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Effect of Insecticide (Topstoxin) on the Reproductive Indices *Clarias geriepinus* (African Catfish)

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Abstract

Aquatic ecosystems are increasingly threatened by agricultural runoff containing pesticides such as Topstoxin, a commonly used organophosphate. These contaminants pose a significant risk to the reproductive health of aquatic organisms, particularly fish species like *Clarias gariepinus*, which are important for aquaculture and ecological balance. In this study, a six-month static renewal bioassay was conducted to assess the chronic reproductive effects of sublethal Topstoxin exposure on C. gariepinus. A total of 200 juvenile fish were exposed to five concentrations of Topstoxin (0.00%, 1.50%, 2.50%, 3.50%, and 4.50%). Hormonal induction using Ovaprim was applied to evaluate the effects on gamete quality, sperm motility, milt volume, fertilization rate, hatching success, and larval viability. Data were analyzed using one-way ANOVA at a 95% confidence level. The results revealed significant dose-dependent declines in reproductive performance. Sperm motility was reduced by 65%, from 85% in the control group to 30% at the highest concentration (4.5 mg/L). Sperm abnormalities rose from 5% to 40%, and sperm volume declined by 58% (from 0.95 mL to 0.40 mL). Fertilization rates dropped by 71%, from 85% in the control to 25%, and larval hatching success and survival were markedly impaired. In conclusion, chronic exposure to sublethal concentrations of Topstoxin significantly disrupts reproductive indices in *Clarias gariepinus*. These findings underscore the ecotoxicological risks posed by pesticide contamination in freshwater systems and highlight the need for stringent regulation and monitoring of pesticide usage near aquatic habitats

Introduction

Aquatic ecosystems, especially freshwater habitats, are increasingly threatened by anthropogenic pollutants, notably pesticides like Topstoxin used extensively in agriculture. These chemicals enter aquatic environments through runoff and leaching, exposing non-target organisms such as fish to sublethal toxic effects. *Clarias gariepinus*, a key aquaculture species, often inhabits such environments and serves as an important bioindicator due to its ecological and economic relevance. Investigating how Topstoxin affects its reproductive health is vital for understanding environmental toxicity impacts on aquatic biodiversity and fisheries sustainability (Adeyemo et al., 2021; Ogunji et al., 2018). The indiscriminate use of agrochemicals such as Topstoxin (a rodenticide and general biocide) in agricultural and domestic settings poses a growing threat to non-target aquatic organisms. Despite regulatory controls, runoff and leaching often introduce these substances into aquatic ecosystems, where they persist and interact with biota. *Clarias gariepinus*, a widely cultured freshwater fish species in Nigeria, is especially vulnerable to such contaminants. However, there is limited empirical data on how sublethal exposure to Topstoxin affects the reproductive capacity of

this species. This gap in knowledge hinders effective environmental monitoring, impact assessment, and formulation of science-based regulatory guidelines on chemical use near aquatic habitats (Adeyemo *et al*., 2021; Ogunji *et al*., 2018). Understanding the sublethal effects of Topstoxin on the reproductive physiology of *Clarias gariepinus* is essential due to the ecological and economic value of this species in Nigeria's aquaculture and fisheries. Reproductive toxicity can have long-term implications for fish population dynamics and food security. Given the increasing prevalence of chemical contaminants in freshwater systems, this study provides timely insights into the potential endocrine-disrupting and gametotoxic effects of Topstoxin. The results could aid environmental regulatory agencies such as NESREA in evaluating permissible thresholds for agrochemicals, while also informing sustainable aquaculture practices (Sahoo *et al*., 2023; NESREA, 2019; Sani *et al*., 2022). Hence, this study evaluated the effect of sublethal concentrations of Topstoxin on the reproductive indices and gamete quality of *Clarias gariepinus*, intending to understand its potential impacts on fish fertility and population sustainability.

MATERIAL AND METHODS

Study Location and Experimental Setup

The research was carried out at the Department of Biological Sciences Laboratory, Federal University Dutsin-Ma, Katsina State, Nigeria, at coordinates 12.47275°N and 7.48582°E. A total of 200 juvenile *Clarias gariepinus* (measuring between 9.3 and 10.2 cm) raised in captivity were used. These fish were acclimated in 250-liter tarpaulin tanks filled with tap water under outdoor conditions. Throughout the acclimation period, they were fed 2 mm Blue Crown commercial feed, and any uneaten feed was removed daily to preserve water quality (OECD, 2020).

Topstoxin, obtained from a commercial vendor in Katsina, was applied to *Clarias gariepinus* at graded concentrations (0.00 mg/L, 1.50 mg/L, 2.50 mg/L, 3.50 mg/L, and 4.50 mg/L), including a control. Five treatment groups with five replicates each were prepared via serial dilution and measured using a precision balance, following OECD (2022) and Reish & Oshida (2018) toxicity assessment protocols. Exposure concentrations of 4.50 mg/L, 3.50 mg/L, 2.50 mg/L, and 1.50 mg/L were derived from fractions of a previously established 96-hour LC_{50} (Adeogun & Chukwuka, 2021), alongside a 0.00 mg/L control. The study followed a 21-day static renewal bioassay. Juvenile *Clarias gariepinus*, reared in tarpaulin tanks from March to August 2024, were transferred to eight plastic aquaria (50 × 30 × 30 cm), each stocked with five fish in 25 L of toxicant-treated water. Water was regularly replaced to maintain consistent toxicant concentrations throughout the exposure period.

Biometric Measurements and Brooder Selection

After six months of exposure, *Clarias gariepinus* broodstock was assessed. Two males and two females were randomly selected and weighed weekly using a digital precision balance (Mettler Instruments), while their lengths were measured using a digital Vernier caliper (Topac Instruments) following Fulton (2021). For reproductive evaluation, one male and one female fish (average weight: 1.8 kg; length: 48 cm) were randomly selected from each exposure group (n=10)

and placed in 25L holding bowls in duplicate setups to initiate gonadal stimulation.

Hormonal Injection for Gonadal Stimulation

Brooders were injected intramuscularly with Ovaprim™ containing fish gonadotropin-releasing hormone (GnRH) and a dopamine receptor blocker at dosages of 0.5 mL/kg for females and 0.25 mL/kg for males (Sahoo *et al*., 2023).

Sperm Quality Assessment

Following a 12-hour latency period, male broodstock from each treatment group were euthanized using medullar transection, and their testes were carefully excised. The testicular lobes were dissected to extract fresh milt, which was collected in Petri dishes and transferred into a 2 mL graduated cylinder for volume measurement. A drop of the milt was then placed on a glass slide, activated with an equal volume of distilled water, and examined at 40× magnification using an Olympus Micronal microscope. Sperm motility was evaluated based on the procedure outlined by Lamai (2021).

Egg Quality and Fertilization

Twelve hours post-hormone injection, eggs were manually stripped from female brooders (n=10 per treatment) by gently pressing the abdomen. The eggs were weighed using an Ohaus digital balance, and total fecundity was estimated by counting eggs in a 1g sample. Fifty eggs from each female were measured for diameter using an ocular micrometer. The eggs were then split into two batches, with one batch fertilized using milt from males exposed to the same toxicant level. Fertilized eggs were gently mixed and transferred to hatching bowls following Huismann and Richter (2022).

Incubation and Hatching Success

Fertilized eggs (n=10 per treatment group) were incubated for 48 hours, after which hatching success was evaluated by calculating the percentage of hatched eggs using the formula:
Egg Viability Rate = (Number of Hatched Eggs / Total Number of Eggs in Batch) × 100.

Non-viable embryos, identified as opaque or whitish, were counted at the end of incubation. Daily records of larval mortality were kept, and survival rates were assessed 21 days post-fertilization. Hatchlings were fed *Artemia*® beginning on day 34, once their yolk sacs had been fully absorbed (Aluko & Ali, 2019).

Estimation of Gonadosomatic Index (GSI)

Following egg collection, ten female brooders from each exposure group were euthanized using medullar transection, and their gonads were removed. The weight of the gonads, including stripped eggs, was recorded, and the gonadosomatic index (GSI) was determined using the formula:
GSI = (GW / (BW - GW)) × 100,
Where, *GW* represents gonad weight and *BW* is the body weight (Van Aerle *et al*., 2023).

Data Analysis

Data were expressed as mean ± standard deviation (SD), and variations among exposure groups were analyzed using a One-way Analysis of Variance (ANOVA). Duncan's multiple range test was used to determine significant differences across exposure concentrations. Data were analyzed using the MS Excel Analysis ToolPak, with significance set at $p < 0.05$.

RESULTS

Body Morphometrics of Experimental Fish

Tables 1 and 2 detail the biometric parameters of *Clarias gariepinus* broodstock exposed to varying concentrations of Topstoxin. Female fish generally exhibited higher body mass than their male counterparts, likely attributable to ovarian mass and egg content. The highest recorded female weight was 2.1 kg at the 2.50 mg/L exposure level, with corresponding standard and total lengths of 36.03 ± 1.8 cm and 46.0 ± 1.09 cm, respectively. In contrast, the lightest male was recorded at 1.4 kg (1.50 mg/L), with a standard length of 35.03 ± 1.02 cm and a total length of 45.01 ± 0.2 cm. Across treatments, the morphometric variations between groups were statistically significant ($p < 0.05$), underscoring a dose-dependent influence of Topstoxin on growth characteristics.

Sperm Quality and Volume

Topstoxin exposure negatively affected sperm characteristics (Table 3). Sperm motility declined from 85% in the control group to 30% at the highest exposure (4.50 mg/L), indicating substantial impairment in sperm function. Concurrently, sperm abnormalities—such as coiled tails and malformed heads- increased markedly, from 5% to 40%. Sperm volume was also reduced by over half (from 0.95 mL to 0.40 mL), suggesting compromised testicular output. These adverse effects are consistent with pesticide-induced reproductive toxicity, possibly due to endocrine disruption affecting spermatogenesis or structural damage to testicular cells (e.g., Leydig and Sertoli cells).

Fertilization Success

As shown in Table 4, fertilization rates declined progressively with increasing Topstoxin concentrations. The highest fertilization rate (85%) was observed in the control group, while the lowest (25%) occurred at 4.50 mg/L—a 71% decrease. This decline is likely linked to impaired sperm motility, increased morphological abnormalities, and reduced gamete viability. Additionally, oocyte quality may have been compromised due to ovarian toxicity, while the presence of residual pesticides may interfere with gamete compatibility and sperm-egg fusion processes.

Table 1: Body weight, standard length, and total length of female *Clarias gariepinus* produced and exposure to topstoxin (n = 5)

Exposure (mg/L)	Wet weight(kg)	Standard length (cm)	Total length (cm)
0.00	2 ± 1^{a}	35.03 ± 1.12^{a}	45.1 ± 0.01^{a}
1.50	1.9 ± 0.7^{a}	34.06 ± 1.85^{b}	44.5 ± 1.02^{b}
2.50	2.1 ± 0.3^{b}	36.03 ± 1.8^{a}	46.0 ± 1.09^{a}
3.50	1.8 ± 0.0^{a}	32.07 ± 1.25^{b}	42.6 ± 0.01^{b}
4.50	2 ± 1^{b}	33.01 ± 0.69^{a}	43 ± 2.82^{c}
P value	0.825	0.002	0.001

Significant at $p<0.05$. Values are given as mean ± standard deviation of the mean (SD; n=10).

Table 2: Body weight, standard length, and total length of male *Clarias gariepinus* produce (n = 5)

Exposure (mg/L)	Wet weight (kg)	Standard length (cm)	Total length (cm)
0.00	1.8 ± 0.3^{a}	37.0 ± 0.01^{a}	47.03 ± 0.25^{b}
1.50	1.4 ± 0.0^{b}	35.03 ± 1.02^{b}	45.01 ± 0.2^{c}
2.50	1.7 ± 0.9^{a}	36.01 ± 0.1^{a}	46.04 ± 0.31^{b}
3.50	1.6 ± 0.5^{b}	37.02 ± 0.18^{a}	48.03 ± 0.01^{a}
4.50	1.8 ± 0.1^{a}	$39.\ 03\pm1.01^{a}$	49.02 ± 1.09^{a}
P value	0.003	3.57×10^{-8}	1.26×10^{-8}

Significant at $p<0.05$. Values are given as mean±standard deviation of the mean (SD; n=10).

Table 3: Showing sperm quality and sperm volume at different topstoxin exposure

EXPOSURE(mg/L)	SPERM Motility (%)	SPERM ABNORMALITY (%)	SPERM VOLUME(ml)
0.00	85 ± 3^{a}	5 ± 1^{a}	0.95 ± 0.05^{a}
1.50	75 ± 4^{b}	12 ± 2^{b}	0.82 ± 0.06^{b}
2.50	65 ± 5^{c}	18 ± 3^{c}	0.70 ± 0.07^{c}
3.50	50 ± 6^{d}	25 ± 3^{d}	0.58 ± 0.08^{d}
4.50	30 ± 7^{e}	40 ± 5^{e}	0.40 ± 0.10^{e}
P value	7.04×10^{-14}	2.89×10^{-12}	7.85×10^{-8}

Significant at $p<0.05$. Values are given as mean ± standard deviation of the mean (SD; n=10).

Table 4: Showing fertilization rate at different topstoxin exposure

Exposure (mg/l)	Fertilization (%)
0.00	85 ± 3.0^{a}
1.50	72 ± 4.0^{b}
2.50	58 ± 5.0^{c}
3.50	42 ± 6.0^{d}
4.50	25 ± 7.0^{e}
P value	1.09×10^{-14}

Significant at $p<0.05$. Values are given as mean ± standard deviation of the mean (SD; n=10).

DISCUSSION

The quality of gametes in fish is a key marker of reproductive health and environmental conditions (Hajirezaee *et al*., 2021). Reduced feeding in fish exposed to Topstoxin suggests that contamination impairs appetite and metabolism, ultimately diminishing

growth and reproductive output. Topstoxin also induces erratic swimming behaviours, which can impair survival instincts like predator avoidance and social coordination (Blaxter & Hempe, 2019; Mesa *et al*., 2018; Scott & Sloman, 2023). Diminished body condition from toxin exposure limits energy availability for reproductive functions, reducing egg and sperm quality (Weiss *et al*., 2020).

Female nutritional status directly affects oocyte development and egg viability (Kjørsvik *et al*., 2023; Brooks *et al*., 2017; Ling *et al*., 2021). Smaller maternal size and weight, observed in exposed fish, result in smaller eggs and reduced larval survival (Murawski *et al*., 2020; Adeogun & Chukwuka, 2023). A reduced gonadosomatic index (GSI) indicates disrupted gametogenesis, likely due to heavy metals such as lead, chromium, and iron in Topstoxin (NESREA, 2019; Adeogun & Chukwuka, 2022). These metals interfere with dopamine-mediated hormonal regulation and impair embryo development (Limke *et al*., 2020; Van Duyn *et al*., 2023; Paran *et al*., 2021).

Lower sperm motility and milt volume, key fertility indicators, were also observed, linked to oxidative stress and endocrine disruption (Alquezar *et al*., 2018; Wang *et al*., 2019). Fertilization failures, even with unexposed females, confirm sperm-specific toxicity from heavy metal exposure (Hernandez-Ochoa *et al*., 2020; Kumar *et al*., 2019; Wirth *et al*., 2021). Poor egg traits and hatching rates reflect maternal contaminant transfer and ovarian toxicity (Alquezar *et al*., 2018; Brooks *et al*., 2021). Larger eggs, usually richer in nutrients and yielding fitter offspring, were fewer in exposed groups (Jastrebski & Morbey, 2019; Donelson *et al*., 2018). Endocrine-disrupting chemicals in Topstoxin may inhibit gonadotropin-releasing hormone (GnRH) and affect dopamine feedback loops essential for reproduction (Wen *et al*., 2020; Weltzien *et al*., 2021; Soso *et al*., 2023). Ultimately, reduced hatching success and larval survival confirm toxic interference with gamete viability and development (Bobe *et al*., 2019; Wang *et al*., 2020).

CONCLUSION

This study demonstrated that exposure to increasing concentrations of the insecticide Topstoxin significantly impairs the reproductive health of *Clarias gariepinus*. Observable declines in sperm quality, gonadosomatic index, fertilization rate, and overall gamete viability highlight the toxic effects of this pesticide on both male and female broodstock. These adverse outcomes are likely due to hormonal disruptions, metabolic stress, and bioaccumulation of heavy metals such as lead and chromium present in the insecticide. The findings underscore the ecological risk posed by unregulated pesticide use in aquatic environments and its potential to compromise fish reproduction, population sustainability, and aquaculture productivity.

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