

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* obtained from Fisheries unit, Akwa Ibom State University obio akpa, farm was studied in two batches (A, and B) using the ethanolic extract 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 ethanolic extract (EE) of *Lantana camara* for the toxicity test. The experimental animals showed different percentage mortalities with toxicant concentrations. The 96 hours LC₅₀ 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 phototoxicity (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 al.*, 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 assess 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 *Lantana camara* on *Clarias 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° 57'52" N and 7°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 were acclimatized in a re-circulatory glass aquaria measuring 96 x 50 x 29 cm containing habitat water for 24 hours 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 (Smith 1982, Udo *et al.*, 2006).

2.3 Collection of Plant Sample

Fresh leaves of (*Lantana 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, Mkpato 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 Kaglo (2001).

2.7 Stocking of Specimen

Each of the Ten (10) plastic aquaria was filled with two liters of habitat water and 10 *Clarias gariepinus* was stocked in each aquarium. The ethanolic extract of (*Lantana 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.

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, 1998; 2005). Parameters that were monitored include dissolve Oxygen (DO), pH, And Temperature (°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 ethanolic extract of (*Lantana 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 *Lantana camara* during the period of study was determine using the formula;

% mortality = $n/N \times 100$ (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 LC₅₀)

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 LC₅₀, 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 *Phragmanthera capitata* 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. Also, the LD₅₀ 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°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)	Temp (°C)	pH
<i>Clarias gariepinus</i>	5.2	29.8	6.77

3.3 Summary of the Percentage Mortality and survivors of *C. gariepinus* in the different concentrations of the ethanoic extract of *Lantana 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 batches.

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* in the different concentrations of the ethanoic extract of *Lantana camara* at the end of the experiment (96 hrs).

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 LC₅₀ Determination

The 96 hours LC₅₀ for *C. gariepinus* exposed to the different concentrations of the ethanolic extract of *Lantana camara* is shown in Table 3 for both batches. The 96 hours LC₅₀ 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: LC₅₀ determination for *C. gariepinus* at the end of the 96-hours bioassay.

Plant	Species	Probit	S.E	LC ₅₀	Log Con.
<i>Lantana camara</i>	<i>Clarias gariepinus</i>	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 *Lantana camara*

The histo-morphological changes observed in the gills of fingerlings exposed to *Lantana 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 *Lantana 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 *Lantana camara* can cause histomorphological changes in the gills of fingerlings, affecting the primary lamella, supporting cartilage, and secondary lamella in varying degrees.

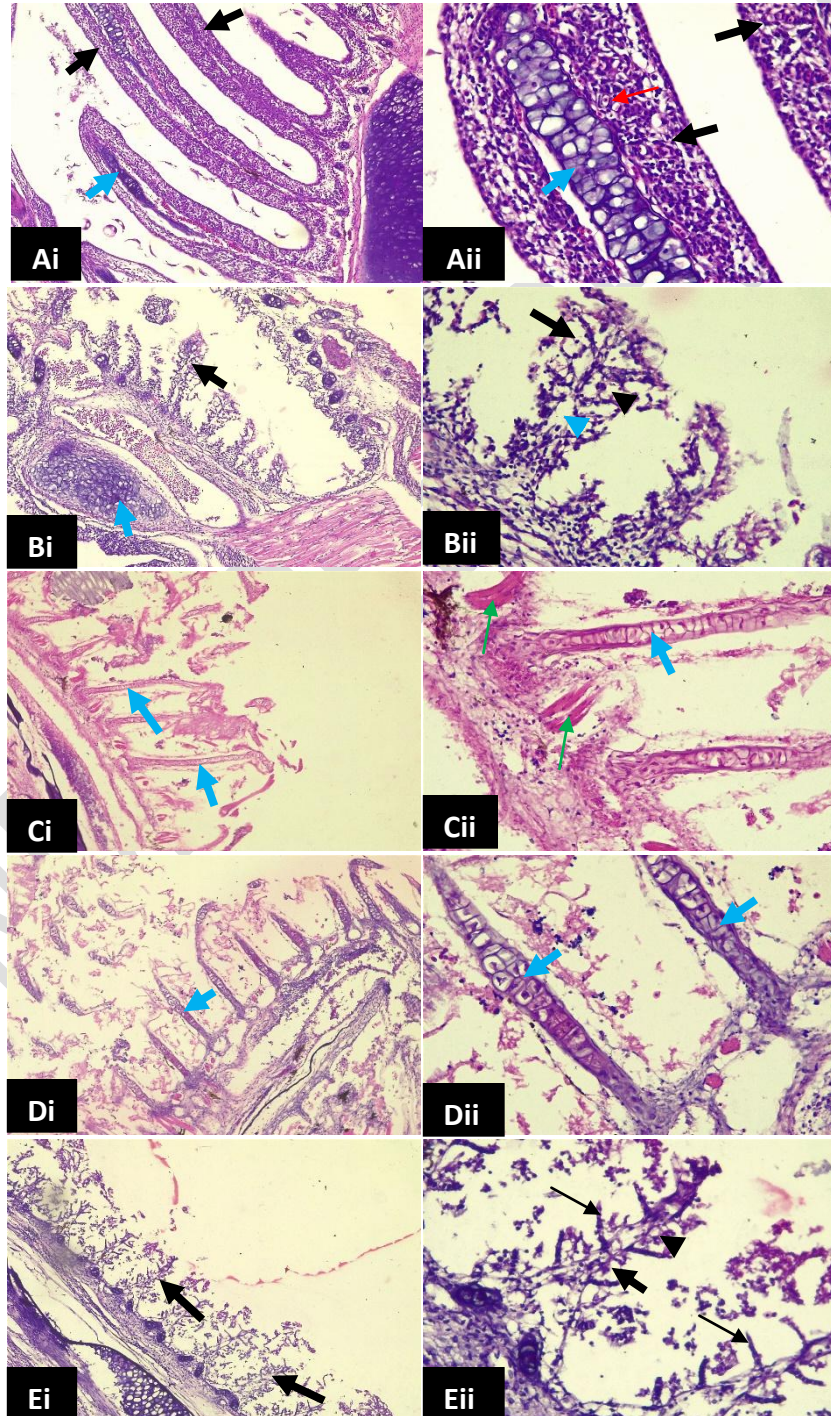


Figure 1: Photomicrograph of the gill arch tissue section across the 5 groups. Haematoxylin and Eosin (H&E) stain, X100 and X400 Magnification

Control Group (Ai&Aii) showed gills section with normal filament epithelium (thick black arrow), the supporting cartilage epithelium (thick blue arrow), muscle fibre (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

4.1.1 Initial Values of the Physico-chemical parameters of the Experimental water

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°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 °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, Samabaswa and Rao, 1985; WHO, 1984). Impurities, pollutants and toxicants are known to play a role either to elevate or reduce the different physico-chemical parameters in aquatic environment (WHO, 1984; Idoho-Umeh, 2002 and Gbem *et.al.*, 2001).

4.2 Percentage Mortality and Survivors

The percentage mortality of *C. gariepinus* in the ethanolic extract of *lantana 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 (*Hevea Brasiliensis*) on the Survival of fingerlings of *Oreochromis niloticus*; George *et.al.*, (2013b) during their studies on the effect of lethal concentrations of rubber extract (*Hevea Brasiliensis*) on the survival on fingerlings of *Clarias gariepinus* under laboratory condition; George *et.al.*, (2015) when working on the toxic effect of crude oil on hatchery reared *Oreochromis 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 *Clarias 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 *Clarias gariepinus*. Calta and Ural, (2004) also reported a dose-dependent toxic response of *Cyprinus carpio* to synthetic pesticides. Ayotunde *et.al.*, (2011) noted that *Clarias 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).

Oh *et.al.*, (1991) gave three factors for the selective toxicity of toxicants for various fish species as: different inhibition of acetylcholinesterase, different detoxification and absorption. 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 (Oh *et.al.*, 1991) which eventually results in the death of the test organisms (Ayotunde *et. al.*, 2011; Adedeji *et.al.*, 2008).

4.3 96 Hours LC₅₀

The 96 hours LC₅₀ of any toxicant is the dose or concentration which kills 50 % of the stocked organisms at the end of the experimental period of 96 hours (4 days) (Samabaswa & Rao, 1985; Akpan *et.al.*, 1999; Udo *et.al.*, 2006; George *et.al.*, 2013a; 2013b, 2014 and 2015).

The 96 hours LC₅₀ is known to vary from toxicant (APHA, 1998; Samabaswa & Rao, 1985) 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 LC₅₀ of toxicants are known to vary as previously reported by the authors earlier cited above. For instance, Ogundiran *et. al.*, (2010) reported 96 hours LC₅₀ of 0.0166 mg/l and 0.0038 mg/l for batch A and B *Clarias gariepinus* fingerlings under the toxicity effects of detergent effluents, 96 hours LC₅₀ 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 *Clarias gariepinus* fingerlings. Again, Ayotunde *et. al.*, (2011) reported the 96 hours LC₅₀ of 0,033 – 0.33 mg/l on *Clarias gariepinus* adults using *Carica papaya* extract. The varied 96 hours LC₅₀ values usually obtained from different toxicants and test organisms is again reported by Ekanem *et. al.*, (2011), when they reported a 96 hours LC₅₀ of 5.0 ± 1.76 and 4.0 ± 1.76 mg/l for *Macrobrachium macrobrachion* and *Macrobrachium vollehovenii*. In this study the 96 hours LC₅₀ 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.

4.4 Effects of the Extract on the gills of the test Organisms

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 deltamethrin 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 *Clarias 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 *Hevea brasiliensis* on the gills of hatchery reared *Oreochromis 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 *Clarias 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 *Clarias gariepinus* following sub-lethal acute exposure to *Hevea brasiliensis*.

4.5 Conclusion

Establishing a dose-response toxicity for *Clarias 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 LC₅₀ 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, *Lantana camara* cannot be used as s raw material in fish feed formulation.

5.0 References

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