



Toxicity of the Herbicide 2, 4-D Dimethyl Amine Salt on Behavioral, Growth and Hematology of *Clarias gariepinus* (Burchell, 1822) Fingerlings

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ABSTRACT

The study aimed to determine the toxicological impacts of the herbicide 2, 4-D Dimethyl amine salt on *Clarias gariepinus* fingerlings. After a series of range finding tests, the fishes were exposed to lethal concentrations of 0.00, 1.34, 1.42, 14.8, 1.54, and 1.60ml/L for 96 hours as well as sub-lethal concentrations of 0.00, 0.069, 0.138 and 0.276ml/L of 2, 4-D Dimethyl amine salt for 8 weeks in a renewal bioassay. The value of 96 hours LC₅₀ was 1.385ml/L. Respiratory disturbance, erratic swimming, loss of equilibrium, lethargy, and sudden fish death were observed in 96hrs of exposed fish, and these varied greatly with differences in concentration of the toxicant, with a trend of significant ($p<0.05$) increase in mortality with increasing concentrations. The physicochemical parameters obtained before and after the experiment were within tolerable limits. The growth rates were significantly ($p<0.05$) reduced in fish exposed to sub-lethal concentrations of the herbicide 2, 4-D Dimethyl amine salt. The toxicant led to significant changes ($p<0.05$) in hematological parameters. Mean red blood cells and packed cell volume reduced with an increase in the concentration of the toxicant, while other parameters, such as white blood cells increased proportionally to the toxicant concentration. The lethal and sub-lethal exposure of *Clarias gariepinus* fingerlings to 2, 4 D dimethyl amine salt with values of 1.6 ml/L, 0.138ml/L 0.276 ml/L indicate significant changes in hematological parameters of acute, sublethal respectively.

Keywords: 2 4-D Dimethyl Amine Salt, *Clarias gariepinus*, Growth, Hematology, Toxicity

INTRODUCTION

The use of herbicides in controlling weeds in agricultural and home fields has increased, which has raised the concern of many environmentalists due to their toxicity in nature to non-targeted organisms (Enetinmi *et al.*, 2020). When herbicides are applied in restricted areas, they are washed and carried away by rains and floods to nearby aquatic systems, affecting aquatic biota, especially fish, which serves as a rich source of protein for the teaming population. Fish live and carry out their activities in the water. Consequently, its performance depends directly or indirectly on the quality of the water it lives in (Amarachneke, 2018).

One of the fastest ways to determine the effect of toxicants on fish is through their behavioral response. Because behavior is the first to show adaptations to environmental variables. The behavioral alteration of the test fish was examined during the exposure of endosulfon, dieldrin and heptachlor for 24-96hr; the behavior of alteration is associated with the physiological responses, which are indications of stress (Islam *et al.*, 2021). Hematological parameters serve as biomarkers for environmental monitoring of fish and their health condition during culture because fish are generally so intimately dependent on the quality of the aquatic environment (Nath *et al.*, 2018). They are indispensable tools to assess the impact of pesticides on fish. Anemic response, which includes decreased red blood parameters such as hematocrit (Ht), Hemoglobin Concentration (Hb), and Erythrocyte Count (RBCC) mostly associated with the alterations in the Mean Cell Volume (MCV) and Mean Corpuscular Hemoglobin indices (MCH and MCHC) was observed after exposure to various herbicides: (Fiorino *et al.*, 2018).

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Growth performance indices are one of the important criteria used in measuring fish responses to environmental Stress. Better growth performance indices were recorded in the control group than in the fish exposed to varying concentration of soap effluent concentrations (Akinkuolie *et al.*, 2021). It has been reported that an increase in levels of copper exposure and Ammonia NH₃ causes a toxic syndrome, including growth depression and increased mortality (Mohseni *et al.*, 2014). This study aims to investigate the acute and sub-acute toxicity of the 2, 4-D Dimethyl amine salt on the behavioral, hematological and growth of *C. gariepinus* fingerlings.

MATERIALS AND METHODS

Experimental Fish

A total number of two hundred and fifty (250) fingerlings of *C. gariepinus* ($4g \pm 0.62$ g mean weight, 6.5 ± 0.5 cm total length) were obtained from Navic Fish farm and transported in a well-aerated jerry can half-cut to the Fisheries A Laboratory A, Ahmadu Bello University Zaria. Before the experiment, the fish's mean weight was kept for a two-week acclimatization period. Fish were fed twice a day on commercial diets at a rate of 3% of their body weight.

Source of Herbicide and Stocking for Acute Bioassay

The herbicide used in this study was a 2, 4-D dimethylamine salt solution at 720g/L manufactured by Binhaj Economic Development Area, Weifang, Shandong, China. Registered by Rainbow Agrosiences Co. Ltd CMD Complex, 183. Five nominal concentrations were prepared geometrically: 1.36, 1.42, 1.48, 1.54 and 1.6ml/L, designated as T₁(control) T₂, T₃, T₄, T₅ and T₆, respectively. Fish were exposed in batches of ten (in 20/L of test medium) to varying concentrations of 2, 4-D dimethylamine salt solution with two replicates for each test concentration along with the control sets. Water was replaced every 48 hours, followed by an addition of the required concentration of the test solution. The mortality at 12, 24, 48, 72 and 96 hours was observed and recorded to determine LC₅₀.

Behavioral Studies

After exposure of the fish to various concentrations of the toxicant, observations were carried out on the behavioral and morphological responses of the fish at 12, 24, 48, 72, and 96 hours (Drummond *et al.*, 1986). The opercular ventilation counts and tail fin movement rates were determined, and timing was carried out using a stopwatch at 12, 24, 48, 72, and 96 hours per minute. Three fish were used for the counting per tank, and the average count was taken.

Stocking for Sub-lethal Bioassay

Fraction of 1/5, 1/10, and 1/20 as T₁=0.069, T₂=0.138, and T₃=0.276ml/L, respectively, along with T₀= 0.00 as control, was taken for the sub-lethal test from the results obtained for LC₅₀ from the acute toxicity of 96 hours exposure. The experiment lasted for 8 weeks. Eight (8) plastic aquaria were used with two replicates per treatment. Ten groups of fish were exposed to 3 sub-lethal concentrations of 2, 4-D dimethyl amine salt. The fish were fed pelleted feeds containing 35% crude protein. Two fish per replicate were used biweekly to withdraw blood for hematological studies.

Growth Performance

Fingerlings of *Clarias gariepinus* were fed with commercial feeds. The fish were fed at 3% body weight two times daily, and observation was made. The final weight of the fish at the commencement of the experiment was taken, and the same was at the end of the experiment (Shalaby *et al.*, 2006). The following growth parameters were computed for each treatment using the formulae:

- i. $Final\ weight = W_f - W_i$
- ii. $Mean\ weight\ gain\ (MWG)g = W_f - W_i$ (Pitcher & Hart, 1982)

- iii. Relative growth rate (RGR) = $\frac{MWG}{Wi} \times 100$ (Davies & Ezenwa, 2010)
- iv. Specific growth rate (SPG) = $\frac{\log W_2 - \log W_1}{t(\text{days})} \times 100$ (Brown, 1957)
- v. Average Daily weight gain ($ADWG$) = $\frac{MWG(g)}{t(\text{days})} \times 100$ (Pitcher & Hart, 1982).

Hematological Determination

The blood sample measuring 0.5ml was collected from the caudal vein, 2cm away (MUAC, 2008) after 96hours of acute exposure and sub-lethal on 14th, 28th, 42nd days of exposure of the fish (2 fish from each of the treatment). The blood was transferred directly to a sample bottle containing EDTA to avoid clotting of samples. The following hematological indices were determined:

Erythrocyte Count

Neubauer's improved hemocytometer was used for the counting of red blood cells (RBC) with the help of Hendricks solution to count red blood cells. It was done by using a hemoglobin pipette to collect Blood samples up to 0.5 mark; the cotton wool was used to wipe and adjust the volume to exactly 0.5 mark. The pipette was filled to 101 marks with the diluting fluid and shaken for some minutes to ensure thorough mixing. The diluted suspension of cells was drawn into the Neubauer's chamber hemocytometer. The hemocytometer was placed under the microscope, and the cells within the boundaries of five small squares of the hemocytometer (Hesser, 1960) were counted for each of the treatments with 4mm objectives and x 40 eyepieces of the microscope. The number of cells was multiplied by x 10 and this gave the total number of cells per cubic millimeter (mm^3) of blood (Hesser, 1960).

Packed Cell Volume

The Packed Cell Volume (PCV) was determined according to the method described by Svobodova *et al.* (2008). The blood samples were collected using a syringe and transferred to capillary tubes. The readings were taken using a micro hematocrit reader after being shaken by a centrifuging machine, which is used for both control fish and toxicant fish samples.

Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were derived from the RBCC data and hemoglobin and PCV using standardized methods (Paulpandian *et al.*, 2022). Mean corpuscular parameters were computed using the formulae:

$$MCV = \frac{PVC}{RVC} \times 10$$

$$MCH = \frac{Hb}{RBC} \times 10$$

$$MCHC = \frac{Hb \text{ in } 100 \text{ mg blood} \times 100}{PCV}$$

Data Analysis

Data obtained were subjected to One-way Analysis of Variance (ANOVA). The significance differences between means were determined using New Duncan's Multiple Range Test (DMRT, 1955) in SPSS (version 23) 95% confidence level ($P=0.05$). The Plots for behavioral data (Tail fin beat and Opercular ventilation rates) were created in R version 4.2. The level of significance was determined at a 95% confidence level ($P=0.05$). Minitab version 15's probit regression tool was used to determine LC_{50} .

RESULTS

Behavioral Response of *Clarias gariepinus* to an Acute Exposure to 2, 4-D Dimethyl an Amine Salt

Clarias gariepinus exhibited some behavioral changes when exposed to 2, 4-d dimethyl amine salt herbicide. After the fish were introduced into the tank containing 2, 4-d dimethyl amine salt herbicide at concentrations of 1.36, 1.42, 1.48, 1.54 and 1.6ml/L. the fish exhibited became restless and agitation. Fishes came to the surface of water much more frequently. Treated fish exhibited increased mucous secretion and progressively became sluggish and lethargic. As the time of exposure increased, the fish stood in a vertical position with their heads above the water's surface; eventually, the fish were found dead.

Opercular Ventilation Beat and Tail Fin Beat

The *C. gariepinus* fingerlings exposed to 2, 4-D dimethyl amine salt showed an increase in opercular ventilation and tail fin beat with the increase in the concentrations of the toxicant for 1.36, 1.42, 1.48, 1.54, and 1.6ml/L respectively. The results of opercular ventilation and caudal fin beat mean with different superscripts across treatments are presented in Figures 1 and 2, respectively. The opercular beats and caudal fin beats of the exposed fish to the toxicant at 12 and 24 hours were higher (103, 96, 107, and 100, respectively) compared to (76 opercular frequency and 82 tail fin beats) in the control fish. As the concentration of 2, 4-d dimethyl amine salt increased, the beats of the opercular and tail fin increased up to the 48th hour when the beats became stable. Further duration of exposure led to more decrease in the opercular ventilation and tail fin beat of the fish. By the 96th hour, the opercular ventilation and tail fin beat rates of the exposed fish were significantly ($P < 0.05$) lower than those of the control group.

Mortality Rate of LC₅₀ and Threshold

Figure 1 shows the LC₅₀ mortality rate, which was determined by the antilog of 0.1413ml/L. The acute toxicity of 2, 4-d dimethyl amine salt was toxic to African catfish fingerlings, with an LC₅₀ value of 1.385ml/L (Figure 3).

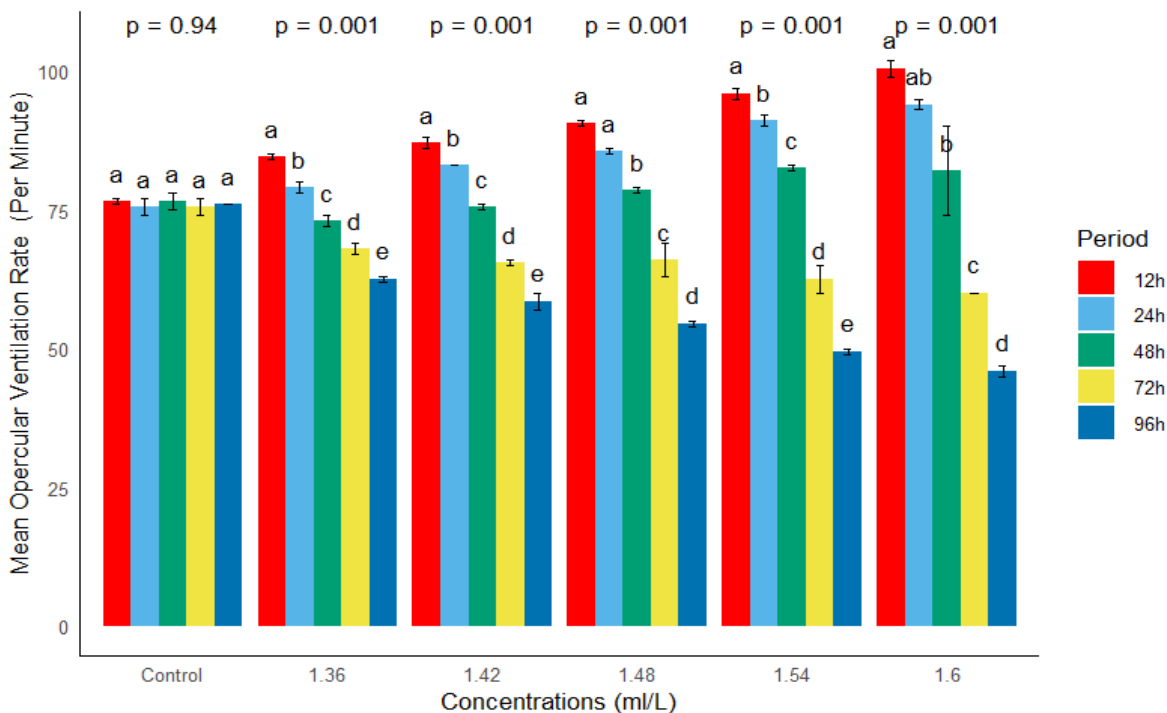


Figure 1: Opercular ventilation rate of *Clarias gariepinus* fingerlings exposed to acute concentration of 2, 4-d dimethyl amine salt.

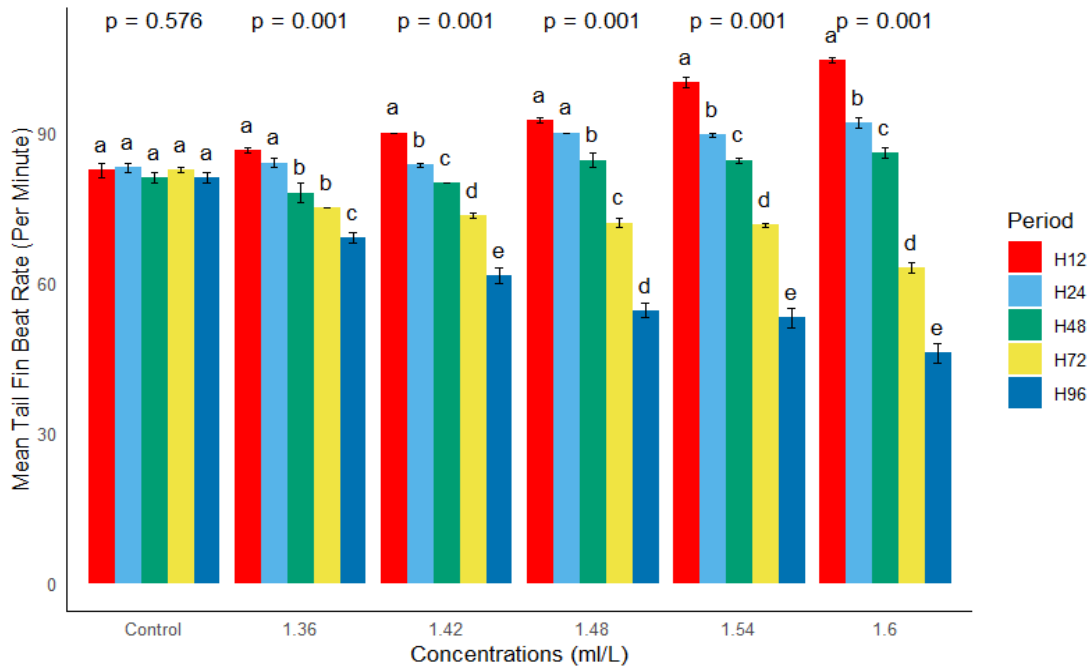


Figure 2: Caudal fin beat of *Clarias gariepinus* fingerlings exposed to acute concentration of 2, 4-d dimethyl amine salt.

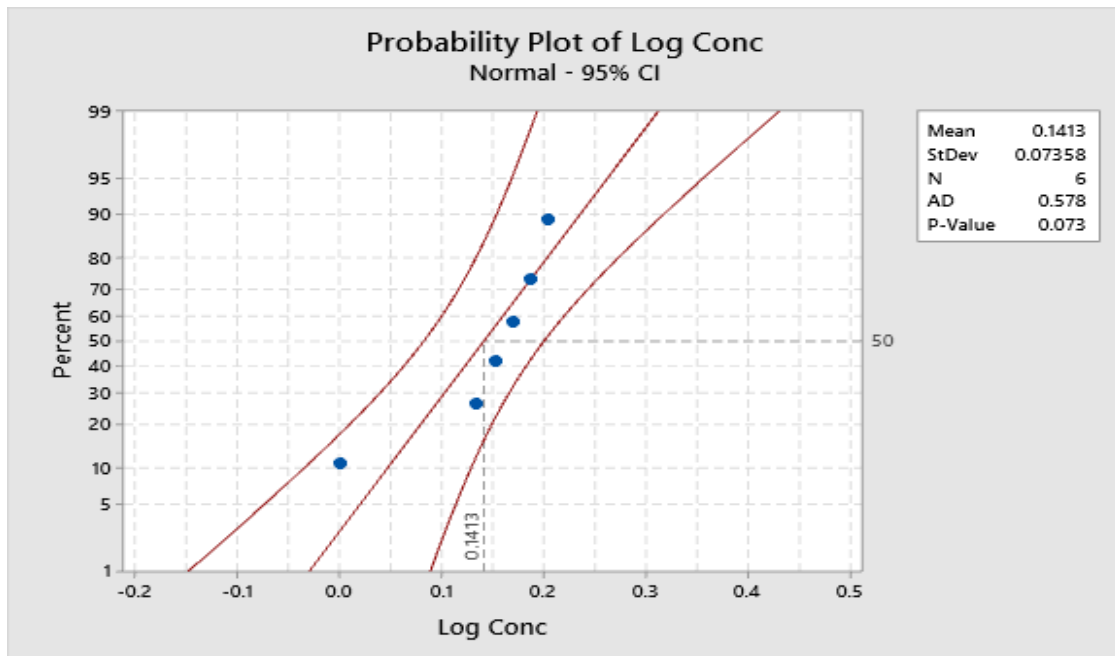


Figure 3: Graph for the 96hrs of exposure to 2, 4-d dimethyl amine salt herbicide on *Clarias gariepinus* fingerlings.

Exposure to the different concentrations of the toxicant significantly affected all growth parameters of the fish compared to the control group (Table 1). Figure 4 shows mean weight gain for the different treatments at intervals of two weeks, which show no significant difference from zero to the sixth week. However, at

week eight, there is a significant difference ($P < 0.05$), with the control gaining more weight compared with the toxicant treatments.

Table 1: Mean Growth of *Clarias gariepinus* Fingerlings Exposed to Sub-lethal concentration to 2, 4-D dimethyl Amine Salt.

Growth Parameters	Concentrations of the 2, 4-D dimethyl Amine Salt ml/L				P value
	0.00	1.36	1.42	1.48	
MW (g)	4.95±0.05	5.35±0.15	5.25±0.05	4.88±0.08	0.056
FW (g)	10.60±0.13 ^a	9.38±0.14 ^b	9.16±0.12 ^b	8.46±0.04 ^c	0.001
WG (g)	5.65±0.08 ^a	4.03±0.29 ^b	3.91±0.07 ^b	3.58±0.11 ^b	0.003
SGR (%/day)	0.006±0.0002 ^a	0.004±0.00003 ^b	0.004±0.00003 ^b	0.004±0.00016 ^b	0.009
MWG (g)	0.56±0.07 ^a	0.40±0.02 ^b	0.39±0.007 ^b	0.36±0.11 ^b	0.003
RGR (%/day)	11.4±0.04 ^a	7.6±0.75 ^b	7.5±0.07 ^b	7.4±0.35 ^b	0.006
ADWG (g/day)	0.01±0.00014 ^a	0.0072±0.0005 ^b	0.007±0.00013 ^b	0.006±0.00021 ^b	0.003

The mean having the same superscript across rows is not significantly different ($P > 0.05$).

Key: IMW= Initial Mean Weight, FM=Final Weight, WG=Weight Gain, SGR= Specific Growth Weight, Relative RGR= Relative Growth Rate, ADWG=Average Daily Weight Gain.

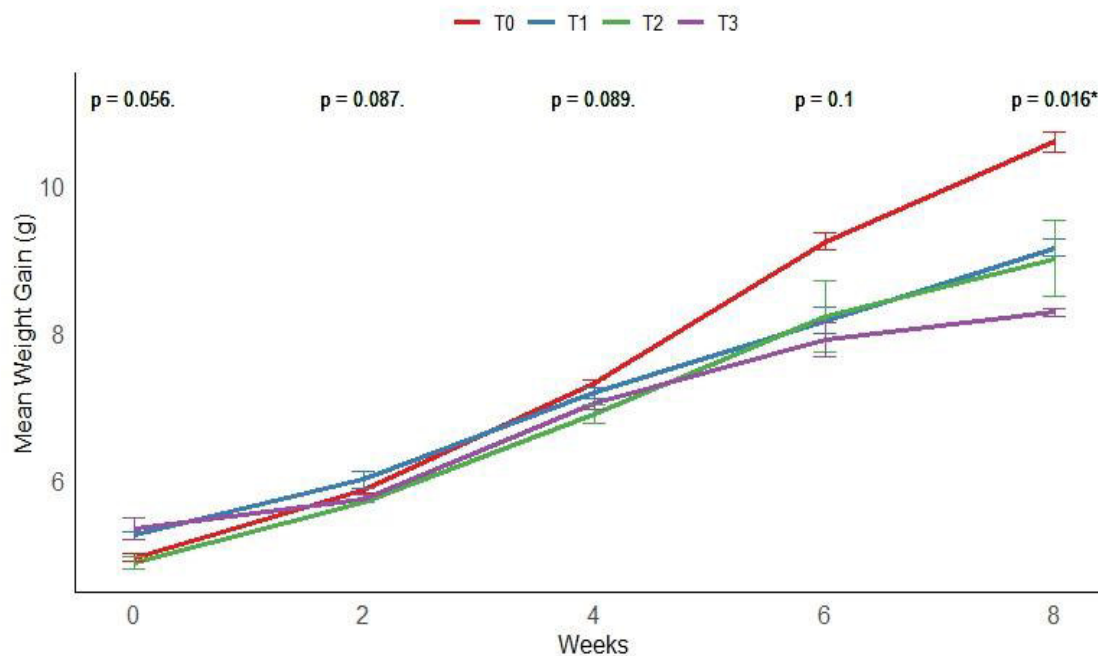


Figure 4. Mean Growth of pattern of *Clarias gariepinus* fingerlings exposed to Sub-lethal Concentration of 2, 4 –D Dimethyl Amine Salt.

Hematological Parameters of *C. gariepinus* Fingerlings Exposed to Lethal Toxicity of 2, 4-D Dimethyl Amine Salt

The acute bioassay on hematological parameters of fish exposed to acute nominal doses of 2, 4-d dimethyl amine salt are presented in Table 2. The RBCC values of 6.9, 5.95, 5.95, 5.55, 5.15, 4.50 ($10^6/UL$) show no significant difference ($P > 0.05$) with 0.00 having the highest value followed by 1.36, 1.42, 1.48, 1.54 and 1.6ml/L concentrations, respectively. This shows that the RBCC of the exposed fish decreased with an increase in concentration. The WBCC values of 4.95, 5.3, 5.2, 5.1, and 6.0 ($10^3/UL$) show no significant difference ($P > 0.05$) compared to a control group with a value of 4.5 ($10^3/UL$). Packed cell volume (PCV) of the exposed fish values of 27.5 and 25.66% show a significant difference ($p < 0.05$) when compared with

the 28.5, 32.5, 34 and 36.5%; as the values decreased, there was an increase in concentrations. There was no significant difference ($P>0.05$) between MCV, MCH, and MCHC values of the fish upon exposure to 2, 4-d dimethyl amine salt compared with the control group.

Hematology Parameters of *C. gariepinus* Exposed to Sub-Lethal Concentrations of 2, 4-D Dimethyl Amine Salt

The sub-lethal bioassay on *C. gariepinus* exposed to sub-acute nominal doses of 2, 4 –D dimethyl amine salt is presented in Table 3. The control group of WBCC, a value of 5.25 (10^6 UL) at week 2, varied significantly from the exposed group value of 6.9 (10^6 UL) at week 6. PCV of the exposed group showed a significant difference when compared with the control group at weeks 2, 4 and 6. MCV of the fish varied significantly ($P<0.05$) at weeks 4 and 6 of the exposed group compared with the control group. RBCC shows a significant difference ($p<0.05$) at weeks 4 and 6 with values of 5.55 and 4.40 (10^3 UL) when compared with the control group of weeks 2, 4, and 6. Only MCH and MCHC did not vary significantly ($P>0.05$) at weeks 2, 4 and 6 when compared with the control group after the fourth and sixth week of exposure. The highest WBCC, 6.90 (10^6 UL), was recorded in the sixth week from 0.296 concentration, RBCC 7.60 (10^3 UL), MCV (97.6 fL), and PCV (42%) from control in the second and sixth week, respectively. The highest (34.8 pg) MCH was recorded in the second week in fish exposed to 0.138 concentration, while the highest (33.9 g/dl) MCHC was recorded in the fourth week in fish exposed in the control group (0.00 and 0.069).

Table 2: Effect of Differential Concentration of 2, 4-D Dimethyl Amine Salt on Hematological Parameters of *C. gariepinus* After 96 hours of Exposure

Hem. Parameters	Concentration of 2, 4-D Dimethyl Amine Salt (ml/L)						P-value
	0.00	1.36	1.42	1.48	1.54	1.60	
RBCC (10^6 UL)	6.9±1.0	5.95±0.45	5.95±0.15	5.55±0.55	5.15±2.50	4.50±0.5	0.168
PCV (%)	36.50±2.0 ^a	34.00±1.0 ^a	32.5±0.5 ^a	28.50±1.0 ^a	27.5±0.5 ^b	25.66±0.5 ^b	0.04
WBCC (10^3 /UL)	4.50±0.5	4.95±0.68	5.30±0.8	5.20±0.3	5.10±0.6	6.00±0.0	0.575
MCV (fL)	82.30±3.0	83.15±4.3	88.9±1.5	86.2±1.2	87.55±0.6	89.8±0.4	0.263
MCH (pg)	29.8±3.0	29.8±3.3	29.10±1.1	32.4±3.4	34.35±4.2	32.75±2.25	0.532
MCHC (g/dl)	30.95±0.4	33.5±00	33.5±95	33.5±95	33.15±0.5	36.5±2.5	0.142

Mean that the same superscript across rows is not significantly different ($P>0.05$).

Table 3: Effect of Differential Concentration of 2, 4 D Dimethyl Amine Salt on Hematological Parameters of *C. gariepinus* after Sub-Lethal Exposure

Parameters	Conc. MI/L	Weeks		
		2	4	6
WBCC (10^6 /UL)	0.00	5.30±0.1 ^b	5.25±0.35 ^b	4.95±0.45 ^b
	0.069	5.20±0.4 ^{ab}	5.85±0.50 ^a	6.00±0.5 ^a
	0.138	6.25±0.50 ^a	6.05±0.2 ^a	6.10±0.80 ^a
	0.276	6.35±0.149 ^{ab}	6.45±0.55 ^a	6.90±0.50 ^a
RBCC (10^3 UL)	0.00	7.60±0.30 ^a	6.6±0.10 ^a	6.25±0.25 ^a
	0.069	6.60±0.40 ^a	5.90±0.10 ^a	5.75±0.049 ^{ab}
	0.138	6.00±0.90 ^a	5.85±0.15 ^{ab}	5.35±0.049 ^b
	0.276	6.30±0.030 ^a	5.55±0.35 ^b	4.40±0.040 ^c
PCV (%)	0.00	39.5±0.99 ^a	39.7±0.099 ^a	42.00±3.0 ^a
	0.069	38.5±0.99 ^a	37.3±1.50 ^{ab}	36.5±0.99 ^b
	0.138	36.7±0.00 ^b	35.2±1.0 ^c	35.0±1.0 ^c
	0.276	36.0±1.0 ^b	35.0±0.99 ^c	34.0±2.0 ^{cd}
MCV (fL)	0.00	97.6±0.40 ^a	90.55±0.15 ^a	91.6±4.21 ^a

	0.069	90.5±24.5 ^a	89.7±3.41 ^{ab}	86.5±7.57 ^b
	0.138	84.3±5.2 ^b	87.4±0.00 ^b	89.6±0.20 ^a
	0.276	86.7±1.25 ^b	84.0±1.0 ^b	83.9±1.12 ^{bc}
MCH (pg)	0.00	33.9±1.9 ^a	31.7±1.5 ^a	29.65±0.45 ^a
	0.069	29.75±0.75 ^a	29.0±1.0 ^{ab}	29.4±3.66 ^{ab}
	0.138	34.8±3.86 ^a	30.1±0.00 ^a	31.3±0.75 ^a
	0.276	30.05±0.5 ^a	31.0±1.5 ^a	31.55±1.45 ^a
MCHC (g/dl)	0.00	32.3±1.7 ^a	33.9±0.45 ^a	32.7±0.70 ^a
	0.069	32.5±0.95 ^a	33.9±0.60 ^a	33.4±0.40 ^a
	0.138	32.8±1.07 ^a	31.3±1.8 ^a	33.65±1.35 ^a
	0.276	33.75±0.25 ^a	32.4±0.8 ^a	31.65±2.557 ^a

The mean with the same superscript across rows is not significantly different (P>0.05).

DISCUSSION

Behavioral Response

The increase in opercular ventilation could be that the fish is trying to adapt to the environment, suppressing toxicant substances by breathing faster or trying to escape toxicants by an increase in caudal fin beat, but a decrease in opercular ventilation and caudal fin beat could be that the fish exhausted its energy. This is in line with the finding of Sharkawy *et al.* (2011), who reported that jumping to the water's surface to gulp air by fish could be attributed to oxygen depletion as a result of pollution caused by herbicides or other toxic chemicals present in the water or irritation caused by dermal contact. The introduction of fish to the test solution usually showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and color fading were prominent after 24 hours exposure of to glyphosate (Ranjan & Kumari, 2022). Fish exposed to higher concentrations of the pesticide showed abnormal behavior and tried to avoid the test water by swimming very fast, jumping and displaying erratic with vigorous jerky movements, faster opercula movement, hyperexcitation, surfacing and gulping of air (Chris *et al.*, 2022).

Hematological Indices

The dose-dependent reduction of red blood cells observed in this study may be due to the reduction of oxygen levels in the experimental aquaria tank, which is in line with the finding of Chen *et al.* (2014), who reported that the reduction of red blood cells is mainly due to hypoxic condition during exposure to stress (lead) which might have destroyed RBC or reduce the rate of its formation. Exposure to herbicide (Atrazine) to African catfish *C. gariepinus* induces anemic condition in the fish due to decrease in total Erythrocyte count (George *et al.*, 2017). Exposure of herbicides chlorpyrifos to *Oreochromis mossambicus* results in anemic conditions due to a significant decrease in hemoglobin (Gayyur *et al.*, 2019). The increased leucocyte count could be a defensive measure taken by the fish to protect its body against the toxic effect of pesticides (Mostakin *et al.*, 2015). Vaiyanan *et al.* (2015) reported a significant increase in total leucocyte Count in common carp *Cyprinus carpio* due to sublethal concentrations of monocrotophos.

Growth Performance

The reduction in growth rate observed in this study could be due to the lower feeding rate of the fish exposed to toxicants or an oxygen reduction in the carrying capacity of the blood, leading to inefficient utilization of assimilated food. Lawee and Imgbian (2017) reported that there is a reduction of growth in *C. gariepinus* fingerlings exposed to sub-lethal concentrations of Bentazon pesticide. The reduction in growth is due to a reduction in the oxygen-carrying capacity of the blood, leading to inefficient utilization of assimilated food or inhibitions of the activities of certain enzymes of the metabolic pathways. It has also been reported that metabolic activities and excretion of toxicants are known to be on the increase in fish at different exposure concentrations. Such an increase requires more energy for homeostatic maintenance than for storage, which could lead to growth reduction (Gbem *et al.*, 2003). Sakala and Musuka (2014) reported that the toxic effect of increased ammonia on tilapia growth rate loss of weight in ammonia treatment is more likely due to the

inhibition of the fish appetite, leading to a significant reduction of feed intake and disturbance in metabolism or an increased activity associated with an attempt to avoid the intoxication. It has been reported that The MWG decreases as the level of NH₃ in water increases, while The FCR of fish is significantly higher ($P < 0.05$) in NH₃ treatments with low concentration (Uchenna *et al.*, 2022).

Conclusions

2, 4- D dimethyl amine salt is toxic to *C. gariepinus* fingerlings at an LC₅₀ value of 1.385 ml/L, eliciting behavioral responses such as restlessness and erratic swimming, opercular beat, tail fin beat, increased gulping of air to cope with the oxygen deficiency. The 2, 4- D dimethyl amine salt at 0.276 ml/L reduced the growth and negatively changed the hematological parameter of *C. gariepinus* fingerlings.

Recommendations

The use of 2, 4-D Dimethyl amine salt-based herbicides should be banned on farmlands near lentic aquatic systems.

REFERENCES

- Akinkuolie, S. O., Adewole, H. A., Ololade, O. D., Lawal, B. M. & Olaleye, V. F. (2021). Assessment of the Growth Performance and Hematological Indices of *Clarias gariepinus* fingerlings Exposed to Soap Effluent. *The Journal of Basic and Applied Zoology*, 82, 56-65.
- Amarachineke, E. F. (2018). -Acute Toxicity of Primextra (Atrazine) on a Freshwater Fish *Heterobranchus bidorsalis*. *Middle-East Journal of Scientific Research*, 26(5), 514-526.
- Audu, B. S., Adamu K. M. & Nonyelu O. N. (2014). Changes in Hematological Parameters Of *Clarias gariepinus* Exposed to Century Plant (*Agave americana*) Leaf Dust. *International Journal of Applied Biological Research*, 6(1), 54-65.
- Brown, M. E. (1957). *Experimental Studies on Growth*. In *The Physiology of Fishes* 1: pp. 361–400.
- Chen, S., Ma K., Wu Y., Ma Y., Qiao H., Fan J. & Wu H. (2014). Dietary Supplementation With Microalgae Enhances the Zebrafish Growth Performance by Modulating Immune Status and Gut Microbiota. *Applied Microbiology and Biotechnology*, 1–16.
- Chris, D. I., Samuel, E. E. & Sokiprim A. (2022). Hematological and Behavioral Response of African Catfish (*Clarias gariepinus*) (Burchell, 1822) Exposed to Sub-lethal Concentration of xylene. *World Journal of Advanced Research and Reviews*, 14(1), 554–565.
- Davis, O. A. & Ezenwa, N. C. (2010). Groundnut Cake an Alternative Protein Source in the Diet of *Clarias gariepinus* fry. *International Journal of Science and Nature*, 1(1), 73–76.
- Drummond, R. A., Russom, C. L. & Gleger, D. L. (1986). Behavioral and Morphological Changes in Fathead Minor (*Pimphales promelas*) as Diagnostic Endpoints for Screening Chemicals According to Mode Of Action; In T.M. Poston & R. Pruddy (eds). *Aquatic Toxicology and Environment Fate*, 9:415-435.
- El-Sharkawy, N. I., Rasha, M., Reda & El-Araby, E. I. (2011). Assessment of Stomp (Pendimethalin) Toxicity on *Oreochromis niloticus*. *Journal of American Science*, 7(10), 568-576.

- Enetinmi, I., Seiyaboh I., Esther B. E. & Sylvester I. S. (2020). Acute Toxicity of *Clarias gariepinus* Fingerlings Exposed to 2, 4-D Dimethylamine Salt. *Journal of Public Health International*, 2641-4538.
- Fioriono, E., Sehenova P., Plhalova L., Blahova J., Svobodova Z. & Faggio C. (2018). Effect of Glyphosate on Early Life Stages: Comparison Between *Cyprinus carpio* and *Danio rerio*. *Environ. Sci. Poll. Res.* DOI 10.1007/s11356-017-1141-5.
- Gbem, T. T., Balogun, J. K., Lawal, F. A., Amune, P. A. & Auta J. (2003). Sub-lethal Effects Of Tannery Effluent on Some Hematological Indices and Growth of *Clarias gariepinus* (Teugels). *Bulletin of Environmental Contamination and Toxicology*, 71: 1200–1206.
- Ghayyur, S., Tabassum, S., Ahmad, M. S., Akhtar, N. & Khan, M. F. (2019). Effect of Chlorpyrifos on Hematological and Seral Biochemical Components of Fish (*Oreochromis mossambicus*). *Pakistan Journal of Zoology*, 51(3), 1047–1052.
- George, A. D. I., Akinrotimi, O. A. & Nwokoma, U. K. (2017). Hematological Changes in African catfish (*Clarias gariepinus*) Exposed to A Mixture of Atrazine and Metolachlor in the Laboratory. *Journal of Fisheries and Science*, 11(3), 48-54.
- Hesser E. F. (1960). *Method for Routine Fish Hematology*. *Progressive Culturist*, 22:164-171.
- Islam, A., Nurul, Amin, S. N., Christopher, L. B., Juraimi, A. & Aziz, A. K. (2021). Determination of Median Lethal Concentration (LC₅₀) for Endosulfon, Heptachlor, and Dieldrin Pesticides to African Catfish (*Clarias gariepinus*) and their impact. *Toxics*, 9 (12), 340.
- Jasmin, J., Rahman, M. M. and Rahman, M. M. (2018). Hematological changes in *Labeo rohita* (H.) Due to Exposure to Pesticides, Difenconazole and Thiamethoxam. *International Journal of Contemporary Research Revision*, 9(1), 20199-20205.
- Kumar, R. S., Sankhla, M. S., Kumar, R., Swaroop, S. & Sonone (2021). Impact of Pesticide Toxicity in Aquatic Environment. *Interface research in Applied Chemistry*, 11(3), 10131–10140.
- Lawee, A. Y. & Imgbian, T. D. (2017). Effects of Sub-Lethal Concentrations of Bentazon Pesticide on The Growth Performance and Some Hematological Indices on Juveniles of *Clarias gariepinus* (Burchell, 1822). *Journal of Fisheries and Aquaculture*, 5(2), 114–121.
- Mohseni, M., Pourkazemi, M. & Baim, S. C. (2014). Effects of Dietary Inorganic Copper on Growth Performance and Immune Response of Juvenile Beluga, *Huso huso*. *Aquaculture and Nutrition*, 20, 547–556.
- Mostakim, G. M., Zahangir, M. M., Mishu, M. M., Rahman, M. K. & Islam, M. S. (2015). Alteration of blood parameters and histo-architecture of liver and kidney of silver barb after chronic exposure to quinalphos. *Journal of Toxicology*, Article ID 415984: 1-8.
- MUAWC (2008). *Blood Collection Guidelines*. Monash University Animal Welfare Committee (MUAWC), 1-10.

- Nath, S., Matozzo, V., Bhandari, D. & Faggio, C. (2018). Growth and Liver Histology of *Channa Punctatus* Exposed to a Common Biofertilizer. *Natural Product Research*. DOI:10.1080/14786419.2018.1428586.
- Paulpandian, P., Beevi, I. S., Somanath, B., Kamatchi, R. K., Paulraj, B. & Faggio, C. (2022). Impact of *Camellia sinensis* Iron Oxide Nanoparticle on Growth, Hemato-Biochemical and Antioxidant Capacity of Blue Gourami (*Trichogaster trichopterus*) Fingerlings. *Biology and Trace Element Research*, pp. 1–13.
- Pitcher, T. J. & Hart, P. J. B. (1982). *Fisheries ecology*. London and Canberra, Croom Helm Ltd. (American Edition): The *Avian Publishing Company Inc.*, Westport, Conn. p. 4.
- Ranjan, K. P. & Kumari, A. (2022). Effects of Herbicide, Glyphosate on Behavior Response and Blood Metabolite of *Clarias batrachus* (Linn.) *International Journal of Fisheries and Aquaculture Studies*, 10(4), 109-112.
- Sakala, M. E. & Musuka, C. G. (2014). The Effect of Ammonia On Growth and Survival Rate of *Tilapia rendalli* in Quail Manured Tanks. *International Journal of Aquaculture*, 4(22), 1-6.
- Salim, A. M., Dauda, A. B. and Yusuf, M. A. (2021). Behavioral Responses of African Catfish, *Clarias gariepinus*, (Burchell, 1822) Juveniles Exposed to Acute Concentrations of Butachlor (Herbicide). *FUDMA Journal of Sciences (FJS) ISSN online: 2616-1370*.
- Shalaby, A. M., Khatab, Y. A. & Abdel-Rahman, A. M. (2006). Effects of Garlic (*Alium sativum*) and Chloramphenicol on Growth Performance, Physiological Parameters and Survival of *Nile tilapia* (*O. niloticus*). *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 12(12), 365–378.
- Svobodova, Z., Kroupova, H., Modra, H., Flajshans, M., Randak T., Savina L. V. & Gela D. (2008). Hematological Profile of Common Carp Spawners of Various Breeds. *Journal of Applied Ichthyology*, 24(1), 55-59.
- Uchenna U. B., Obiahu H O., Zhifeng Y. & Chikeziri C. O. (2022). Evaluating The Effect of Acute Ammonia Toxicity In a Freshwater Ecological Environment on The Growth and Hematological Functioning of *Clarias gariepinus*. *Water Infrastructure, Ecosystems and Society*, 00(0), 1. Doi: 10.2166/aqua.2022.138.
- Vaiyanan, V., Sridharan G., Raveendran, S. & Chairman, K. (2015). Impact of Pesticides on Hematological Parameters of *Cyprinus capio*. *World Journal of Pharmacy and Pharmaceutical Science*, 4(8), 1424-1430.