Original Research Article

Evaluation of SharpshooterLethal and Morphometric Indices Effects in *Clariasgariepinus*

Comment [JI1]: Title is incomplete

ABSTRACT

Aim

The present study evaluated the lethal and morphometrics indices effects in *Clarias gariepinus.*

Comment [JI2]: Lethal or morphometric indices for what?

Study Design

This study employs experimental design and statistical analysis of data and interpretation.

Comment [JI3]: Is this study design???

Place and duration of studies

This study was carried out in Applied Biology Special Laboratory Agbani, Enugu State University of Science and Technology Enugu State (ESUT), Enugu State Nigeria. It lasted thirty days.

Methodology

The effect of sharpshooter on the physicochemical parameters of the water used for the study was analysed using standard methods. The 96h LC_{50} value estimated by Probit Analysis was $0.03mgl^{-1}$. Based on the 96h LC_{50}, the sublethal concentrations of sharpshooter ($1/10^{th}$ of 96h $LC_{50,}$ and $1/5^{th}$ of 96h LC_{50}= 0.01mg/L, 0.03mg/L). The morphometric indices especially hepatosomatic index (HSI) and condition factor (K) were also estimated. Using standard methods.

Comment [JI4]: Very poor writing

Results

The physico-chemical parameters of the test water showed no significant difference ($p > 0.05$) compared with the control. Mortality caused by the pesticide increased with increase in concentration. Mortality rate increased with increase in concentration with the highest recorded 0.05 mgl^{-1} at 96h (90% (27 fishes out of 30 fishes). The safe levels determined for the pesticide showed some variations. Whereas there was no significant difference ($p > 0.05$) between 0.01 MgL^{-1} treatments and control, 0.03 MgL^{-1} caused a significant decrease ($p < 0.05$) in HIS compared with control. Similarly, sharpshooter treatment caused a duration dependent significant increase ($p < 0.05$) at day 15. The treatment with sharpshooter caused concentration and duration significant increase ($p < 0.05$) in condition factor (K) compared with control.

Comment [JI5]: Poorly described

Conclusion

This study has demonstrated that sharpshooter is toxic to *Clarias gariepinus* even at low concentrations. Therefore, the use of this pesticide in the environment especially farm lands and areas close to aquatic environment should be applied with caution to avoid the risk of contamination.

Key words: Sharpshooter;mortality; morphometric; *Clarias gariepinus*

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INTRODUCTION

The growing demand for increased food production to meet the need for the ever increasing global population has sophisticated agricultural technology in which pesticides especially insecticides play a crucial role. The aquatic living resources are very vulnerable to herbicides contamination as run-offs from farms and industries end up in water bodies [1]. Stability or variations in physicochemical parameters in water bodies' depends on human activities and the analyses of these parameters is useful for assessing the vulnerability of the water body and the organisms inhabiting there [2].

Sharpshooter is a broad spectrum pesticide consisting of both cypermethrin and profenofos in a formulation (profenofos 40 + cypermethrin 4 EC). *Profenofos* is a persistant and toxic organophosphorus insecticide widely used in agriculture for crop protection and pest control, thus marketed for these purpose [3-4]. Cypermethrin is a synthetic pyrethroid that has found wide acceptability. It is extensively used in agriculture and forestry because of its high activity against a broad spectrum of insect pest [5]. Nevertheless cypermethrin has been found to be highly toxic to fish [6] even in very low concentrations varying from 0.2 to 2.2 µg/L in 96hr [7]. Generally fishes exposed to toxicants have higher average concentration of bilirubin than ones not exposed [8].[9] highlight the importance of evaluating growth response and oxidative stress in commercially important fish species.
African sharp tooth catfish *Clariasgariepinus* is a typical air-breathing catfish with scaleless bony elongated body with long dorsal and anal fins and a helmet like head. According to [10], it is probably the most widely distributed fish in Africa. They have an ubquitious distribution in rivers, streams, ponds, dams, and lakes in Africa [11]. They are important commercial fish, widely consumed and cheap source of animal protein for low-income earners. The present study evaluated the effects of sharpshooter on the oxidative stress biomarker of *C.gariepinus*.

Comment [JI6]: ?????

Comment [JI7]: I did not find anything about oxidative stress biomarker in this manuscript.

Comment [JI8]: Background of this study is not properly addressed

MATERIALS AND METHODS

Procurement of fish specimen and test chemical

A total of 90 juveniles of *C. gariepinus* were procured from Sacen Fish Farm, Enugu, ... and transported in well aerated 500 litres capacity aquaria tanks to Applied Biology Special Laboratory Agbani, Enugu State University of Science and TechnologyEnugu State (ESUT), Enugu State Nigeria. The experimental fish were acclimatized for two weeks under laboratory condition, fed with top feed (a commercial feed) daily at 3 % body weight. Fecal matter and other waste materials were siphoned off and water was changed daily to reduce ammonia content in the water. Dead fishes were removed with forceps to avoid possible deterioration of the water quality. Ethical clearance was obtained from the Fishery Department, Ministry of Agriculture and Natural Resources committee on experimental animal care (MANR/FD/2017/EC101).Commercial formulation of profenofos 40g and cypermethrin 4g, with trade name "sharpshooter" supplied by West African cotton Ltd.,Lagos Nigeria with CAS NO- 41198-08-7 and 52315-07-8 respectively were purchased in agrochemical shop in Ogbete Main Market Enugu.

Comment [JI9]: No indication of nutritional composition

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Determination of water quality parameter
Water quality parameters such as temperature, pH and dissolved oxygen were checked by direct reading methods using thermometer, pH meter and dissolved oxygen meter [12].

Acute toxicity test
The test was conducted using a semi-static bioassay in 40litre glass aquaria (60x30x30cm). In the range finding test, the percentage mortalitiesof 0% and 100% lie between 0.01mg/l and 0.05mg/l. Therefore the definitive test was conducted consisted five concentrations of sharpshooter (0.01, 0.02, 0.03, 0.04, 0.05mg/l).During the exposures, each concentration were set in triplicate. Juveniles of *Clarias gariepinus* were randomly exposed to different concentrations of sharpshooter. Another set of juvenile fish were simultaneously maintained in water without test chemical - control. Precaution was taken in the stocking of the fish by dropping them gently into the plastic aquaria. The experiment lasted for 96 hours (4 days). After 48 hrs of exposure, the test solution was changed so as to counter-balance the decreasing pesticide concentration.
The median lethal concentration (LC_{50}) value was determined following the probit analysis method described by [13].

Determination of safe levels
The Safe levels of the test pesticidewere estimated by multiplying the 96 hr LC_{50} with different application factors (AF) and was based on [14-19].

Determination of sublethal concentration
The 96h LC_{50} values of sharpshooter on *C. gariepinus* was 0.03mg/l following the probit analysis method as described by [13]. Based on the 96h LC_{50} value, the test concentration of sharpshooter was exposed to sublethal concentration (SL-1; $1/10^{th}$ of 96h $LC_{50,}$ and $1/5^{th}$ of 96h LC_{50} = 0.01mg/l, 0.03mg/l). Ninety fishes were exposed to different sublethal concentrations and a control. Each treatment group were further randomized into three replicates of 10 fishes per replicate in 10 litres of water. The exposure lasted for 15days during which the fish were fed with small quantity of food approximately 1% of total body weight about an hour before the test solution was renewed to avoid catabolism and subsequent mortality. On each sampling day (1, 5, 10 and 15), three fishes from each triplicate experiment including control were sacrificed.

Determination of morphometric indices
The body weight and standard length of each fish were determined after each exposure interval. Thereafter, the liver dissected out, weighed so as to calculate the hepatosomatic index (HSI) and condition factor (K). The indices HSI and K were calculated according to according to[20].

$$HSI = \frac{Liver\ weight}{body\ weight}\ x\ 100$$

$$K = \frac{Body\ weight}{Total\ length\ (cm)}\ x\ 100$$

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Statistical analysis
The data obtained from the experiment were statistically analyzed using SPSS version 22. The data were subjected to two-way analysis of Variance (ANOVA) at significance difference of 5% probability level while Duncan multiple range test was used to determine the differences among treatment groups.

RESULTS AND DISCUSSION
Water quality parameters and percentage mortality rate of juveniles of *C. gariepinus* exposed to different concentrations of sharpshooter for 24, 48, 72 and 96 hours exposure period
The physico-chemical characteristics of the test water is shown in Table 1. The pH of water varied within 8.45-8.60 in the treatments.The water temperaturevalues ranged from 25.54^{o}C – 25.81^{o}C in the treatment. The dissolved oxygen varied within 5.0-5.5. Stability or variations in physicochemical parameters in water bodies' depends on human activities and the analyses of these parameters is useful for assessing the vulnerability of the water body and the organisms inhabiting there [2].

Table 2 showed the percentage mortality juveniles of *C. gariepinus* exposed to sharpshooter examined at different exposure periods (24, 48, 72 and 96 h) depending on different concentrations. The pesticide concentration of 0.05 mgl^{-1} at 96h exposure recorded highest mortality of 90% (27 fishes out of 30 fishes) while the least value at 0.01 mg^{-1}at 96h exposure recorded the lowest mortality of 30% (9 fishes out of 30). The control recorded 0% mortality. Mortality rate increased with increase in concentration. The 96h LC_{50} of 0.03 mg/l obtained for sharpshooter was lower than 0.38 and 1.25 mg/l reported for *O. niloticus* exposed to butachlor [21], respectively. Also, it is lower than 0.07 mg/l reported by [22] when *O. niloticus*was exposed to organophosphate commercial formulation pesticide. The 0.03 mg/l LC_{50} in 96h obtained for sharpshooter in the present investigation indicates that the pesticide was very toxic to *C. gariepinus* juveniles. The toxicity of the pesticide was both exposure duration and concentration dependent, thus accounting for differences in $LC_{10\text{-}90}$ values obtained at different concentrations and durations of exposure. The toxicity of compounds to organisms has however been known to be dependent on concentration, pH, temperature, developmental stages and exposure periods [23].

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Table 1.ThePhysico-Chemical Parameters of the Experimental Water Exposed to Different Concentration Levels of Sharpshooter

S/N	Treatment (mg/L^{-1})	Temperature ^{o}C	DO (mg/l)	pH
1	Control	25.00±0.05	5.00±0.00	8.04±0.01
2	0.01	25.54±0.05	5.00±0.00	8.45±0.05
3	0.02	25.68±0.05	5.10±0.00	8.45±0.01
4	0.03	25.70±0.05	5.30±0.03	8.55±0.15
5	0.04	25.75±0.05	5.40±0.03	8.57±0.14
6	0.05	26.81±0.05	5.50±0.01	8.60±0.17

DO = Dissolved Oxygen

Table 2. Percentage Mortality Rate of Juveniles of *Clariasgariepinus* Exposed to Different Concentrations of Sharpshooter for 24, 48, 72 and 96 Hours Exposure Period

Conc. (µg/L)	Total Death	Survival / % mortality	Mortality			
			24 h	48 h	72 h	96 h
Control	0	100(0)	0	0	0	0
0.01	9	70(30)	0	3	3	3
0.02	11	63(37)	1	3	4	5
0.03	15	50(50)	2	3	5	5
0.04	20	33(67)	3	3	6	8
0.05	27	10(90)	3	6	9	9

Treatment size (n = 30)

Safe levels estimation

The safe levels were estimated following different methods (Table 3).In the present study, the safe levels determined for the pesticide showed some variations. However, due to large variation in safe levels as determined by different methods, the estimates of safe levels cannot be guaranteed. The estimated safe levels obtained for sharpshooter in *Clarias gariepinus* in the present study, as calculated by multiplying the 96hr LC_{50} with application factor (AF) as recommended by different methods, varied from 3.00×10^{-3} to 3.00×10^{-7} mgl^{-1}. However, the large variation in safe levels determined by various methods has resulted in controversy

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over its acceptability [24-25]. Dependence on LC_{50} values could be a notable weekness in determining AF.

Table 3. Estimated Safe Levels of Sharpshooter for *C. gariepinus* after 96 Hours

Pesticides	96h LC_{50} (mg/L^{-1})	Method	Application Factor	Safe Level (mg/L^{-1})
Sharpshooter	0.03	Hart et al (1948)	-	1.875×10^{-03}
		Sprague (1971)	0.1	3×10^{-03}
		CWQC (1973)	0.01	3×10^{-04}
		NAS/NAE (1973)	0.01 – 0.00001	3×10^{-03} – 3×10^{-07}
		CCREM (1991)	0.05	1.5×10^{-03}
		IJC (1977)	5% of 96h LC_{50}	1.5×10^{-03}

Comment [JI10]: Are these methods?

Lethal concentration (LC_{50}) of sharpshooter pesticide depending on exposure time for *C. gariepinus*

Table 4 showed the lethal concentration of sharpshooter in *C. gariepinus*. The LC_{50} values with 95% confidence limits of different concentrations of sharpshooter were 0.05 (0.04-0.05), 0.04 (0.04-0.05), 0.03(0.03-0.04) and 0.03 (0.03-0.04) for 24h, 48h, 72h, and 96h respectively. This showed that as the exposure time increases from 24h to 96h, the median lethal concentration decreases.

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Table 4. Lethal Concentration of Sharpshooter Depending on Exposure Time for Juvenile of *C. gariepinus*

Pesticides	Lethal concentration	Exposure Time (Hours) 24	48	72	96
Sharpshooter	LC10	0.03 (0.02– 0.04)[a]	0.03 (0.02– 0.03)[a]	0.03 (0.02– 0.04)[a]	0.02 (0.01– 0.02)[a]
	LC20	0.04 (0.03– 0.04)[a]	0.03 (0.02– 0.04)[a]	0.04 (0.03– 0.04)[a]	0.02 (0.02– 0.03)[b]
	LC30	0.04 (0.03– 0.04)[b]	0.03 (0.03– 0.04)[b]	0.04 (0.03– 0.04)[b]	0.02 (0.02– 0.03)[b]
	LC40	0.04 (0.04– 0.05)[b]	0.04 (0.03– 0.04)[a]	0.04 (0.04– 0.05)[b]	0.03 (0.02– 0.03)[a]
	LC50	0.05 (0.04– 0.05)[a]	0.04 (0.04– 0.05)[a]	0.05 (0.04– 0.05)[a]	0.03 (0.03– 0.04)[a]
	LC670	0.05 (0.04– 0.06)[a]	0.05 (0.04– 0.06)[a]	0.05 (0.04– 0.06)[b]	0.04 (0.03– 0.05)[a]
	LC80	0.06 (0.05– 0.04)[a]	0.05 (0.05– 0.07)[b]	0.06 (0.05– 0.04)[a]	0.05 (0.04– 0.06)[a]
	LC90	0.07 (0.06– 0.12)[a]	0.06 (0.05–0.07)[b]	0.07 (0.06– 0.12)[a]	0.06 (0.05– 0.08)[a]

Determination of morphometric indices

The hepatosomatic index (HSI) of juveniles of *Clarias gariepinus* exposed to sharpshooter.

The hepatosomatic index (HSI) is shown at Figure 1. Whereas there was no significant difference ($p > 0.05$) between $0.01MgL^{-1}$treatments and control, $0.03MgL^{-1}$caused a significant decrease ($p < 0.05$) in HIS compared with control. Similarly, sharpshooter treatment caused a duration dependent significant increase ($p < 0.05$) at day 15.

The condition factor (k) of juveniles of *Clarias gariepinus* exposed to sharpshooter

The condition factor (K) in Figure 2. The treatment with sharpshooter caused concentration and duration significant increase ($p < 0.05$) in condition factor (K) compared with control.

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Morphometric indices serves as exposure index to environmental contaminants. The condition factor, a somatic biomarker is indicative of health and reflects feeding conditions as well as energy consumption and metabolism. Liver is the metabolic organ, it is a target for the metabolism in the fish body, the liver index (HSI) is a useful biomarker detect hazardous effects of the environmental stressors [26]. In sharpshooter, there were significant increase ($p<0.05$) in the HSI of the exposed fish as compared to the control. Increase in HSI have been reported in *Oreochromis niloticus* exposed to paraquat herbicide® [27]and *Oreochromis mossambicus* exposed to azinphos-methyl® [28].

The Condition Factor (k) not only gives an indication of the fish health condition but can be used to elucidate the effects of contaminants in animals [29]. In the present study, fish exposed to all sharpshooter, showed significant decrease ($p<0.05$) indicating that the pesticide have effect on the condition factor of the exposed fish. Similar results have been reported in fish exposed to other toxicants [28]. This study thus, indicates that liver organ can be used as bio-indicator biomarker of pollutant effects of pesticide toxicity on fish and shows that fish are very sensitive to environmental changes.

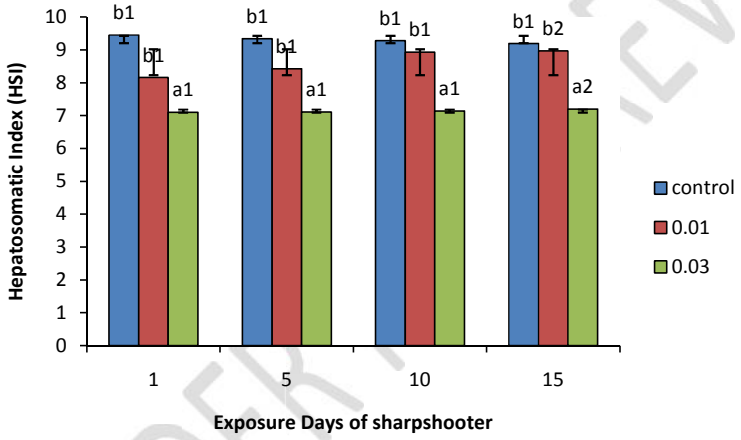

Fig 1. Hepato-somatic indices (HSI) of *C. gariepinus* exposed to sharpshooter for 15-days. Letters indicated significant difference ($p<0.05$) in mean values among pesticide concentrations, and numerals indicated significant difference ($p<0.05$) in mean values among durations of exposure.

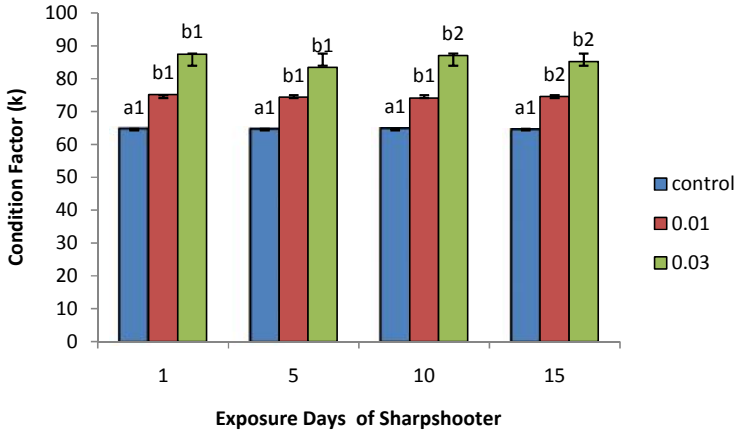

Fig 2. Condition Factor of *C. gariepinus* exposed to sharpshooter for 15-days. Letters indicated significant difference ($p<0.05$) in mean values among pesticide concentrations, and numerals indicated significant difference ($p<0.05$) in mean values among durations of exposure.

Conclusion

From the research carried out on the juveniles of *C. gariepinus*, it was deduced that sharpshooter is toxic *C. gariepinus*. Also, short term exposure of juveniles of *C. gariepinus* to Sharpshooter at even low concentrations was sufficiently effective in disrupting physiological processes of *C. gariepinus*.

However, the use of this pesticide in the environment or near farm lands or in an area close to aquatic environment should be applied with caution to avoid the risk of pesticides contamination.

Recommendations

We recommend that indiscriminate uses of insecticide should be monitored by government and non-Governmental organizations. Similar research to determine various effects of insecticide on fresh water and lakes should be carried out. Biological methods of controlling insects and pest should be adopted by farmers especially those around rivers and coastal regions.

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