ESTABLISHING A DOSE-RESPONSE TOXICITY FOR *Clarias gariepinus* FINGERLINGS EXPOSED TO ETHANOLIC EXTRACT OF *Lantana camara*

ABSTRACT

The toxicity of *lantana camara* on the survival and histopathology of gills of *Clarias gariepinus* fish from Fisheries unit, Akwa Ibom State University Obio Akpa, farm was studied in two batches (A, and B) using the ethanolic extract (EE) of *Lantana camara* for 96 hours under laboratory conditions. Five concentrations ranging from 0, 2, 3, 4, and 5 mg/L were prepared from the (EE) of *L. camara* for the toxicity test. The experimental animals showed different percentage mortalities with toxicant concentrations. The 96 hours LC_{50} for *C. gariepinus* for both batches (A, and B) was given at 2.983 mg/l representing a log transformed concentration of 0.475 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment. The different batches of *C. gariepinus* ($P>0.05$) had no significant difference in mortality indicating that the ethanolic extract had same toxic effects on the test organisms. Toxicant exposure induced behavioral changes such as abnormal and uncoordinated swimming movement, restlessness, respiratory difficulties and attempt at jumping out was observed during the study. The results of the present study suggest that the ethanolic extract of *L. camara* had severe impacts on the test organism resulting in mortality. The effects of the plant extract on the gills of *C. gariepinus* fingerlings had severe impacts on the test organisms. Samples were taken from each of the concentrations to examine the effects of the extract on the gills of *C. gariepinus*. There was no observable change in the gills of the test organism in the control group. In the 2 mg/l concentration, the gill showed diffused epithelial degeneration of the primary lamella., in the 3 mg/l and 4mg/l concentration it was observed that the gills showed complete diffused epithelial degeneration of the lamella. Although cartilaginous filament of the epithelium was preserved between the two adjacent filament in skeletal muscle. Finally, in the 5 mg/l concentration the gill section showed diffused epithelial degeneration of the primary lamella and few retained processes of the secondary lamella. From the findings, it is observed that extract obtained from *L. camara* is toxic to aquatic life. Therefore, effective management strategies should be put in place to ensure safety compliance.

Keywords: Dose-Response, Toxicity, Ethanolic Extract, *Latana camara*, *Clarias gariepinus*, Histopathology, Gills

1.0 Introduction

Lantana sp. (Verbenaceae) is a highly invasive tropical weed that attacks more than 60 % of forests worldwide (Sharma, *et.al.*, 2005). The genus harbors 150 species and is native to the tropical and subtropical areas of South America, Asia and Africa. *L. camara* L. is the most dominant species (Negi, *et. al.*, 2019). Although Lantana sp. is used in many countries as a decorative ornamental, the presence of pentacyclic triterpenoids, including lantadenes A and B in their leaves and seeds, has been correlated with the plant's adverse effects, especially when ingested by animals, causing cholestasis, hepatotoxicity, and photo-toxicity (Negi, *et. al.*, 2019).

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potentials of medicinal plants used in various traditional systems (Mouokeu, *et. al.*, 2011, Njateng, *et. al.*, 2010, Kuete, 2010). The World Health Organization (WHO) estimates that 70 to 80% of the people in developing countries use traditional medicine as a major source of health care. However, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs. So far, many plants have been reported to be toxic to both human and animals (Mouokeu, *et. al.*, 2011, Aguinaga, *et. al.*, 2014). It should therefore, be emphasized that the traditional use of any plant for medicinal purposes, by no

means, warrants the safety of such plant. Plants in folk medicine should therefore, be evaluated for safety or toxicity and necessary recommendations made on their use.

Natural products have always played a major role in the development of organic chemistry. The various structural types of natural products contribute not only to new findings or pose challenging synthetic problems but also provide hope that they may become the basis for new biologically active substances of commercial significance. Biological testing has played an important role in toxicity studies of plant extracts. There are numerous bioassay studies on plant extracts. The first of such work involving some local plants was reported as early as 1965 (Nakanishi *et at*., 1965).

The goal of toxicity testing is to identify possible adverse effects of exposure to environmental agents, to develop dose-response relationship that can elucidate the severity of effects associated with known exposures and ultimately to predict the effects of exposures on human populations. In order to research and access the effects of chemicals, toxicologists perform carefully designed studies and experiments. These experiments help identify the specific amount of chemical that may cause harm and potential risks of being near or using products that contain certain chemicals. Therefore, the aim of this study was to assess the toxic effect of *L. camara* on *C. gariepinus* fingerlings, in order to provide critical information on the toxicity of the plants.

2.0 Materials and Methods

2.1 Collection of Test Organism

Fingerling of *Clarias gariepinus* were collected from Akwa Ibom State University fish farm, Obio Akpa Akwa Ibom State, Nigeria located within 4^0 57'52" N and $7^0$45'29'' E. The climate of the area is tropical and is characterized by distinct wet and dry seasons. The vegetation of the study area is generally rainforest close to the mangrove belt. Human activities in the area include farming, hunting, boat building and sand mining. A total of two hundred (200) fingerling were collected and used for the study.

2.2 Acclimatization of Specimen's

The fingerlings where acclimatized in a re-circulatory glass aquaria measuring 96 x 50 x 29 cm containing habitat water for 24hours in the fisheries and aquaculture laboratory of Akwa Ibom State fish farm. This enhanced the stability of the fingerlings from stress of collection and transportation (Udo *et al*, 2006).

2.3 Collection of Plant Sample

Fresh leaves of (*L. camara*) was collected for the study. The collection site of the plant was at Oron road in Uyo Local Government Area, Akwa Ibom State. The date of Collection was 20th January, 2023. The plants material was taken for identification and authentication by a plant systematics at the Department of Botany Herbarium, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin Local Government Area.

2.4 Preparation of Plant Material

After the identification, the leaves were washed and sun dried. The leaves were shredded and spread on cellophane and allowed to dry for 72 hours under room temperature. The dried leaves were pulverized (grinded) into fine powder using wooden pestle and mortar.

2.5 Preparation of Ethanolic Extract (Maceration and Extraction)

Cold extraction method (Maceration) was used in this research according to Hidayat and Wulandari (2021), in the extraction procedure, 1000ml of 99% Concentrated Ethanol was used to Macerate 240g of the plant materials in an airtight container and kept in the laboratory under room temperature for 72 hours (3 days). In the due date of filtration, the mixture was filtered with Muslim cloth to acquire the filtrate. The extract was stored in 250ml conical flasks. The conical flask was well labelled, the mouth of the conical flask was covered with foil paper and masking tape rapped around the mouth to ensure that it is tightly covered.

2.6 Preparation of Experimental Aquaria

Ten (10) rectangular plastic aquaria measuring 25 ×10 × 15 cm were thoroughly washed with tap water and properly rinsed with fresh water of similar salinity and allowed to drain dry for 24 hours on the laboratory bench based on Dede and Kagbo (2001).

2.7 Stocking of Specimen

Each of the Ten (10) plastic aquaria was filled with two liters of water and 10 *C. gariepinus* was stocked in each aquarium (George, et. al., 2013a). The EE of *L. camara*) with varying concentrations was added to each stocked aquaria and allowed to stand for 96 hours for mortality examination. A preliminary test was conducted to give the actual variations in concentration to be used for the bioassay. Each of the aquarium had a replicate to ensure accuracy (George, et. al., 2013a).

2.8 Monitoring of Water Quality

Water Quality Parameters was monitored prior to commencement of the experiment and also periodically according to Standard Method (APHA, 2009; 2005). Parameters that were monitored include dissolve Oxygen (DO), pH, And Temperature (^{0}C). Temperature and pH was measured using portable pH /Ec/ TDs/ Temperature HANNA, H1 991301 Model instrument while oxygen was measured using digital portable analyser JPB - 607A from "Search Tech Instrument".

2.9 Monitoring of Specimen for Mortality

The effects of the various concentration of the EE of (*L. camara*) on the fingerlings was monitored on a 24 hours' basis for 96 hours as recommended by Udo *et. al.,* (2006) and Ekanem and Ekpo (2008).

2.9.1 Determination of Mortality and Survival rates of Fingerlings

The percentage mortality and survival rates of the fingerlings in the different concentrations of the ethanolic extract of *L. camara* during the period of study was determine using the formula;

% mortality =n/N × 100 (Chan, 1977).

Where;

n = number of dead fish per aquarium per concentration

N = Total Individual Stocked

The difference between dead fish and survivors will give the percentage survival of the fingerlings at the end of the experiment (96 hours) (Udo *et. al*., 2006).

2.9.2 Determination of Mortality Lethal Median Concentration (96 hours LC_{50})

The effects of the various concentrations of the ethanolic extract of plant (*Lantana camara*) on the fingerlings was determined by graphical method (Probit Level Determination as recommended by Omoregie (2002), Omoregie and Ufodike (2000), Ekanem and Ekpo (2008) and Udo *et.al*. (2006). At Lethal Median Concentration LC_{50}, after 96 hours of test, the number of fingerlings that are expected to die was determined from the graph. Similarly, the concentration that will kill 5% of the stocked fingerlings at the end of the test (96 hours) was determined at the probit level (Omoregie, (2002) Omoregie and Ufodike (2000), Udo *et. al*., (2006); Ekanem and Ekpo (2008).

2.10 Collection of Sample for Histopathology Extractions

The gill's tissues were isolated from the test animal and fixed in formalin -saline for 48 hours. The fixed tissue was processed manually through graded ethanol, cleared in xylene impregnated and embedded in paraffin wax, sections of the tissue sample were cut with a rotary microtome, stained by hematoxylin and eosin technique, prepared tissues were finally observed using a microscope for pathological changes at x100 and x400 magnification.

2.11 Data Analysis

The results of the respective concentration effects of the ethanolic extract of *L. camara* was presented in tables. Two-way analysis of variance (ANOVA) was used to test for significant ($P<0.05$) difference considering the extract concentration and mortality time. p-value will allow to affirm if the observed differences in the variation of the studied parameters is significant. Also, the LC_{50} was determined using Probit analysis. All statistics were carried out using SPSS version 20.0.

3.0 Results

3.1 Initial Water Quality Parameters

The initial water quality parameters prior to stocking are shown in Table 1. Dissolved oxygen had a value of 5.2 mg/l, with a value of 29.8^{o}C for Temperature and 6.77 for pH.

Table 1: Initial Physico-chemical parameters of the test water prior to stocking of test organism

Fish Species	Initial physico-chemical parameters prior to stocking		
	DO (mg/l) Concentration	Temp (^{o}C) value	pH value
Clarias gariepinus	**5.2**	**29.8**	**6.77**

3.3 Summary of the Percentage Mortality and survivors of *C. gariepinus* Exposed to the EE of *L. camara* at the end of the experiment (96 hours).

The percentage mortality and survivors of *C. gariepinus* at the end of the test period in each of the concentrations are shown in Table 2 for the two batches of the experiment.

In the 0 mg/l concentration of the extract, no mortality was recorded throughout the test period in both batches A and B. in the 2 mg/l concentration of the extract, 70 % mortality was recorded leaving behind 30 % survivors in both bathes.

At the end of the 96-hour bioassay 100 % mortality was observed in the 3, 4 and 5 mg/l concentration of the extract leaving behind no test organisms in the test media for both batches (Table 2). Statistical Analysis using two way Anova (SPSS 20.0) showed that there was no significant difference ($p<0.05$) in mortality between the two batches in the different concentration and time.

Table 2: Summary of the Percentage Mortality and survivors of *C. gariepinus* Exposed to the EE of *L. camara* at the end of the experiment (96 hours).

Conc. of extract (mg/l)	BATCH A				BATCH B			
	Mortality (M)	% M	Survivors (S)	% S	Mortality (M)	% M	Survivors (S)	% S
0	0	0	10	100	0	0	10	100
2	7	70	3	30	7	70	3	30
3	10	100	0	0	10	100	0	0
4	10	100	0	0	10	100	0	0
5	10	100	0	0	10	100	0	0

3.4 96 Hours LC_{50} Determination

The 96 hours LC_{50} for *C. gariepinus* exposed to the different concentrations of the ethanolic extract of *L. camara* is shown in Table 3 for both batches. The 96 hours LC_{50} is given at 2.983 mg/l representing a log transformed concentration of 0.475 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment.

Table 3: LC_{50} determination for *C. gariepinus* at the end of the 96-hours bioassay.

Plant	Species	Probit	S.E	LC_{50}	Log Con.
L. camara	*Clarias gariepinus*	P= 3.403 -- 7.170X	2.741	2.983	0.475

4.7 Histopathology of the gill of *C. gariepinus* Exposed to the different concentrations of the ethanoic extract of *L. camara*

The histo-morphological changes observed in the gills of fingerlings exposed to *L. camara* indicate that exposure to the plant can cause degenerative changes in the gill structure. The control (0mg/l) group 1 (**Ai & Aii**) displayed normal highly cellular primary lamella epithelium, indicating that the gills were healthy and without any significant histological changes. Group 2 (2mg/l) exhibited diffused epithelial degeneration of the primary lamella and supporting cartilage epithelium as shown in figure 1 (Bi & Bii). Groups 3 (3mg/l) and 4 (4mg/l) figure 1 (Ci & Cii) (Di & Dii) respectively displayed complete

diffused epithelial degeneration of the filament, but preserved supporting cartilage epithelium. This indicates that the degenerative changes caused by *L. camara* were limited to the epithelium of the lamella and did not extend to the cartilage. The highest dose Group 5 (**Ei & Eii**) showed diffused epithelial degeneration of the primary lamella and few retained processes of the secondary lamella. The results suggest that exposure to *L. camara* can cause histomorphological changes in the gills of fingerlings, affecting the primary lamella, supporting cartilage, and secondary lamella in varying degrees.

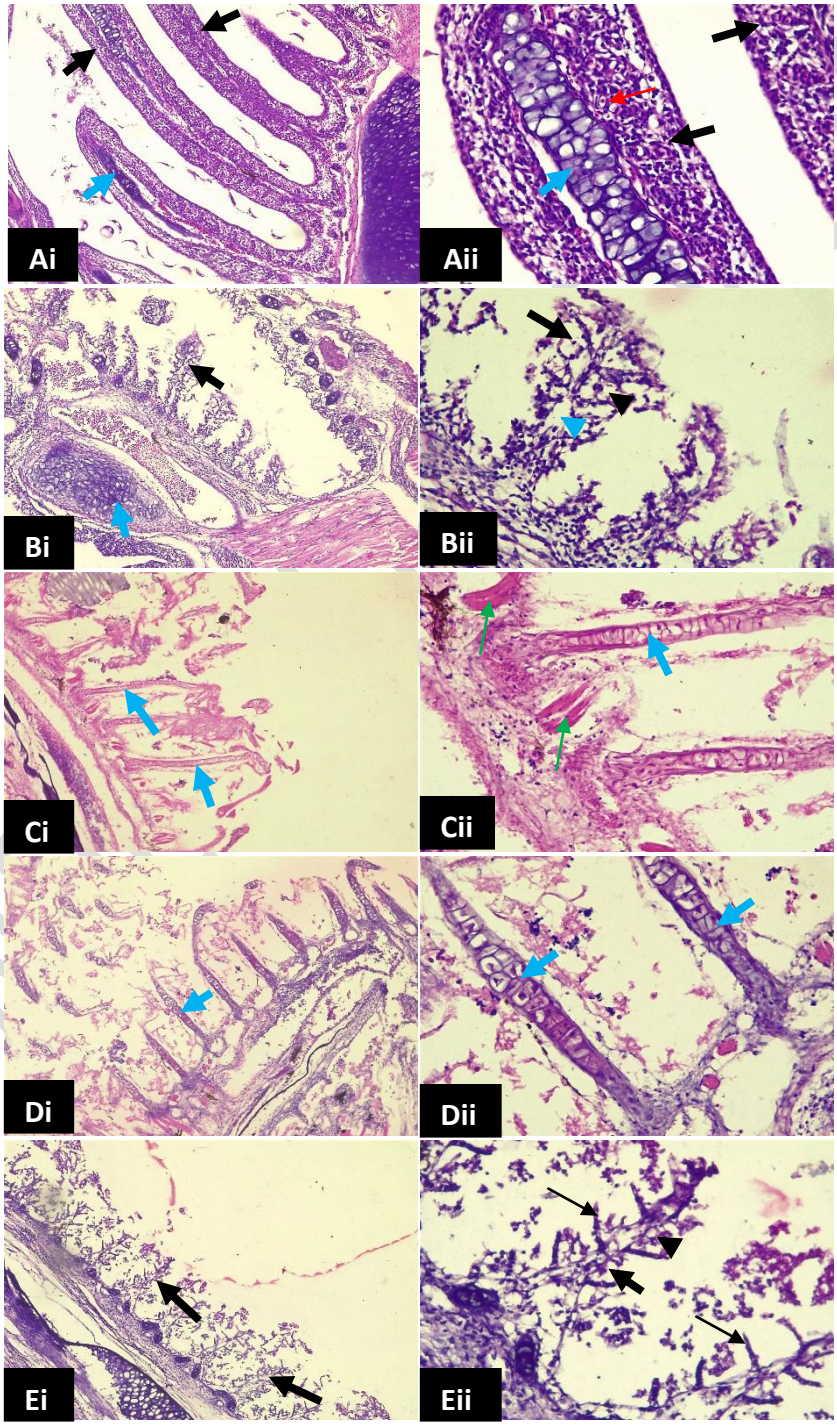

Figure 1: Photomicrograph of the gill arch tissue section across the 5 groups. Haematoxylin and Eosin (H&E) stain, X100 and X400 Magnification

Control Group (Ai&Aii) showed gills section with normal filament epithelium (thick black arrow), the supporting cartilage epithelium (thick blue arrow), muscle fiber (arrowhead) and the capillaries (thin red arrow). The 2mg/l (Bi&Bii) showed moderate epithelium degeneration of the filament and the supporting cartilage, while 3mg/l (Ci&Cii) & 4mg/l (Di&Dii) showed retained cartilage epithelium but diffused degeneration of filamentous epithelium and 5mg/l (Ei&Eii) depicted diffused epithelium degeneration of both primary (thick black arrow) and secondary (thin black arrow).

4.0 Discussion

Three basic physico-chemical parameters were taken in line with standard practice in toxicological studies prior stocking of the experimental fish. Dissolved Oxygen value of 5.2 mg/l with a value of 29.8^{o}C recorded for temperature and a value of 6.77 was recorded for pH. In aquaculture operations, there are recommended values for these parameters. For dissolved oxygen a range of between 4.0 – 6.0 mg/l is suitable, 6.7 – 8.6 for pH and 25.0 – 30.0 ^{o}C for temperature are recommended values for standard operation of aquaculture (Udo, 2007, Ajah 2007, George *et.al*., 2013a; George *et.al*., 2013b; George *et.al*., 2014; George *et.al*., 2015).

The ranges of the physico-chemical parameters of the experimental water were found to fall within the acceptable limits prior to the commencement of the experiment as previously reported by the authors under reference.

The preservation of the standard values of the physico-chemical parameters by the experimental water prior to the commencement of the experiment might have been as a result of absence of impurities or the toxicant and the organisms themselves (Vogels, 2000, WHO, 2022). Impurities, pollutants and toxicants are known to play a role either to elevate or reduce the different physico-chemical parameters in aquatic environment (WHO, 2022; Idoho-Umeh, 2002 and Gbem *et.al*., 2001).

The percentage mortality of *C. gariepinus* in the ethanolic extract of *L. camara ranged* from 0 – 100 % in both batches A and B at the end of the 96-hours bioassay. No mortality was recorded in the 0 mg/l concentration of the toxicant. However, 70 % mortality was recorded in the 2 mg/l concentration in each of the batches while 100 % mortality was recorded in each of the 3 mg/l, 4 mg/l and 5 mg/l concentration of the toxicant. The results of the present findings which shows that mortality was concentration-dependent are in consonance with the earlier reports by George *et.al*., (2013a) when reporting on the laboratory bioassay of the potential effect of rubber extract (*H. brasiliensis*) on the Survival of fingerlings of *O. niloticus*; George *et.al*., (2013b) during their studies on the effect of lethal concentrations of rubber extract (*H. brasiliensis*) on the survival on fingerlings of *C. gariepinus* under laboratory condition; George *et.al*., (2015) when working on the toxic effect of crude oil on hatchery reared *O. niloticus* fingerlings and earlier assertion made by George *et.al*., (2014) when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *C. gariepinus* juveniles.

In the present study, percentage mortality was concentration dependent. The higher the concentration, the higher the percentage mortalities. Similar results have been reported by different authors; Ayuba and Ofojekwu (2002), Adedeji *et.al*., (2008) and

Ogundiran *et.al.,* (2010) while investigating on the toxicity of different plant extracts on *C. gariepinus*. Calta and Ural, (2004) also reported a dose-dependent toxic response of *C. carpio* to synthetic pesticides. Ayotunde *et.al*., (2011) noted that *C. gariepinus* generally tolerate and adapt to environments with rapid changes in water chemistry and increasing contaminants concentration. This explains the response of the species to different concentration. However, the differential mortality observed in the different concentration of the extract over the 96 hours' period, vindicates the law of tolerance according to (Shellford, 1941).

Mechanism for the selective toxicity of toxicants for various fish species depend on four factors which include, nature of the toxicant, varying concentration of the toxicant, Exposure time and fish species. The above factors might have probably been responsible for the different toxic reaction showed by the fish in the different concentration and time during the period of experiment. The reactions are usually more pronounced at higher concentrations due to increased inhibition of acetylcholinesterase which eventually results in the death of the test organisms (Ayotunde *et. al*., 2011; Adedeji *et.al*., 2008).

The 96 hours LC_{50} of any toxicant is the dose or concentration which killed 50 % of the stocked organisms at the end of the experimental period of 96 hours (4 days) (Udo *et.al*., 2006; George *et.al*., 2013a; 2013b, 2014 and 2015).

The 96 hours LC_{50} is known to vary from toxicant (APHA, 2009) and from concentration to concentration of the toxicant (Cagauan *et.al*., 2004; Ayotunde *et.al*., 210).

In the present study the 96 hours LC50 was 2.983 mg/l representing a log concentration of 0.475 for both batches (A and B). The 96 hours LC_{50} of toxicants are known to vary as previously reported by the authors earlier sited above. For instance, Ogundiran *et. al*., (2010) reported 96 hours LC_{50} of 0.0166 mg/l and 0.0038 mg/l for batch A and B *C. gariepinus* fingerlings under the toxicity effects of detergent effluents, 96 hours LC_{50} of 0.1 mg/l and 0.03 mg/l was reported by Adewoye *et.al*., (2010) when working on the effects of soap and detergent effluents on *C. gariepinus* fingerlings. Again, Ayotunde *et. al*., (2011) reported the 96 hours LC_{50} of 0,033 – 0.33 mg/l on *C. gariepinus* adults using Carica papaya extract. The varied 96 hours LC_{50} values usually obtained from different toxicants and test organisms is again reported by Ekanem *et. al*., (2011), when they reported a 96 hours LC_{50} of 5.0 ± 1.76 and 4.0 ± 1.76 mg/l for *Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii*. In this study the 96 hours LC_{50} of 0.475 mg/l obtained for both batch A and B might have depended on the ranges of the toxicant finally used for the bioassay after conducting a preliminary test.

The effects of the ethanolic extract of *Lantana camara* showed pathological effects on the gill lamellae of *Clarias gariepinus* fingerlings. However, the gill lamellae in the control (0 mg/l) were not affected. Pathological effects were pronounced at 2 mg/l, 3 mg/l, 4 mg/l and 5 mg/l concentration of the extract which shows evidence of diffused epithelial degeneration of the lamella.

Gill lamellae cell disintegration has been reported by several authors, Diana *et.al*., (2007) when investigating on the biochemical and histological effects of deltamethrim on *Carassius auratus gibelio* with different effects such lamellae cells hypertrophy and nuclear pycnosis in the basal cells, Gabriel *et. al*., (2007) reported histopathological changes in the gills of *C. gariepinus* exposed to refined petroleum oil and kerosene under laboratory conditions.

The histological changes observed in the present study were concentration dependent with severe alteration been pronounced at higher concentration. The results of this findings are similar to earlier assertion reported by George *et. al*., (2015) when reporting on the acute toxic effects of *H. brasiliensis* on the gills of hatchery reared *O. niloticus* fingerlings and observed histological changes in the gills of the exposed organisms which were concentration dependent, George *et.al*., (2014a) when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *C. gariepinus* juveniles; Idowu *et. al*., (2019) when studying the effect of *Euphorbia hirta* leaf extract on histopathology of juveniles *Clarias gariepinus* and George *et.al*., (2014b) when reporting on the histopathological alterations in gills of fingerlings of *C. gariepinus* following sub-lethal acute exposure to *Hevea brasiliensis.*

4.1 Conclusion

Establishing a dose-response toxicity for *C. gariepinus* fingerlings exposed to ethanolic extract of *Latana camara* were investigated using static bioassay under laboratory condition. Prior to the toxicity test of the extract on the fish species physico-chemical parameters were taken before stocking of the experimental fish. The percentage mortality recorded in this study was concentration dependent with higher mortality recorder at higher concentrations in both batches. This assertion is true based on the law of tolerance as proposed by Shelford, (1941). From the mortalities ratio the 96 hour LC_{50} was 0.475 mg/l for both batches. The results of histopathology showed similar observation of concentration dependent, the higher the concentration, the pronounced the effects of the extract on gill lamellae showing degeneration with no pathological changes seen on the gills of the control group in both batches. Based on the results of the study which showed high percentage mortalities when exposed to the ethanolic extract of *Lantana camara,* it is imperative that ecologically friendly methods should be put in place to checkmates invasive species within our environment. Further research is recommended on the toxicity of locally available plants within our environment. This recommendation stems from the fact that the toxicity level observed during the study was very high even at low concentrations. Based on the findings from the present studies, *L. camara* cannot be used as s raw material in fish feed formulation.

5.0 References

Adedeji, B. A., Adedeji, A. O., Adeyemo, O. K. and Agbede, S. A. (2008). Acute toxicity of diazinon to the African catfish *Clarias gariepinus*. *African Journal of Biotechnology*, 7(5): 651-654.

Adewoye, S. O. (2010). A Comparative study on the behavioural responses of *Clarias gariepinus* on exposure to Soap and detergent effluents. *Advances in Applied Research*, 1(1):89-95.

Ajah, P. O. (2007). Fish feeding and hatchery management. Calabar, Nigeria. Jerry Commercial Productions, 178Pp.

American Public Health Association (APHA). (2005). Standard Methods for Examination of Water and Waste Water.18th edition of the American Public Health Association. 1422.

APHA (American Public Health Association) (2009). Standard Methods for the examination of water and waste waters. New York, 541 Pp.

Ayotunde, E. O. and Ofem, B. O. (2005). Acute and Chronic toxicity of pawpaw (*Carica papaya*) seed provide to Nile tilapia (*Oreochromis niloticus* Lime 1757). *Advance Environmental Biology*, 7:101-107.

Ayotunde, E. O., Ofem, B. O. and Bekah, A. F. (2011). T0xixcity of *Carica papaya* Linn: Haematological and piscidal effects on adult's catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Science*, 6(3):291-308.

Ayotunde, E. O., Ofem, B. O., Okey, I. B., Ikpi, G.U., Ochang, S. N., Agbam, N. E. and Omini, D. O. (2010). Toxicity of pawpaw (*Carica papaya*) seed provide to sharp-tooth catfish (*Clarias gariepinus*) fingerlings and effects on haematological parameters. *International Journal of Fisheries and Aquaculture*, 2:71-78.

Ayuba, J. O. and Ofojekwu, P. C. (2002). Acute toxicity of the root of Jimson weed (D*atura innoxia*) to the African catfish (*Clarias gariepinus*) fingerlings. *Journal of Aquatic science*; 17:131-133.

Cagauan, A.G., Galaites, M.C. and Fajardo, L.J. (2004). Evaluation of botanical piscicides on nile tilapia (*Oreochromis niloticus*) and mosquito fish (*Gambeesia affini*, Baird and Girard). Proceedings of the6 th International Symposium on tilapia in aquaculture, September 12-16, Manila, Philippines, *American Tilapia Association and Philippine Bureau of Fisheries and Aquatic Resources*, pp. 179-187.

Calta, M. and Ural, M. S. (2004). Acute toxicity of the synthetic pyrethroid deltamethrin to young mirror carp, Cyprinus carpio. *Fresen Environmental Bulletin*; 13(11a):1179–1183

Chan, E. I. (1977). Oil pollution and tropical littoral communities. Biological effects of the 1975 Floride key oiul spills. Pp 187-192. In: Procedures of oil spill Conference. Api publication, Washington DC.

Dede, E. B. and Kagbo, H. D. (2001) Aqua toxicological effects of water soluble fraction (WSF) of diesel fuel on *Oreochromis niloticus* fingerlings. *Journal of Applied Science and Environmental Management*, 5(1): 93-96.

Diana, C., Andreea, S. C., Diana, D., Huculeci, R., Marieta, C. and Anca, D. (2007). Biochemical and histological effects of deltamethrim exposure on the gills of *Carassius auraus* gibelio (Pisces: Cyprimidee) *Biotechnology,* 40(1):65-72.

Ekanem, A. P and Ekpo, I. A. (2008). Effects of commercial detergents on the juvenile of *Herterobranchus longifilis* (Curvier and Valiennies). *African Journal of Environmental Pollution and Health*, 6(1): 18 – 23.

Ekanem, A. P, Asuquo, F. E. and Ndiok, E. J. (2011). Toxicity of crude oil to freshwater shrimp *Macrobrachium macrobrachion* and *Macrobrachium Vollenhovenii* from Nigeria Coastal water. *Bulletin Environmental Contaminant and Toxicology*, 86:394 – 397.

Gabriel, U. U., Ezeri, C. N. and Amakiri, E. U. (2007). Haematology and gill pathology of *Clarias gariepinus* exposed to refined oil and kerosene under laboratory conditions. *J. Anim. Vet. Advances*, 6:461-465

Gbem, T. T., Balogun, J. K, Lawal, F. A., and Annune, P. A. (2001). Trace metal accumulation in *Clarias gariepinus* (Teugels) exposed to sub-lethal levels of tannery effluent. *Science Total Environmental*, 271: 1 - 9.

George U. U**.,** Etim**,** I. N., Ekanim, M. P. and Akpan, M. K. (2015). Toxic Effect of Crude Oil on Hatchery Reared *Oreochromis niloticus* Fingerlings. *Journal of Academia and Industrial Research,* 3 (11): 573-576.

George U. U., Etim, I. N., Ekanim, M. P. and Akpan, M. K. (2015). Acute Toxic Effects of *Hevea brasiliensis* on the Gills of Hatchery Reared *Oreochromis niloticus* Fingerlings. *Journal of Academia and Industrial Research,* 3 (11): 562-566.

George, U. U., Asuquo, F. E., Idung, J. U. and Andem, A. B. (2013a). Effects of Lethal Concentration of Rubber Extract (*Hevea brasiliensis)* on the survival on fingerlings of *Clarias gariepinus* Under Laboratory Conditions. *Journal of Natural Sciences Research*, 3 (9): 56-60.

George, U. U., Asuquo, F. E., Idung, J. U. and Andem, A. B. (2013b). A Laboratory Bioassay of the Potential Effects of Rubber Extract (*Hevea brasiliensis)* on the survival of fingerlings of *Oreochromis niloticus. Journal of Biology, Agriculture and Health Care,* 3 (11): 70-74.

George, U. U**.,** Joseph A. and Andy, J. A. (2014a). Histopathological Alterations in Gills of Fingerlings of *Clarias gariepinus* (Burchell, 1822) Following Sublethal Acute Exposure to *Hevea brasiliensis. International Journal of Scientific & Technology Research,* 3(9):252- 255.

George, U. U**.,** Urom, S. E. and Etanketuk, N. (2014b**).** Acute Toxic Effect of Qua Iboe Light Crude Oil on the Gills of *Clarias gariepinus* Juveniles. *International Journal of Environment and Pollution Research,* 2(2):16-30.

Heijenick, D. G., Janssen, C. R. and Decren, W. M. (2003). The combined effects of pH, hardness and dissolved organic carbon on the chronic toxicity of zinc to *Daphina magna*: Development of the surface response model. *Archives Environmental Contamination Toxicology*, 44: 210 – 217.

Hidayat, R. and Wulandari, P. (2021). Methods of Extraction: Maceration, percolation and Decoction. Eureka Herba Indonesia; 2(1):68-74.

Idoho-Umeh, G. (2002). Pollution Assessment of Olamoro water bodies using physical, chemical and biological indices. Ph.D Thesis, University of Benin, Benin City, Nigeria. 485 Pp.

Idowu, A. A., Soetan, M.O, Akinde, A. and Popoola, O.C. (2019). Effect of *Euphorbia hirta* leaf extracts on histopathology of juvenile *Clarias gariepinus, Nigerian Journal of Animal Science,* 21 (1): 96-109

Kovisto, S. (1995). In *Daphina magma*: An ecologically representative zooplankton species toxicity test. *Environmental pollution*, 90: 263 – 267.

Ogundiran, M. A., Fawole, O. O., Aderoye, S. D. and Ayandiran, T. A. (2010). Toxicological impacts of detergents effluent on juveniles of African catfish (*Clarias gariepinus*) (Burchell, 1822). *Agriculture and Biology Journal of North America*, 1(3): 330 – 342.

Omoregie, E. (2002). Acute Toxicity of Water Soluble Fraction of Crude Oil to the Nile Tilapia (*Oreochromis niloticus*). *Bulletin of Environmental Contamination and toxicity*, 68: 623 – 629.

Omoregie, E. and Ufodike, B. C. (2000). Effects of water soluble fraction of crude oil on growth of the Nile Tilapia (*Oreochromis niloticus*) (L). *Bulletin of Environmental Contamination and toxicity*, 64: 601 – 605.

Udo, P. J. (2007). Techniques in Fish farming (Practice and Management). Wusen Publishers, Calabar, Nigeria, 100 Pp.

Udo, P. J., Ekanem, A P. and Eze, E E. (2006). Toxicity of crude oil to early life stages of *Heterobranchus longifilis* (Cruveier and Valiennces) Pisces: Bagridae). *Tropical Environmental Research*, 1:450-459.

Vogels, S. (2000). Quantitative chemical analysis. In Mentham , J., Denney, RC., Barnes, JD and Thomas, MSK (Eds.), 806 Pp.

World Health Organization (WHO) (2022). Guidelines for drinking water quality: Fourth edition incorporating the first and second addenda. WHO Geneva, 614 Pp.