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Comparative Study of Acute Toxicity of Diquat Dibromide Herbicide Formulation on Fingerling and Juvenile Developmental Stages of *Clarias gariepinus* (Burchell).

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Abstract:

Introduction: Water is an important resource essential for health, household use, agriculture, transportation, ecological as well as many other anthropogenic activities. But as essential as it is, water is also one of the most poorly managed resources, often contaminated with varieties of pollutants, with potential threat to human and aquatic wildlife. These pollutants include those from industrial activities as well as agrochemicals and environmental pesticides including insecticides, fungicides and herbicides that are majorly from agriculture. Currently, the toxicity status of many of these pesticides on wildlife is unknown and this lacuna is important to be filled.

Aims: This study assessed the 96 hour acute toxicity of diquat dibromide against both fingerlings and juvenile stages of *Clarias gariepinus*

Materials and Methods: : Following OECD, (2012) fish toxicity protocol, 96-hour acute toxicity of Diquat dibromide formulation was assessed against African catfish (*Clarias gariepinus*) at both fingerlings and juvenile stages. The exposure concentration ranged from 2.5-11 mgL^{-1}.

Results: The result of this experiment showed that percentage mortality increased relative to concentration at both developmental stages. The 96-hour lethal concentration was 8.66 mgL^{-1} and 2.65 mgL^{-1} for fingerlings and juvenile stage respectively. This means that although Midstream formulation is moderately toxic to both the juvenile and the fingerling stage, the fingerlings is more susceptible to the formulation.

Conclusion: This separate level of susceptibility within the developmental stages could have long term impacts on the population dynamics of this fish species. This result is essential as it shows the importance of selecting appropriate developmental stage for toxicity evaluation. Therefore, application of Midstream formulation should be restricted from aquatic system where this fish species or others with similar physiological and ecological characteristics could be found.

Keywords: **Diquat, herbicide toxicity, differential susceptibility, fingerlings, juvenile.**

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Water is an essential resource for agriculture, transportation and many other anthropogenic activities [1] Despite the importance of water as an indispensable resource, it is the most poorly managed resource in the world [2, 3]. Over the last decade, the contamination of fresh water with a wide range of pollutants has become a matter of great concern, not only because of the threat to public water supply but also the damage caused to aquatic wildlife [4]**.** The agro-pesticide chemicals including insecticides, fungicides, molluscides and herbicides, have become integral parts of agriculture, not just in the developed countries, but also increasingly in the developing countries[2]**.** For instance, the annual global agrochemical usage is estimated at 11.2 billion kg, most of which are herbicides[5, 6]. These pesticides are now one of the most important contaminants in the natural habitat [7,8]. Many of them have demonstrated adverse effects on the environment [9, 10, 11, 12]. It has been estimated that only 1% of the applied pesticides reach the target pests, leaving the bulk of the pesticides (99%) to impact the environment [13, 14].

Pesticides get into the environment through run-offs as well as several other natural and anthropogenic processes [15], while wind can also carry them to other grazing areas, human settlements and undeveloped areas [16], potentially affecting non-target species [17]**.** All these can lead to loss of biodiversity and elimination of key species and health hazards such as cancer, endocrine disruption, developmental abnormalities among many others [18].

The increasing awareness of aquatic contamination and pollution necessitates chemical toxicity tests to extrapolate their safe level that is permissible in the environment [19, 20]. Lethal toxicity (24-96 hours) for various pesticides have been reported for different fish species including organochlorine, organophosphate, Roundup and atrazine [21, 22, 23, 24, 25, 19, 26, 12]. Numerous studies have reported differential responses at different developmental stages of various fish against many pesticides [27].

Midstream formulation is made from Diquat (9, 10-dihydro-8a, 10a-diazonia phenanthrene ion), which is a post-emergent, non-selective contact herbicide and crop desiccant, also used in aquatic weeds control [28, 29]. Diquat first received U.S Federal registration for control of submerged and floating aquatic weeds in 1962 and completed the Registration Eligibility Decision (RED) in 2000 [28]. Diquat is used widely across the United States of America, Europe, Australia and Japan as well as many Southern African countries like Botswana, Mozambique and South Africa [30]. Midstream formulation contains nonyl phenol ethoxylate as surfactant [31, 32].

To date, not much is known regarding the toxicity of Midstream formulation (diquat dibromide) herbicide to catfish *Clarias gariepinus,* particularly at various developmental stages. The catfish are found throughout Africa and the Middle East, and live in freshwater ecosystem, including lakes, rivers and swamps, as well as human-made habitats such as oxidation ponds or even sewage systems. This study therefore assessed the 96-hour toxicity of this herbicide formulation against the *C. gariepinus at* both juvenile and fingerling developmental stages.

2. MATERIAL AND METHODS

2.1. Experimental fish species

C. gariepinus also sometimes called African sharp tooth belongs to the family *Clariidae*, the air-breathing catfishes. They are carnivorous, feeding on a wide variety of prey items, from zooplankton, to small crustaceans to other fish, on commercial systems they have been habituated to an omnivorous feeding behavior [33]. *C. gariepinus* respire bimodally. It is very hardy since it tolerates both well and poorly oxygenated waters. It is widely cultivated and found in water bodies in Nigeria, hence they are widely used as biological indicator in ecotoxicological studies [34].

2.1.1. Source /Maintenance of Test Fish.

A total of 200 each of fingerlings (with mean body mass and length of 3.48 g and 6.38 cm respectively) and juveniles (mean body mass and length of 6.48 g and 8.25 cm respectively) of the African catfish. The mean body mass and body length is the total body mass or length of the 200 fish divided by the 200 numbers of the fish). *C. gariepinus,* were obtained from the fish farm of Agricultural Science Department of Lagos State University of Education, Oto Ijanikin, Lagos state, Nigeria (Table 1). The fishes were transported in well aerated containers to the Ecotoxicology and Endocrine Disruptors' Laboratory at the Department of Zoology and Environmental Biology, Lagos State University, Ojo (6.4628° N, 3.2017° E). The fishes were maintained for one week in several glass aquaria with stocking density of 30 fish/10L aquarium to be acclimated to the laboratory conditions at 24.3ºC and 74% humidity, using charcoal filtered natural well water. The fishes were kept under natural room temperature and photoperiod and were fed once daily with a balanced commercial fish pelleted diet of 45% protein content [35]. Unconsumed feed and faecal materials were suctioned out daily, in order to reduce the bacteria build up and concomitant physiological stress impacts on the fishes. The feeding of the fishes was discontinued 24 hours prior to the exposure. The experiment was conducted in line with aquaculture water quality management guidelines and practice [36].

2.2. Test chemical

The herbicide: Midstream (373 g/L) (diquat dibromide) (Syngenta Ltd, South Africa).

2.3. Exposure Methodology

Following Standard fish toxicity protocol [37], 96-hour acute toxicity was conducted using static renewable system. The range finding test was carried out prior to determining the concentrations of the test solution for definitive test. In the test, a set of 10 healthy fishes were randomly exposed to graded concentrations of the herbicide formulation as well as the control. The mean body mass and mean body lengths of the fishes were taken prior to the exposure (Table 1). The exposure was conducted using 10-litre tanks containing 5 L of aerated borehole water. The test solution was replaced every 24 hours to counter-balance the decreasing concentrations of the herbicide formulation. The experiment was set in duplicate and mortality was monitored every 12 hours. The behavioral response of the test organisms to toxic stress was also observed and recorded. Only mortality less than 10% in the control was accepted for the experiment. The fishes were confirmed dead when they failed to respond to probing stimulus. The LC_{50} of the herbicide formulation was determined using USEPA Probit 1996 software version 1.5 [38].

Table 1. The mean mass and length of exposed *C. gariepinus*

Developmental Stages	**Exposure Concentration (mg/L)**	**Mean Body Mass (g)**	**Mean Body Length (cm)**
Fingerlings	0, 2.5, 3.0, 3.5, 4.0, 4.5	3.48 ± 0.05 g	6.38 ± 0.07 cm
Juveniles	0, 7, 8.5, 9, 10, 11.	6.48 ± 0.04 g	8.25 ± 0.05 cm

2.4. Exposure Concentration

Based on the initial toxicity test, definite exposure concentrations of 7.0, 8.5, 9.0, 10.0, and 11.0 mgL^{-1} were adopted for the fingerling developmental stage, while final exposure concentrations of 1.0, 3.0, 3.5, 4.0, and 4.5 mgL^{-1} were adopted for the juvenile developmental stage (**Table 1**).

2.5. Data Analysis

The mortality data collected were used in calculating the median lethal concentration at 96 hours using Probit USEPA software version 1.5

3. RESULTS AND DISCUSSION

3.1. Behavioral Responses

The formulation induced behavioral and morphological responses including lateral/upward body-bending, increased mucus secretion on their body surface, erratic/spiral swimming, darting behavior, and respiratory distress.

3.2. Morphological Alteration

Bleached body with lesion was observed in the fingerlings at higher exposure concentrations. The test fish also showed peculiar bloated lungs and stomach (Figure 1 a-c). The bloated lungs and stomach were also accompanied by ulceration and bleeding (Figure 2).

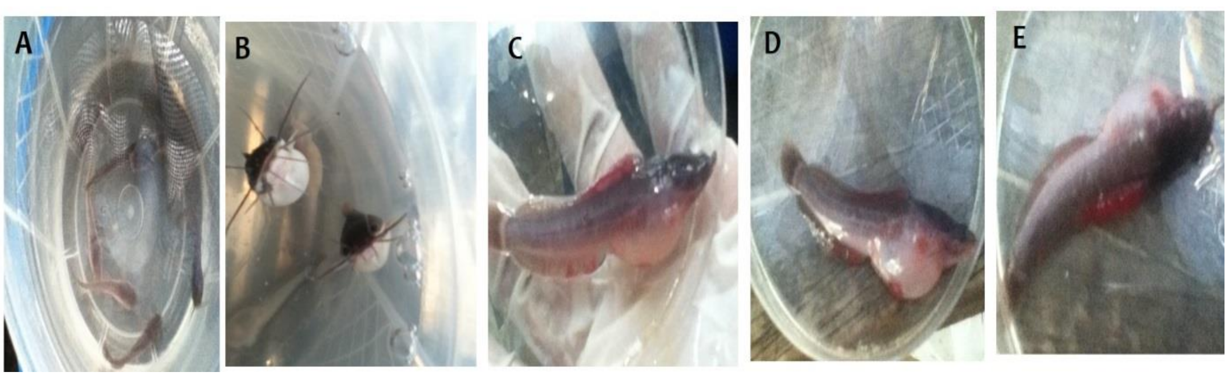

Figure 1: Fish with swollen gut (B) compared to the control (A), with the graduated degree of ulceration and bleeding(C-E) after exposure to Midstream formulation for 96 hours.

3.3. Acute Toxicity

3.3.1. Fingerlings developmental stages

The observed % mortality of *C. gariepinus* fingerlings increased relative to the concentration of Midstream formulation. The 96-hour % mortality which was 30% at 7 mgL^{-1} increased to 40% at 8.5 mgL^{-1} before increasing to 60-90% at 9-11 mgL^{-1} concentrations (Table 2). The percentage mortality data then produced 96-hour LC_5, LC_{10} and LC_{50} of 5.17 mgL^{-1}, 6.51 mgL^{-1} and 8.66 mgL^{-1} (Table 3).

Table- 2- Percentage mortality of fingerling developmental stage of *C. gariepinus* against Midstream formulation at 24-96 hours

Conc/Hour	24 hrs.	48 hrs.	72 hrs.	96 hrs.
7.0	0	10	20	30
8.5	0	10	20	40
9.0	0	0	40	60
10.0	0	0	50	70
11.0	0	10	60	90

Table 3- Estimated LC values and confidence limits of Midstream formulation against fingerlings developmental stage of *C. gariepinus*

Point LC	Exposure Concentrations	95% Confidence Limit Lower- Upper
1.0	5.162	3.258- 6.413
5.0	6.006	3.273- 7.078
10.0	6.511	3.984- 7.471
50.0	8.656	7.604- 9.476
85.0	10.899	9.805- 14.960
90.0	11.509	10.249- 17.209
99.0	14.517	11.978- 29.958

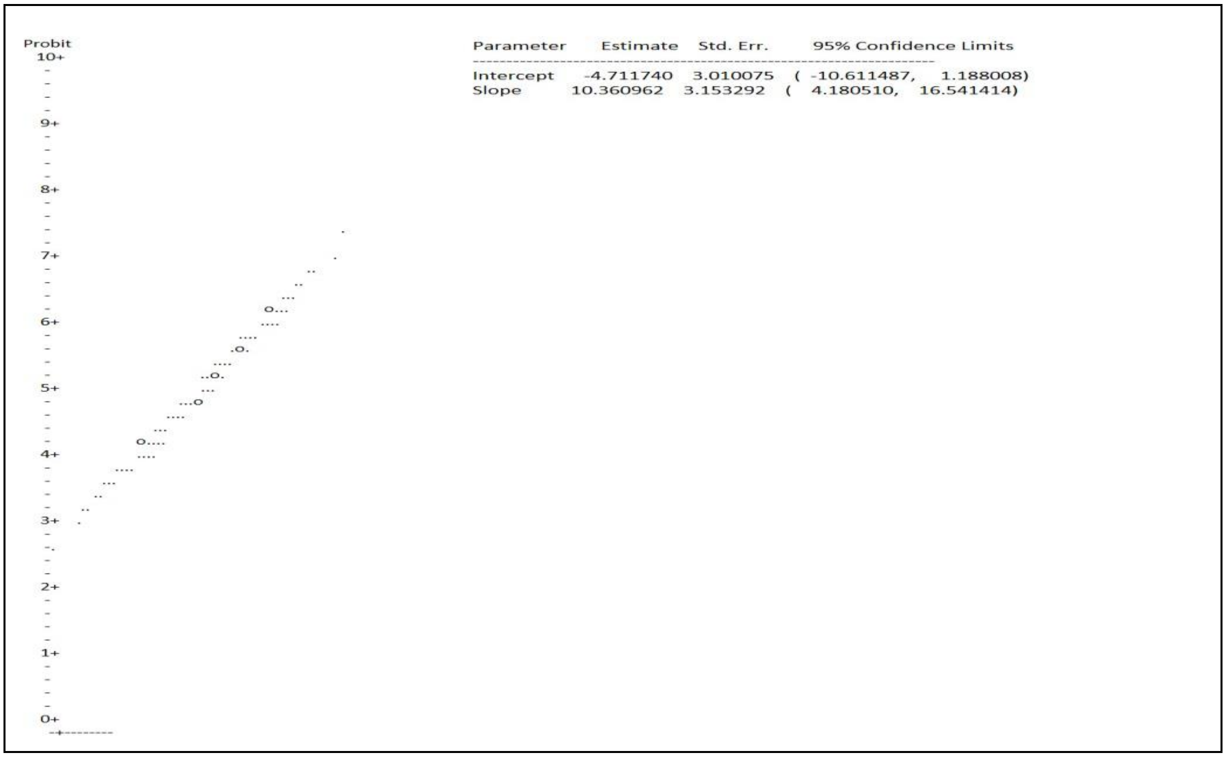

Figure 2: 96 hours LC $_{50}$ for Midstream formulation on *Clarias gariepinus* fingerlings with the estimated LC/EC values and confidence limits

3.3.2. Juvenile Developmental Stage

The percentage mortality of juvenile developmental stage increased with the concentration. At 96-hour, the mortality which was 10% at 1.0 mgL^{-1} increased to 50% at 3.0 mgL^{-1} before increasing to 60-80 % mortality at concentrations of 3.5 mgL^{-1} and 4.5 mgL^{-1} (Table 4). The percentage mortality data at 96-hour for the juvenile developmental stage then produced estimated LC_5, LC_{10} and LC_{50} of 0.84 mgL^{-1}, 1.08 mgL^{-1} and 2.65 mgL^{-1} respectively (Table 5)

Table- 4- Percentage mortality of Juvenile stage of *C. gariepinus* at 24-96 hours

Conc	24- hour	48- hour	72- hour	96-hour
1.0	0	0	10	10
3.0	10	20	40	50
3.5	10	30	40	60
4.0	20	50	60	80
4.5	30	60	70	80

Table 5- Estimated LC values and confidence limits of Midstream formulation against Juvenile developmental stage of *C. gariepinus*

Point LC	Exposure conc.	95% Confidence Limit Lower- Upper
1.0	0.520	0.031-1.062
5.0	0.837	0.102-1.444
10.0	1.079	0.194-1.708
50.0	2.645	1.635-3.483
85.0	5.459	4.018-14.163
90.0	6.480	4.564-21.493
99.0	13.453	7.461-133.821

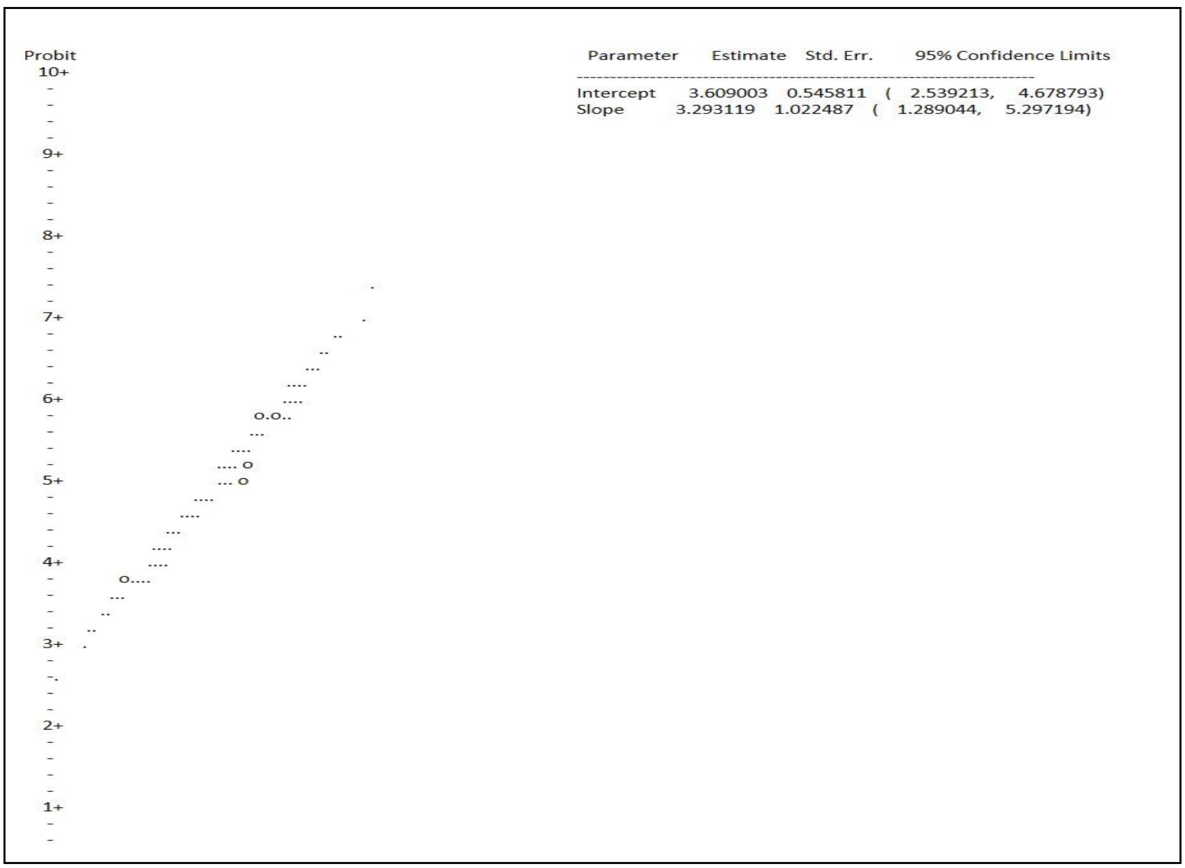

Figure 3: 96-hour LC_{50} for Midstream formulation on *C. gariepinus* juvenile with the estimated LC/EC Values and Confidence Limits.

4. Discussion

Agriculture has become strongly dependent on the use of various chemical pesticides, which aids in pest and weeds management. These pesticides are contributing to the deterioration of water and soil resources [39], potentially impacting environmental health and thereby raising concern on their non-target impacts [40, 41]. The presence and widespread of agrochemicals in aquatic systems around the world for example, has been proposed as a contributing factor to biodiversity decline [42]. Herbicides for example has been recognized as a major source of water pollution with potential harmful effect on wildlife [43]. Therefore, toxicity effects, especially at embryonic development and juvenile life cycle phases, is becoming a concern.

4.1. Fingerlings

Using the USEPA toxicity classification, the current 96-hr LC 50 of 8.66 mgL^{-1} is moderately toxic to the fingerlings of *C. gariepinus* compared to other formulations of diquat. The 96-hour LC_{50} value obtained for fingerlings in the present studies (8.66 mgL^{-1}) was higher than 96-hour LC_{50} obtained for Walleye fish (0.74 mgL^{-1}) and the small mouth bass fish (4.9 mgL^{-1}) with another commercial formulation of diquat [44]. This 96-hour LC_{50} value (8.66 mgL^{-1}) of this formulation at *C. gariepinus* fingerlings developmental stage was however lower than the 96-hour LC_{50} values obtained for fingerlings developmental stage exposed to Roundup (19.58 mgL^{-1}) [45], atrazine (10.2 mgL^{-1}) and mesotrione (532.0 mgL^{-1}) [46]. This means that the midstream formulation is more toxic to the fingerling developmental stage of *C. gariepinus* compared to Roundup, Atrazine and mesotrione formulation.

4.2. Juvenile Stages

The 96hour LC_{50} value obtained for this formulation at juvenile developmental stage of *C. gariepinus* is 2.645 mgL^{-1}, is moderately toxic. This is lower than the reported value obtained for tihan formulation (8.8 mgL^{-1}) and Flubendiame (active ingredient) (7.7 mgL^{-1}) [47] as well as Buprofezin (4.4.92 mgL^{-1}) [48] at the same developmental stage. This result showed that midstream formulation is more toxic to the juvenile stage of this fish than tihan formulation**,** flubendiamide and Buprofezin formulations. In contrast, the current 96-hour LC_{50} value of the Midstream formulation against Juvenile stage is higher than those of thionex formulation (Endosulfan) (0.22 mgL^{-1}) **[**47], Cypermethrin (0.06 mgL^{-1}) [49] and Roundup formulation (0.295 mgL^{-1}) [50]. This indicates that Midstream formulation is less toxic to the juvenile stage of *C. gariepinus* than the thionex**,** Cypermethrin and Roundup formulations.

4.3. Differential Response in Developmental Stages

The 96-hour LC_{50} value of this formulation to the fingerling stage (8.66 mgL^{-1}) is greater than that of the juvenile developmental stage (2.645 mgL^{-1}). This result means that the fingerling stage of *C. gariepinus* is less susceptible to Midstream formulation than the juvenile stage. This observation is similar to the result of Agbohessi *et al.* [47], (when the fish were exposed to Tihan, flubendiamide, spirotetramat and thionex) and Marimuthu *et al* [48], where they observed that fingerlings stages are less susceptible than the juvenile stages (when the fish were exposed to Buprofezin)

That the Midstream formulation shows higher toxicity to the juvenile than the fingerling means that there is a differential response between the developmental stages and that the juvenile stage must be the basis for any ecological protection for this species in order to protect this fish.

4.4. Behavioral and Morphological alterations

The various behavioral responses observed in this study including lateral and upward body-bending, erratic swimming and darting behavior are symptoms of acute physiological stress from the formulation, particularly the toxic attack on the respiratory epithelium, leading to reduction in gills function and depression in respiratory capacity [35, 51,52, 53, 54]. According to Harlt *et al.,* [55], changes in permeability of the gills due to xenobiotic depressed it functions, leading to low oxygen absorption and depressed metabolism. The mucus secretion for example, is usually a response to a chronic irritation on the skin of the fish [54].

The observed lesions and bleached body revealed severe impacts already affecting deep into the body tissues, causing mucosa hemorrhage. This indicates a serious interaction of the formulation on the body cells tissues, and damaged the nerve tissues [56]. The observed bloated neck and stomach also called gastric edema is also a symptom of acute impact on the kidney and liver [56]. This bloated stomach is a symptom of acute irritation to the intestine and generalized symptoms of hepatoxicity and nephrotoxicity of the formulation, which must have depressed the functionality of these organs [56, 57].

5. CONCLUSION

It can be concluded that the Midstream formulation is moderately toxic to the African catfish in a concentration dependent manner. Hence, precautions must be taken where it becomes highly necessary to be used in any aquatic habitat, where this fish species or others with similar physiological and ecological characteristics are found. This study also showed that the juvenile stage is more susceptible to the toxicity of the Midstream formulation than the fingerlings stage. This is important to be taken into consideration in the environmental assessment of other pesticides. Further studies should be carried out on the mechanism of oxidative stress pathway of the diquat on *Clarias gariepinus*.

COMPETING INTERESTS

Authors have declared that no competing interests of any kind exist

AUTHORS' CONTRIBUTIONS

Oluwaseun Babalola and Oluwatobi Amosu designed the study, performed the statistical analysis Oluwaseun Babalola wrote the protocol, and wrote the first draft of the manuscript. Oluwatobi Amosu managed the analyses of the study. 'Loveth Ibrahim managed the literature searches and performed most of the experiment. All authors read and approved the final manuscript."

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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