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INVESTIGATION INTO THE CURATIVE POTENTIAL OF DIETARY VITAMIN C SUPPLEMENTATION ON ATRAZINE TOXICITY: BEHAVIOUR AND GROWTH PERFORMANCE OF *Clarias gariepinus* JUVENILES (BURCHELL, 1822)

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ABSTRACT

Atrazine is one of the most widely used pesticides in Nigeria. Several studies have shown the toxic effects of this herbicide on aquatic organisms, such as fish. Thus, it is critical to identify mitigation options to protect the health of fish, particularly commercially important aquaculture species exposed to atrazine from agricultural runoffs. This study investigated the toxic effects of 10, 20, and 30 μg L^{-1} of atrazine on the behaviour and growth performance of *Clarias gariepinus* juveniles and the curative potentials of dietary Vitamin C supplementation. The fish samples were taken weekly from different tanks to determine the specific growth rate, condition factor, and behavioural pattern following sub-chronic exposure to atrazine. The exposed fish groups showed a significant decrease ($p < 0.05$) in their specific growth rate (SGR) and condition factor with increasing concentrations of atrazine. Atrazine exposure also resulted in behavioural anomalies, including erratic swimming, clinging to the water surface, loss of equilibrium, lethargy, and skin discolouration. Co-exposure with Vitamin C did not significantly improve the growth performance or ameliorate the observed behavioural impairments. Therefore, findings from this study indicate that atrazine interferes with the behaviour and growth performance of *C. gariepinus* juveniles. However, the antioxidant property of Vitamin C supplementation in the fish diet did not significantly mitigate the toxicity of atrazine in the exposed fish.

Keywords: *Clarias gariepinus,* Atrazine, Curative, Vitamin C, Growth, Behaviour

INTRODUCTION

Aquaculture is a crucial industry catering for the sustenance and livelihoods of millions across the globe. The rising demand for fish, renowned for its protein-rich content and wildlife production, has propelled the overall expansion of this sector. Recent attention has focused on the adverse effects of environmental contaminants on the well-being and development of aquatic organisms, with herbicides like atrazine identified as a significant threat to water bodies (Opute *et al.,* 2022).

Atrazine, classified as a class III herbicide from the triazine family, ranks among the widely used pesticides by Nigerian farmers (Olatoye *et al.,* 2021). The National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria approved the use of atrazine in 2010 (atrazine 50%). However, the registration process of this herbicide possibly overlooks its potential impacts on non-target organisms. This oversight highlights the need for thorough descriptive and mechanistic toxicological investigations (Opute and Oboh, 2021). Atrazine and other herbicides are critical in the control of weeds in agriculture. However, their application can affect various physiological processes, potentially disrupting key aspects such as photosynthesis, pigment, lipid, and amino acid production, and seedling growth (Kim *et al.,* 2017; Lushchak *et al.,* 2018).

Fish, known for their well-understood characteristics and importance in supplying animal protein, are frequently selected as test organisms in toxicological studies (Opute and Oboh, 2021). Recent research has extensively explored the effects of atrazine on fish health and growth, revealing the herbicide impacts on reproduction, metabolism, and immune function (Singh *et al.,* 2018; Pérez *et al.,* 2022). However, the combined effects of atrazine and Vitamin C on fish behaviour and growth performance remain relatively unexplored (Mohammed *et al.,* 2023).

Vitamin C, or ascorbic acid, plays a vital role in fish growth and health, participating in collagen synthesis, immune function, and antioxidant defense mechanism (Trichet *et al.,* 2015). Studies suggest that Vitamin supplementation could mitigate the adverse effects of environmental pollutants on fish (Wu *et al.,* 2015; Gomes *et al.,* 2022;). For instance, Gomes *et al.* (2022) reported altered biochemical parameters in *Rhamdia quelen* fish when atrazine interacted with Vitamin C. Therefore, diets enriched with sufficient Vitamin content over extended periods could offer an economically viable antioxidant solution for inclusion in fish diets.

This study explored the curative potentials of Vitamin C on atrazine's effects on the growth and behaviour of *C. gariepinus* juveniles, a commercially important freshwater fish widely cultivated in Africa and other parts of the world.

MATERIALS AND METHODS

Test Chemicals

AtraForce® (New Haven, CT, USA), which contained 50% Atrazine SC and Em-Vit-C® (a Vitamin C product by Emzor Pharmaceuticals Nigeria), , were obtained from authorized outlets in Benin City, Edo State. A fresh stock solution of atrazine, with a concentration of 7.5 µg L^{-1} in water, was prepared every two days. Subsequently, 20, 40, and 60 mL of this stock were added to 15 L of water, resulting in targeted treatment concentrations of 10, 20, and 30 µg L-1, respectively. Twenty-four tanks, comprising the exposure groups and two controls, were used for the experiment. One control had only water, but the other had water and 0.8 g of Vitamin C. Dechlorinated tap water exposed for 36 hours was used as the test medium. (Ezemonye and Enuneku, 2005). The water underwent analysis for pH, electrical conductivity, dissolved oxygen, and temperature. The temperature and pH of the test media were maintained at 29°C and 6.0, respectively.

Experimental design and treatment

Five hundred (500) four-week-old *Clarias gariepinus*, of mixed sexes with an average length of 8.32 ± 0.81 cm and an average weight of 6.93 ± 1.52 g, were obtained from a registered fish farm in Benin-City, Edo State, Nigeria. The fish were transported in a plastic container to the fish tanks in the Animal House, Department of Animal and Environmental Biology, University of Benin. The fish were allowed to acclimatize for one week (7 days) in a 100 L tank filled with 50 L of dechlorinated borehole water. The study maintained a photoperiod of 12 hours of light and 12 hours of darkness. During acclimatization, the fish were fed commercial feed (Blue Crown, 2mm, Olam Group, Kwara, Nigeria) at 4% of their body weight daily, divided into two rations (morning and evening) (Ajani *et al.,* 2007). Feeding stopped 24 hours before initiating the exposure, and a measured amount of Vitamin C (0.8 g) was introduced twice daily to the designated treatment tanks labelled as AT_I, AT_{II}, AT_{III}, AT_IVT, $AT_{II}VT$, $AT_{III}VT$, CVT, and C. These labels corresponded to three atrazine concentrations without Vitamin C, atrazine concentrations with Vitamin C, control with Vitamin C, and control treatment without Vitamin C, respectively. Each experiment group consisted of triplicates with 20 juvenile catfish per tank. During the 28-day experiment, 50% of the test media was replaced every other day in all the exposure groups to reduce turbidity and monitored for behavioural responses, growth, and mortality.

Measurement of growth performance

Three fish samples from each treatment tank and the control tanks were measured weekly (7 days). The growth rate was calculated using the formula:

$$\text{Growth rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Time (days)}} \times 100$$

The Specific Growth Rate (SGR) was calculated as using the formula:

Specific Growth Rate (SGR) =

$$\frac{\text{In (final weight)} - \text{In (Initial weight)}}{\text{time (days)}} \times 100$$

Fulton's condition factor (K) was calculated using the formula given below:

$$K = \frac{\text{Total weight of fish}}{(\text{length})^3} \times 100.$$

Physicochemical analysis of treatment water

The pH, temperature, alkalinity, conductivity, total dissolved solids (TDS), and dissolved oxygen (DO) of the various treatment water samples were consistently measured throughout the study using standardized methods (APHA, 2005).

Data analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS version 21). The results are presented in all the tables as treatment means ±

standard deviation of the mean (SD). A one-way analysis of variance (ANOVA) was employed to assess significance among exposure chemicals and concentrations, with significance set at $P < 0.05$. Tukey's post hoc test compared the means of different treatments. Furthermore, regression analysis was employed to investigate the linear relationships between standard length (SL) and body weight (BW).

RESULTS AND DISCUSSION

Behavioural performance of juveniles

Clarias gariepinus juveniles exhibited varied responses and tolerance levels to sub-lethal atrazine exposure at different concentrations. Abnormal behavioural changes observed in the treatment tanks include erratic or agitated swimming, loss of equilibrium, frequent surfacing and hanging on the water surface, and discolouration (Table 1). The behavioural responses were concentration-dependent, wherein higher concentrations correlated with greater intensity of the responses. The result from this study aligns with the report of Marzouk *et al.* (2012) on female *Clarias gariepinus* that acute and chronic exposures atrazine resulted in clinical abnormalities such as diminished appetite, lethargy or restlessness, rapid opercular movements, and aberrant skin pigmentation in the form of faded skin. Observations in this study are similar to the findings of Chattopadhyay *et al.* (2006), who reported erratic behavioural patterns of fish exposed to herbicides. Elias *et al.* (2018) observed comparable alterations like various typical swimming behaviours and heightened deformities in fish subjected to sub-lethal concentrations of thiobencarb. These behaviours encompassed loss of balance, restless or erratic swimming, air gulping, sudden rapid movements, and excessive mucus secretion. In the present study, an increase in respiratory movements was observed during the entire period of the bioassay, while fish became inactive and almost non-motile, with clinical signs of fading of body colour, erosion of scales, lesions, and hemorrhagic patches all over their body, especially on the ventral side. The discolouration was pale and persisted throughout the exposure duration (28 days); however, the intensity of paleness reduced in the lower concentrations, with dark spots observed on the bodies of the exposed catfish juveniles. Ikele *et al.* (2011) report the same for juveniles of *C. gariepinus* exposed to diethyl phthalate. Opute and Oboh (2021) provided a plausible explanation for these behaviours, suggesting that pesticide poisoning may cause nervous system failure, impacting the physiological and biochemical activities of the fish.

Table 1: Behavioural observations of *Clarias gariepinus* juveniles for 28 days exposure to varying concentrations of Atrazine and Atrazine + Vitamin C

Behaviour	Control	AT_1	AT_2	AT_3	CVT	AT_1VT	AT_2VT	AT_3VT
Erratic swimming	-	-	+	++	-	-	+	+
Loss of Balance	-	+	++	+++	-	-	+	+
Discolouration	-	+	++	+++	-	-	+	++
Lethargy	-	+	+++	+++	-	-	+	++
Hanging on water surface	-	+	++	+++	-	-	-	+

(+) low response, (++) moderate response, (+++) high response, (-) no response

Growth Performance of Juveniles

It was observed that Atrazine toxicity affected the growth of the *C. gariepinus* juveniles in this study. Factors such as the difference in feed intake or the difference in food metabolism (Lal *et al.*, 2013) could explain the retardation observed in the growth of the exposed fish. The standard length (SL) and total body weight (TBW) of *C. gariepinus* juveniles were observed weekly for four weeks and varied, ranging from 9.37–11.59 cm (SL) and 8.03–15.69 g (W) in the control group (Tables 2-5). The standard length and body weight in the 30 μgL^{-1} treatment concentration ranged from 8.43–9.66 cm (SL) and 6.54–9.28 g (W), respectively (Tables 2-5).

Toxicants can impact growth directly or indirectly through their influence on feeding, as these processes are interconnected. Diminished physical activity can indirectly affect feeding, leading to consequences for growth. For instance, fish seem to elevate their metabolic efforts for expelling toxins, consequently redirecting more energy towards homeostatic maintenance rather than storing it for growth (Elias *et al.,* 2018). A significant factor that may be responsible for the reduction in growth rate could be the transformation of a portion of nutrients from the digestion of food consumed into energy to cope with chemical stress following exposure to atrazine. Lal *et al.* (2013) noted a decrease in plasma levels of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in Asian stinging catfish (*Heteropneutes fossilis*) following exposure to malathion. The reported decline was associated with reduced fish growth, diminished food intake, and the impact of pesticides on the metabolism of feed into somatic growth. Huynh and Nugegoda (2012) also reported similar weight reduction in Australian catfish (*Tandanus tandanus*) following exposure to chlorpyrifos at concentrations ranging from 2 to 10 mg/L-1. Additionally, high metabolic costs associated with heavy metal pollution have been linked to a decreased growth rate, as highlighted in the study by Xie *et al.* (2014).

Heavy metal pollution is associated with a reduction in growth rate and decreased feed conversion rate in marine organisms, possibly resulting from the tissue burden of heavy metals, thereby increasing metabolic costs (Xie *et al.,* 2014). Thus, the observed slowed growth in exposed juveniles of *C. gariepinus* could be attributed to the inhibition of acetyl cholinesterase.

Acetylcholinesterase is an enzyme that deactivates the neurotransmitter acetylcholine at nerve endings in the enteric nervous system. However, if interrupted, acetylcholine will accumulate, stimulating smooth muscle M3 muscarinic receptors and increasing motor activity in the gastrointestinal tract. Inhibition of acetylcholinesterase is suspected to decrease efficiency in the utilization of dietary proteins in fish feeds, resulting in growth retardation in atrazine-exposed C. gariepinus juveniles (Opute and Oboh, 2021).

On the other hand, length-weight relationships are for estimating fish condition factors, and these values are used for comparing the condition (fatness or well-being) of fish (Jisr *et al.,* 2018). The standard length and weight of exposed fish significantly ($p<0.05$) decreased across all treatment concentrations in a dose-dependent trend (Tables 4-5). Consequently, specific growth rate (SGR) and relative growth rate (RGR) showed significant decline with increasing concentrations of atrazine treatment. The decrease in weight gain and specific growth rate across the treatment groups in this study could have resulted from the allocation of energy for homeostatic processes and tissue repair rather than being solely directed towards storage and growth.

Table 2: Summary of growth performance of *C. gariepinus* juveniles exposed to varying concentrations of atrazine (Week 2)

Parameter	Control	AT_1	AT_2	AT_3
W(g)	8.03 ± 2.02^{a}	6.92 ± 2.17^{b}	6.91 ± 2.43^{b}	6.54 ± 1.29^{b}
TL (cm)	10.93 ± 1.24^{a}	9.86 ± 1.08^{b}	9.84 ± 1.19^{b}	9.42 ± 0.76^{c}
SL (cm)	9.37 ± 1.13^{a}	8.53±1.06^{b}	8.43 ± 0.94^{b}	8.43 ± 0.48^{b}
GR (%)	-0.53 ± 0.10^{a}	-0.67 ± 0.05^{b}	-0.11 ± 0.21^{c}	0.94 ± 0.50^{d}
SGR (%)	-0.50 ± 0.00^{a}	0.10 ± 0.30^{b}	0.71 ± 0.30^{c}	0.93 ± 0.20^{d}
K	0.61 ± 0.30^{a}	0.72 ± 0.11^{b}	0.73 ± 0.10^{b}	0.78 ± 1.00^{c}

Data are presented as mean ± SD. Different letters indicate significant differences between treatments. TL-Total length; SL-Standard length; W-Weight; GR-Growth rate; SGR-Specific growth rate; K-Condition factor; Superscript (a, b, c, d): Significant difference between treatment groups.

Table 3: Summary of growth performance of *C. gariepinus* juveniles exposed to varying concentrations of atrazine + Vitamin C (Week 2)

Parameter	Control	AT_1VT	AT_2VT	AT_3VT
W(g)	9.68 ± 3.68^a	5.88 ± 1.31^b	6.98 ± 1.48^b	7.44 ± 2.54^c
TL (cm)	11.49 ± 1.39^a	9.82 ± 0.80^b	9.74 ± 0.59	10.08 ± 0.92
SL (cm)	9.87 ± 1.21^a	8.36 ± 0.62^b	8.39 ± 0.57^b	8.72 ± 0.75^b
GR (%)	-0.34 ± 0.30^a	-0.78 ± 0.24^b	0.90 ± 0.38^c	0.83 ± 0.10^c
SGR (%)	-0.36 ± 0.32^a	-0.43 ± 0.43^a	0.86 ± 0.10^b	0.79 ± 0.91^b
K	0.63 ± 0.11^a	0.62 ± 0.12^a	0.76 ± 0.13^b	0.73 ± 0.13^b

Data are presented as mean ± SD. Different letters indicate significant differences between treatments. TL-Total length; SL-Standard length; W-Weight; GR-Growth rate; SGR-Specific growth rate; K-Condition factor; Superscript (a, b, c, d): Significant difference between treatment groups.

Table 4: Summary of growth performance of *C. gariepinus* juveniles exposed to varying concentrations of atrazine (Week 4)

Parameter	Control	AT_1	AT_2	AT_3
W(g)	12.60 ± 4.29^a	8.77 ± 3.63^b	7.85 ± 0.70^c	7.04 ± 2.00^d
TL (cm)	12.25±1.28^a	10.80 ±1.31^b	10.68 ±0.39^b	10.28 ± 0.93^b
SL (cm)	10.61 ± 1.17^a	9.05 ± 1.04^b	9.34 ± 0.45^b	8.6 ± 0.78^c
GR (%)	2.45 ± 1.20^a	0.53 ± 0.30^b	0.54 ± 0.33^b	0.78 ± 0.56^c
SGR (%)	1.86 ± 0.57^a	0.50 ± 0.21^b	0.50 ± 0.28^b	0.71 ± 0.34^c
K	0.69 ± 0.37^a	0.70 ± 0.90^a	0.64 ± 0.82^b	0.65 ± 0.71^b

Data are presented as mean ± SD. Different letters indicate significant differences between treatments. TL-Total length; SL-Standard length; W-Weight; GR-Growth rate; SGR-Specific growth rate; K-Condition factor; Superscript (a, b, c, d): Significant difference between treatment groups.

Table 5: Summary of growth performance of *C. gariepinus* juveniles exposed to varying concentrations of atrazine and Vitamin C (Week 4)

Parameter	Control	AT_1VT	AT_2VT	AT_3VT
W(g)	15.69 ± 4.66^a	8.516 ± 2.33^b	7.99 ± 2.57^c	9.28 ± 2.80^d
TL (cm)	13.38 ± 1.68^a	10.97 ± 0.77^b	10.74 ± 0.65^b	10.90 ± 1.14^b
SL (cm)	11.59 ± 1.36^a	9.17 ±.8.00^b	9.12 ± 0.59^b	9.66 ± 1.03^c
GR (%)	1.94 ± 0.23^a	1.03 ± 0.44^b	1.03 ± 0.23^b	1.41 ± 0.25^{ac}
SGR (%)	1.54 ± 0.89^a	0.89 ± 0.44^b	0.93 ± 0.11^b	1.18 ± 0.13^c
K	0.65 ± 0.15^a	0.65 ± 1.26^a	0.64 ± 0.36^a	0.72 ± 0.37^b

Data are presented as mean ± SD. Different letters indicate significant differences between treatments. TL-Total length; SL-Standard length; W-Weight; GR-Growth rate; SGR-Specific growth rate; K-Condition factor; Superscript (a, b, c, d): Significant difference between treatment groups.

The total length and body weight significantly decreased with increasing concentrations of atrazine during the four weeks of exposure. At the end of the two weeks of exposure, the standard length ranged from 9.37 to 10.02 cm in the control group and 8.30 to 8.42 cm in the 30 μgL-1 treatment group. A parallel trend in body weight was also documented, with a range of 9.50 to 9.60 g in the control group and 6.50 to 6.54 g in the 30 μg/L treatment group. During the third week of exposure, the average weight of fish in the treatment groups ranged from 7.63 g to 5.78 g, showing a high decrease from the control average weight of 8.93 g to 15.25 g.

Length – Weight Relationship (LWR) of *C. gariepinus* Juveniles

The length-weight relationship of the control and exposed *C. gariepinus* showed b-values (regression coefficients) of 0.3173, 0.2964, 0.3039, and 0.0777 for control, AT_1, AT_2, and AT_3, respectively (Figures 1-4). However, dietary supplementation with Vitamin C did not significantly differ from exposure to atrazine alone. The b-values (regression coefficients) were 0.3039, 0.3103, -0.0088, and 0.2576 for control, AT_1Vt, AT_2Vt, and AT_3Vt, respectively (Figures 5-8).

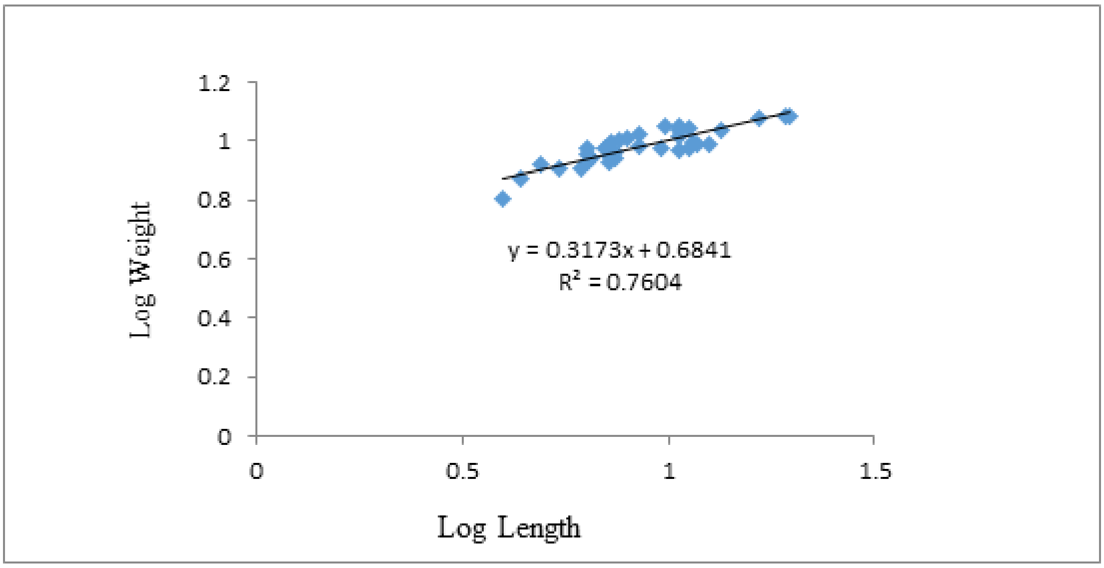

Figure 1: Regression between length and body weight of *C. gariepinus* catfish juvenile for 4 weeks (Control)

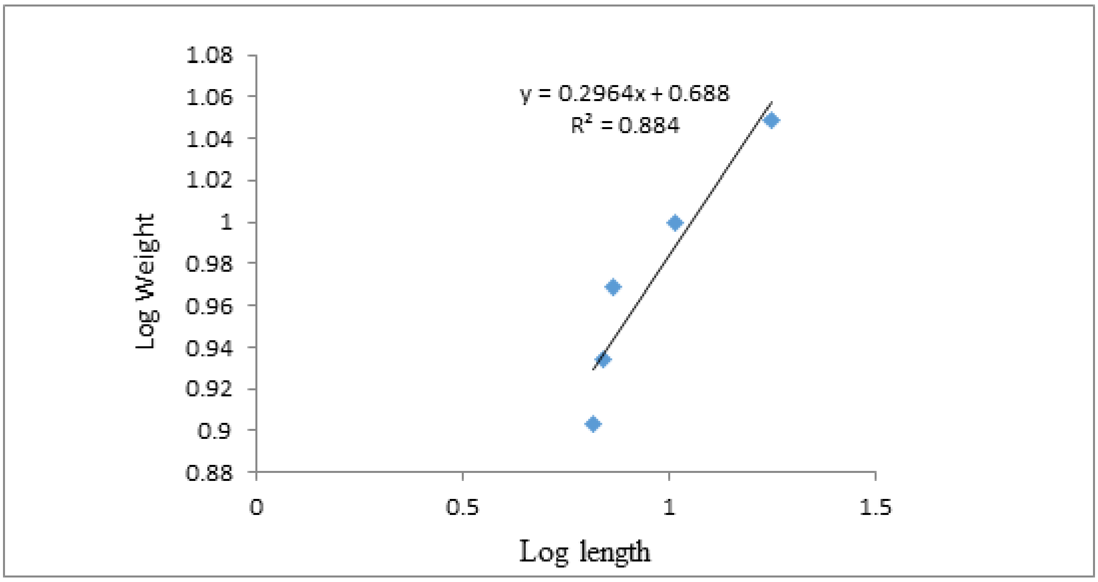

Figure 2: Regression between length and body weight of *C. gariepinus* catfish juvenile for 4 weeks (AT_1)

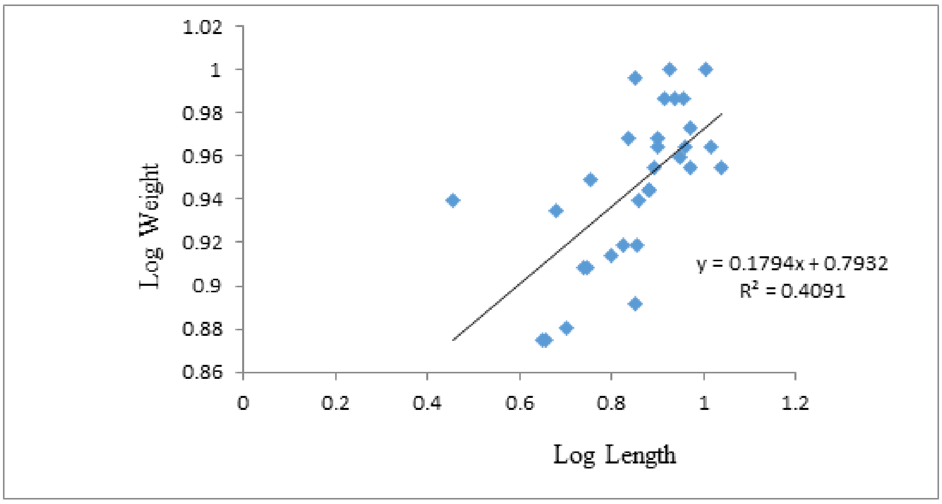

Figure 3: Regression between length and body weight of *C. gariepinus* catfish juvenile for 4 weeks (AT_2)

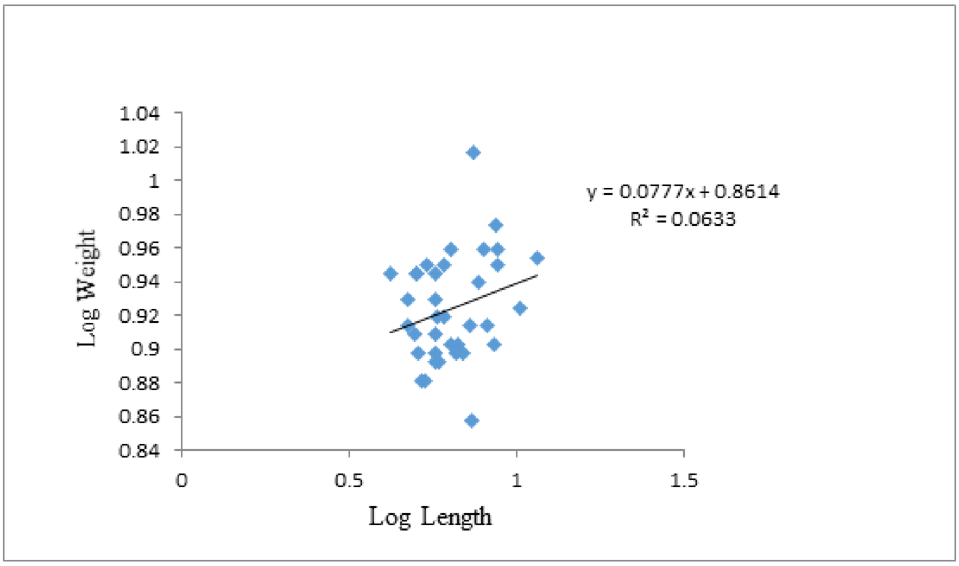

Figure 4: Regression between length and body weight of *C. gariepinus* catfish juvenile for 4 weeks (AT_3)

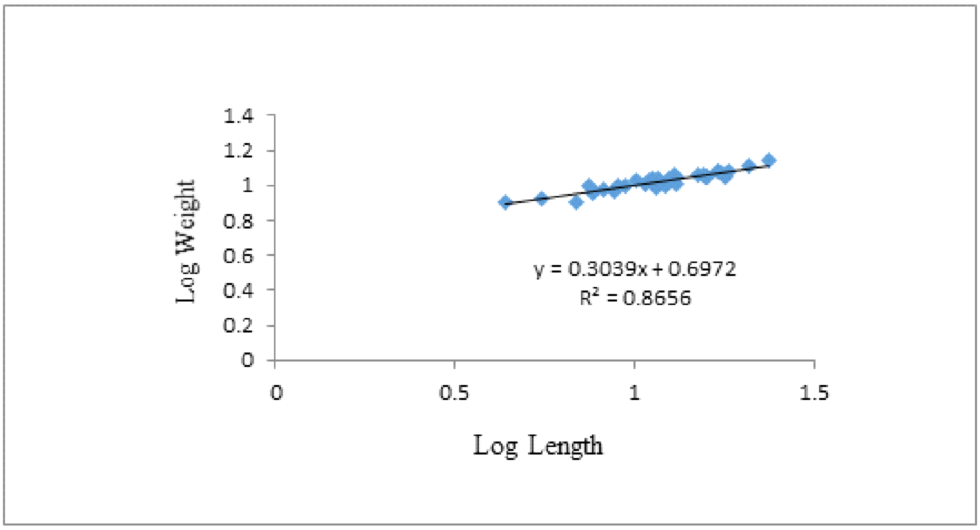

Figure 5: Regression between length and body weight of *C. gariepinus* catfish juvenile for 4 weeks (Control with Vitamin C)

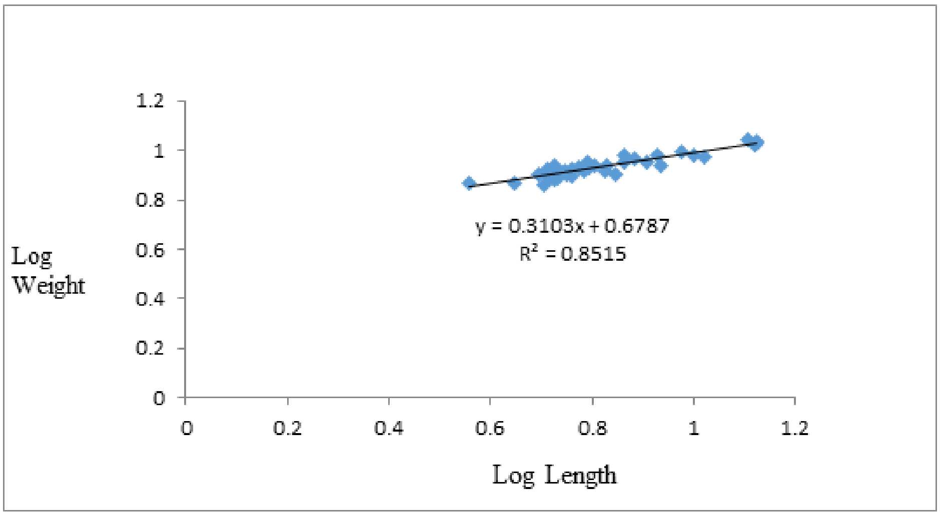

Figure 6: Regression between length and body weight of C. *gariepinus* catfish juvenile for 4 weeks (AT_1VT)

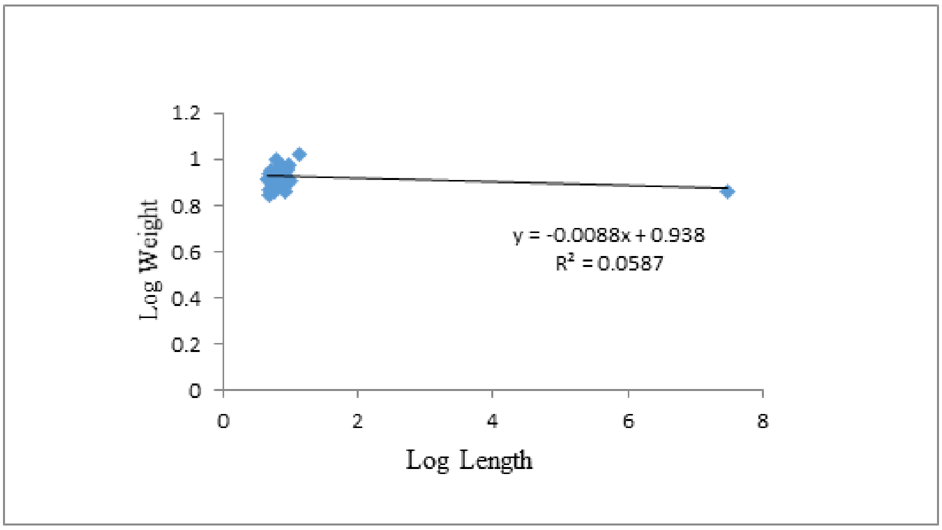

Figure 7: Regression between length and body weight of C. *gariepinus* catfish juvenile for 4 weeks (AT_2VT)

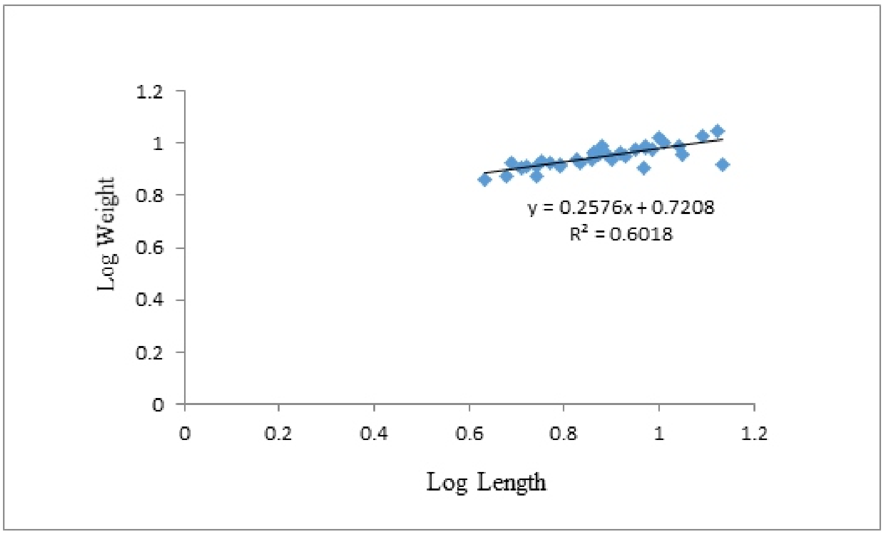

Figure 8: Regression between length and body weight of *C. gariepinus* catfish juvenile for 4 weeks (AT_3VT)

The length-weight relationships among the control and exposed groups, including the group with Vitamin C, exhibited negative allometric growth patterns (b < 3), which implies a preference for an increase in length above weight under the experimental conditions. Atrazine not only impacted the growth rate and condition factor but also influenced the overall growth pattern. In fisheries biology, length-weight relationships are critical in converting length measurements into weight and understanding the growth characteristics associated with these variables (Opute and Oboh, 2021). Additionally, these relationships are employed to estimate the fish condition factor, a measure used to assess fish condition (fatness or well-being) (Jisr *et al.,* 2018). The condition factor of a fish reflects its physiological state, influenced by intrinsic factors like gonadal development, organic reserves, and the presence or absence of food in the gut, as well as extrinsic factors such as food availability and environmental variability (Flura *et al.,* 2015). These findings suggest that atrazine negatively influences the growth patterns of fish, as reflected in the length-weight relationships, and the addition of Vitamin C does not seem to mitigate this effect. These observations also underscore the broader significance of these relationships in assessing the condition and well-being of fish in the context of environmental factors.

CONCLUSION

The study findings reveal that exposing *Clarias gariepinus* juveniles to atrazine disrupts their normal behaviour and growth performance, and can be attributed to the increased energy directed towards detoxification processes, consequently limiting the energy available for growth. The observed abnormal behaviours, including hanging on the surface, lethargy, skin discolouration, and erratic swimming, likely contribute to reduced competitive ability, increased vulnerability to predation, and impaired feeding. Co-exposure with Vitamin C showed minimal to no significant role in alleviating atrazine's adverse effects on growth performance and behaviour in the fish species. The 28-day laboratory exposure might not accurately represent field or natural conditions, especially considering the influence of climate change. Therefore, future studies should be done in controlled environmental chambers to mimic near-natural conditions. Employing new approach methods (NAMs) can help further elucidate the subtle effects of atrazine. The fixed concentration used in this study may have limited the Vitamin C supplementation experiment. To better understand the abatement potential of Vitamin C against pesticide toxicity, future studies should explore varying concentrations of atrazine in conjunction with Vitamin C supplementation.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this research.

AUTHORS' CONTRIBUTIONS

P.A.O. conceptualized the research; E.I. conducted the research; P.A.O. and E.I. analyzed and interpreted the findings; P.A.O. and E.I. drafted the manuscript; P.A.O. reviewed and edited the manuscript.

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