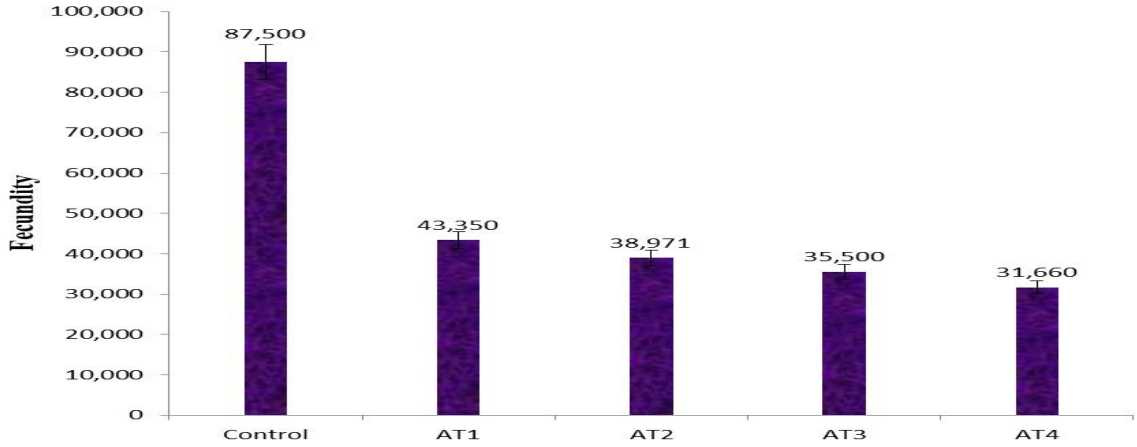

Figure 1: Mean fecundity of adult *C. gariepinus* exposed to atrazine for 4 weeks.

Percentage (%) fertilization and hatchability (36 h post-fertilization): A significant reduction in the fertilization rate of eggs from the adult fish exposed to atrazine across all the treatment groups was recorded. The average fertilization rate at 60min-PFS was 93.33% in the control group and this decreased to 69.23 % and 56.47% in the groups exposed to 80µg/l and 100µg/l respectively of atrazine (Figure 2). The decrease in the fertilization rate with increase in the concentrations of atrazine was significant at $p<0.05$. There was a dose-depended decrease in the hatchability rate of eggs from exposed adults of *C. gariepinus.* Significant ($p < 0.05$) decrease in hatchability of up to 52.34% and 37.89% in the fish treated with 80µg/l and 100µg/l atrazine respectively were observed (Figure 3). The number of unhatched/dead eggs increased significantly ($p < 0.05$) in all the treated groups, but the higher concentration treatment groups had significantly more dead eggs in comparison to the control.

The inhibition of fish egg fertilization by pesticides has been documented at various doses relevant to aquatic environments (Martyniuk *et al.*, 2020; Parra-Arroyo *et al.*, 2022). Additionally, the survival rate of developing eggs and the percentage of hatchings in catfish eggs show a correlation with increasing concentrations of different glyphosate-based herbicides (GBH) up to 1.0 part per million (Kale *et al.*, 2923). Westernhagen (1988) proposed two distinct mechanisms for the sublethal effects of pollutants on early developmental stages. The first mechanism involves the exposure of parent fish, leading to a subsequent reduction in deposited eggs. This is particularly evident when fish are chronically exposed to low levels of metals or pesticides, resulting in a notable decrease in egg production, potentially up to 80%. Similar effects are observed with short-term exposure to cyclic hydrocarbons. The second mechanism focuses on the exposure of the extruded egg. Before cleavage

initiation, calcium waves are triggered at fertilization, stimulating the contraction of the actin cytoskeleton. This contraction causes the non-yolky cytoplasm to be squeezed into the animal pole of the egg, transforming the spherical egg into a more pear-shaped structure with an apical blastodisc (Leung *et al.,* 1998). The delay in the initiation of cleavage observed at higher atrazine concentrations during embryonic exposure has been attributed to the interference of atrazine with the stimulation process of the actin cytoskeleton (Opute and Oboh, 2021). Jezierska *et al.* (2009) attributed structural and functional disruptions as responsible for the reduced number of hatched larvae during embryonic development. According to their findings, the hatchability of common carp under optimal conditions exceeded 70%, while that of metal-exposed embryos was significantly lower, at 34%.

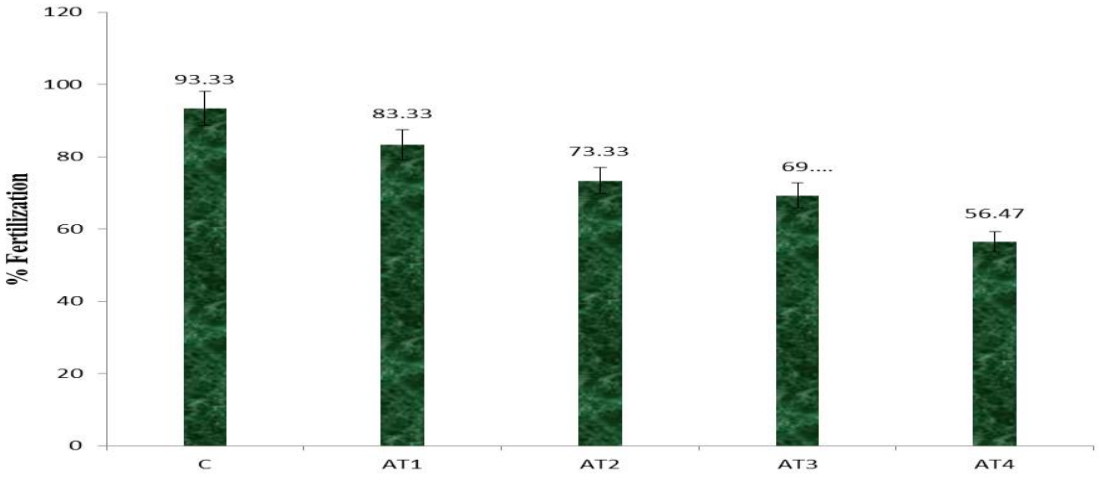

Figure 2: Percentage (%) Fertilization of the eggs of adult *C. gariepinus* exposed to atrazine for 4 weeks.

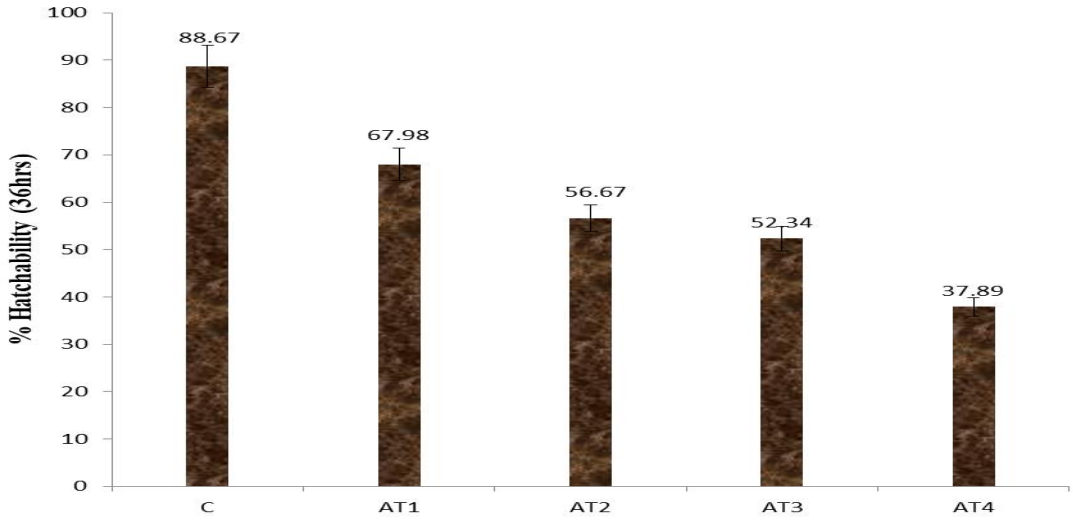

Figure 3: Percentage (%) hatchability of the eggs of adult *C. gariepinus* exposed to atrazine for 4 weeks.

Percentage (%) survival (7 days post-hatching): Embryonic exposure of adult *C. gariepinus* to atrazine at different concentrations significantly ($p<0.05$) resulted to reduced percentage survival at each developmental stage (Figure 4). The lowest (43.33%) larval mortality at the end of the experiment (7 days post-hatching) occurred in the control group, while the highest (86.88%) occurred in the 100µg/l atrazine treatment group. Mortality in AT_1 and AT_2 was 83.33% and 83.46 %, respectively and 85.11% in 80µg/l treatment group.
Exposure of *C. gariepinus* eggs to atrazine at very low concentrations has been found to interfere with successful embryonic development, resulting in delayed and aborted hatching, growth retardation, and severe developmental defects, as well as post-hatching mortalities (Opute and Oboh, 2021). They noted a significant decrease in embryo survival, impacting their overall hatching success. According to their findings, in most deformed embryos, the development did not progress, while only a few embryos in the control group developed normally. The maximum embryo mortality before hatching occurred at the blastula stage of development across all atrazine treatment groups. At this stage, mortality could be attributed to the embryo's inability to continue the developmental process.

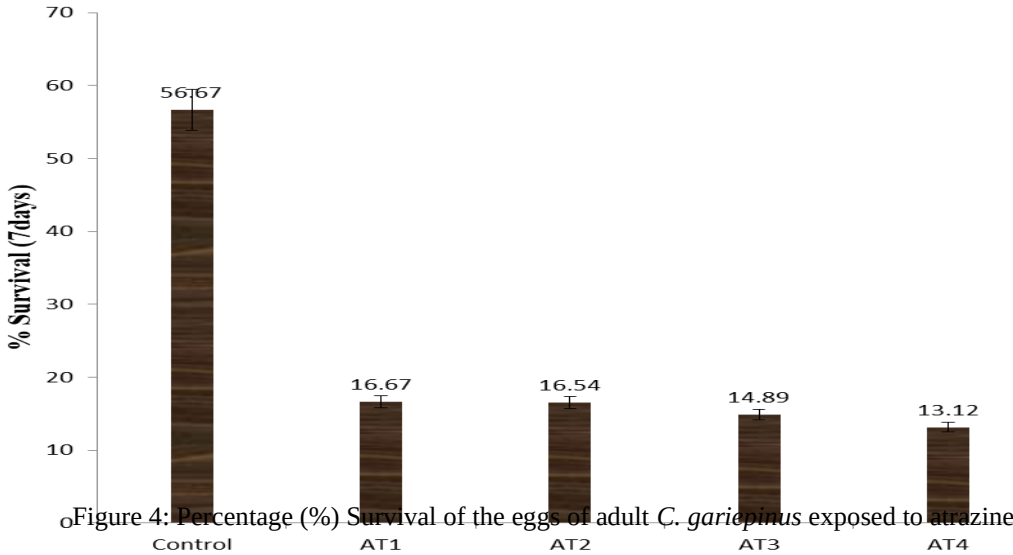

Figure 4: Percentage (%) Survival of the eggs of adult *C. gariepinus* exposed to atrazine for 4 weeks.

Conclusion

The results of this study clearly demonstrate that adult exposure of *Clarias gariepinus* to atrazine significantly affects fertilization, fecundity, hatching, and the survival of the first filial generation, potentially causing disruptions at various stages of embryonic development. Despite inconsistent reports on the capacity of atrazine to affect reproductive processes such as fecundity, the findings from this study highlight serious reproductive implications that could lead to a decline in fish population in the wild if the unchecked application of atrazine continues. Additionally, further studies at the molecular level should be conducted to better understand the subtle effects of this commonly used pesticide on fish reproduction.

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