

DOSE-RESPONSE RELATIONSHIP AND HISTO-MORPHOLOGICAL ALTERATIONS ON *Oreochromis niloticus* JUVENILES FOLLOWING EXPOSURE TO ETHANOLIC EXTRACT OF *Latana camara*

Abstract

Five concentrations ranging from 0, 5, 10, 15, and 20 mg/l were prepared from the ethanolic extract (EE) of *L. camara* for the toxicity test. The experimental animals showed differential percentage mortalities with toxicant concentrations. The 96 hours LC₅₀ for *O. niloticus* for both batches (A and B) was given at 7.346 mg/l representing a log transformed concentration of 0.866 mg/l. The different batches of *O. niloticus* (P>0.05) had no significant difference in mortality indicating that the EE of *L. camara* had same toxic effects on the test organisms. The results of the present study suggest that the EE of *L. camara* had severe impacts on the test organism resulting in mortality. The effects of *L. camara* on the gills of *O. niloticus* Juveniles showed severe impacts on the test organisms. Five samples were taken from each of the concentrations to examine the effects of the extract on the gills of *O. niloticus*. There were no observed changes in the gills of the control group as compared to other treatment which showed evidence of histological alterations. From the findings, it is observed that extract obtained from *L. camara* is toxic to aquatic life.

Keywords: Dose-Response, Relationship, Histomorphological Alterations, Exposure, Ethanolic Extract, *Latana camara*

1.0 Introduction

Lantana is a genus of both herbaceous plants and shrubs comprising of about 150 species and belongs to the family Verbenaceae (Ghisalberti, 2000). *Lantana camara* is an evergreen climbing aromatic shrub of the genus Lantana and is considered to be one of the most important therapeutic plants of the world (Sharma, *et al.*, 2000; Srivastava, *et al.*, 2005). It can grow up to 2–4 m in height under normal conditions but has the capacity to climb up to 15 m in height with the support of adjacent vegetation (Day, *et al.*, 2003). *L. camara* is native to tropical regions of America and Africa, but now, it has been introduced as an ornamental plant in most countries worldwide including Saudi Arabia and has been completely naturalized in most tropical and subtropical parts of the world as it can easily grow and survive in variety of agro-climatic conditions (Sharma, 1981).

L. camara have been extensively used in traditional medicine for the treatment of various ailment such as, malaria, ulcers, cancer, high blood pressure, tetanus, tumors, eczema, cuts, catarrhal infections, chicken pox, measles, rheumatism, asthma and fevers (Day, *et al.*, 2003; Ghisalberti, 2000; Lenika, *et al.*, 2005). It is an excellent plant that contained several classes of bioactive natural products including triterpenoids, flavonoids, steroids, iridoide glycosides, oligosaccharides, phenylpropanoid glycosides, and naphthoquinones (Begum, *et al.*, 2014; Sousa, *et al.*, 2012).

Plants with therapeutic actions have been used by rural communities and even urban population in the treatment of several diseases, and their beneficial effects have been attributed to their chemical constituents. This is at least in part, because their chemical constituents may have antioxidant activity, thereby, preventing the oxidative damage resulting from oxidative stress, which has been implicated in many diseases including stroke, cancer and other neurodegenerative diseases (Ghisalberti, 2000).

Although, *L. camara* is well studied for its pharmacological properties (Ghisalberti, 2000), it is recognized so far among the most toxic plants (Ghisalberti, 2000). Its toxic

effects to human and livestock has been reported in Australia, India, New Zealand, South Africa and America, and were attributed to the presence of lantadenes A, B and D (Ghisalberti, 2000). In addition, methanolic extract of the leaves showed anti-inflammatory, anti-pyretic, analgesic, antioxidant and antibacterial activities (Sousa, *et al.*, 2013).

Considering the lack of information in the literature regarding the toxicological effects of *L. camara*, the present study aimed to evaluate the toxicity of ethanolic extracts from the leaves of *L. camara* on *Oreochromis niloticus*. The need to provide fish via aquaculture for the growing human population has become a global issue due to declining stock from wild populations consequently as a result of human perturbations. The major setback to the aquaculture industry is the cost of feed, since 70% of the entire production solely depends on feeding. This research intends to evaluate the toxicity of *lantana camara* and ascertain if it can be useful as a plant-based additive in the formulation of fish feed. Also, as an invasive species it's pertinent to understand its toxicity and make accurate recommendations based on the results that will be obtained from the study.

2.0 Materials and Methods

2.1 Collection of Test Organism

Fingerling of *Oreochromis niloticus* 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, Mkpata 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 *Oreochromis niloticus* 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, APHA, 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;

$$\% \text{ mortality} = n/N \times 100 \text{ (Chan, 1977).}$$

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 *Oreochromis niloticus* 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>Oreochromis niloticus</i>	5.2	29.8	6.77

3.2 Variation in water Quality (Physico-chemical parameters) in the test media with *Oreochromis niloticus* as test organism (Batches A and B) during the experimental period (96 Hours)

Table 2 shows the variation recorded in the different physico-chemical parameters for *Oreochromis niloticus* (Batches A and B) in the different concentration of the extract and time.

Dissolved oxygen concentrations ranged between 4.00 – 5.2 mg/l in the 0mg/l concentration of the extract. The highest DO value was recorded at the 24 hour of test with the least value recorded during the 96 Hour of Test.

In the 5 mg/l concentration of toxicant, dissolved oxygen ranged between 2.8 – 4.8 mg/l. the least value was recorded during the 96 hour of test while the highest value was recorded during the 24 hour of test. In the 10 and 15 mg/l concentration of toxicant, dissolved oxygen ranged 2.4 – 4.6 mg/l and 1.4 – 4.4 mg/l respectively. The highest and least value of DO were observe at the 24 hours of test and 96 hours of test for both concentrations during the study duration. In the 20 mg/l concentration of toxicant, dissolved oxygen value ranged from 1.3 – 4.2 mg/l. The highest value was recorded during the 24 hour of test and the least value was observed during the 96 hour of test.

Temperature value were observed to range between 27.1 – 27.6 °C during the 96 hours' bioassay. The least value of 27.1 °C was recorded during the 72nd and 96th hours of test in the 5 mg/l concentration of toxicant while the highest value of 27.6 °C was recorded during the 24 hour and 96 hour of test in the 0 mg/l concentration of toxicant (control).

The value of pH was observed to range between 5.80 – 6.31 during the 96 hours' experimental bioassay. The least value of 5.80 was recorded during the 96th hours of test in the 3 mg/l concentration of toxicant while the highest value of 6.31 was recorded during the 24 hour of test in the 0 mg/l concentration of toxicant (control).

Table 2: Summary of the variations in the physico-chemical parameters in the test media during the experimental period.

Parameters	Conc. (mg/l)	BATCH A				BATCH B			
		24 hr	48 hr	72 hr	96 hr	24 hr	48 hr	72 hr	96 hr
Dissolved Oxygen (mg/l)	0	5.2	4.7	4.4	4.0	5.2	4.7	4.4	4.0
	2	4.8	4.0	3.2	2.8	4.8	4.0	3.2	2.8
	3	4.6	2.8	2.6	2.4	4.6	2.8	2.6	2.4
	4	4.4	1.6	1.5	1.4	4.4	1.6	1.5	1.4
Initial: 5.2 mg/l	5	4.2	1.5	1.4	1.3	4.2	1.5	1.4	1.3
Temperature (°C)	0	27.6	27.5	27.5	27.6	27.6	27.5	27.5	27.6
	2	27.4	27.4	27.3	27.2	27.4	27.4	27.3	27.2
	3	27.5	27.5	27.3	27.3	27.5	27.5	27.3	27.3
	4	27.4	27.4	27.2	27.3	27.4	27.4	27.2	27.3
Initial: 29.8 °C	5	27.4	27.3	27.1	27.1	27.4	27.3	27.1	27.1
pH	0	6.31	6.25	6.22	6.12	6.31	6.25	6.22	6.12
	2	6.26	6.22	6.12	6.00	6.26	6.22	6.12	6.00
Initial: 6.77	3	6.21	6.20	6.00	5.80	6.21	6.20	6.00	5.80
	4	6.21	6.22	6.22	6.12	6.21	6.22	6.22	6.12
	5	6.16	6.05	6.02	6.00	6.16	6.05	6.02	6.00

3.3 Summary of the Percentage Mortality and survivors of *O. niloticus* in the different concentrations of the ethanoic extract of *Latana camara* at the end of the experiment (96 hours).

The percentage mortality and survivors of *O. niloticus* at the end of the test period in each of the concentrations are shown in Table 3 for both batches during the experimental period.

In the 0 mg/l concentration of the extract, no mortality was recorded throughout the test period in both batch A and B. In the 5 mg/l concentration of the extract, 40 % mortality was recorded leaving behind 60 % survivors in both batches. At the end of the 96-hour bioassay 100 % mortality was observed in the 10, 15 and 20 mg/l concentration of the extract leaving behind no test organisms in the test media for both batches (Table 3). 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 3: Summary of the Percentage Mortality and survivors of *O. niloticus* in the different concentrations of the ethanoic extract of *Latana 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
5	4	40	6	60	4	40	6	60
10	10	100	0	0	10	100	0	0
15	10	100	0	0	10	100	0	0
20	10	100	0	0	10	100	0	0

3.4 96 Hours LC₅₀ Determination

The 96 hours LC₅₀ for *Oreochromis niloticus* exposed to the different concentrations of the ethanoic extract of *Latana camara* is shown in Table 4 for both batches. The 96 hours LC₅₀ is given at 7.346 mg/l representing a log transformed concentration of 0.866 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment.

Table 4: LC₅₀ Determination for *O. niloticus* at the end of the 96-hours bioassay.

Plant	Species	Probit	S.E	LC ₅₀ (mg/l)	Log Con. (mg/l)
<i>Latana camara</i>	<i>Oreochromis niloticus</i>	P= 2.295 - 2.650X	2.006	7.346	0.866

3.5 Histopathology of the gill of *O. niloticus* Exposed to the different concentrations of the ethanoic extract of *Latana camara*.

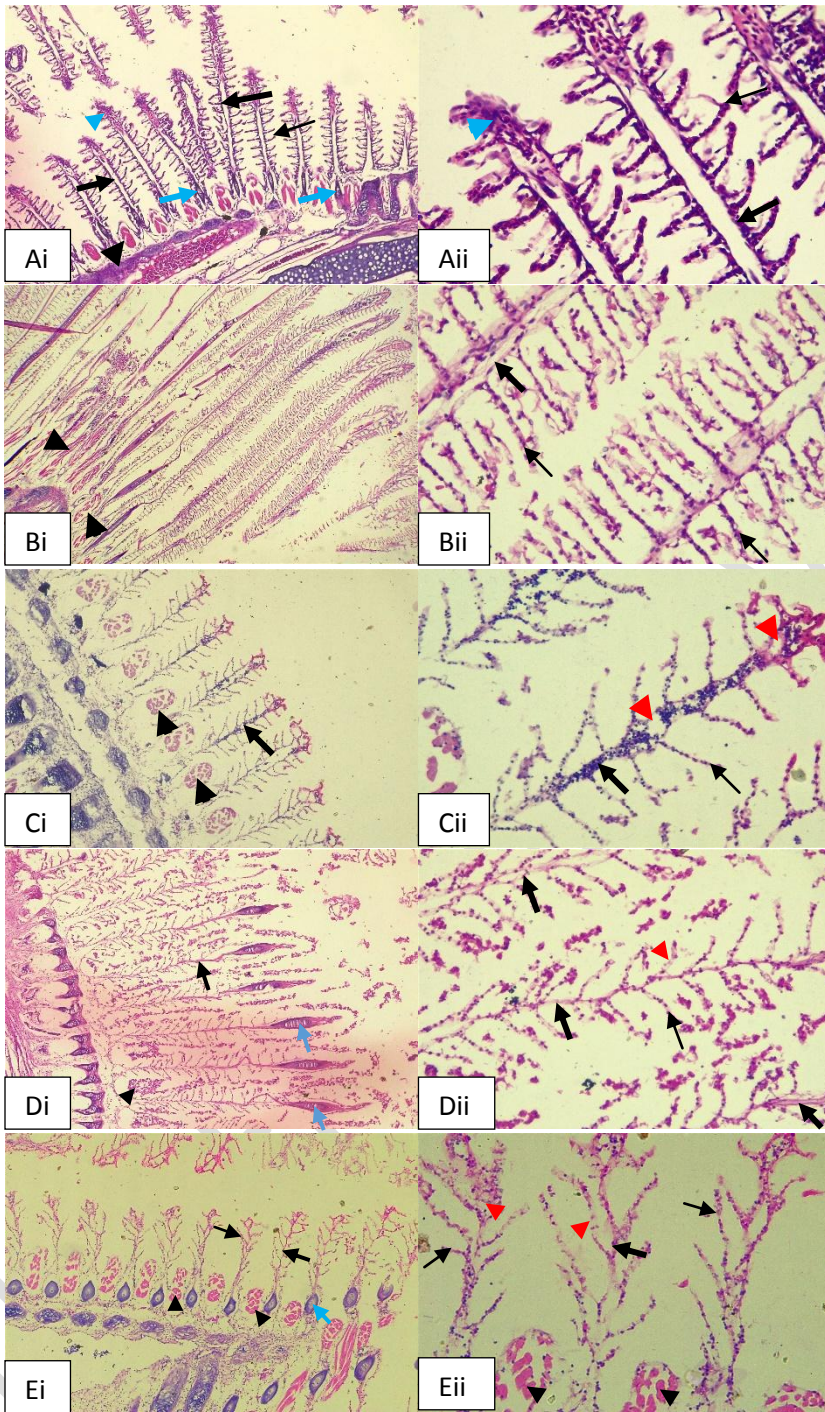


Figure 1: Photomicrograph of Gill arch tissue sections of Group 1-5. Haematoxylin and Eosin (H&E) stain. Each group was shown x100 and x400 magnification.

Control Group (Ai&Aii) showed gills with normal primary filament epithelium (thick black arrow), secondary lamella (thin arrow), the supporting cartilage epithelium (thick blue arrow), muscle fibre (black arrowhead) and the capillary-rich apical part of the filament (blue arrowhead). (Bi&Bii) showed reduced cellularity of the filaments. (Ci&Cii), (Di&Dii) and (Ei&Eii) depict grossly reduced tissue epithelial cells in form of multifocal epithelial degeneration (red arrowhead) along the primary and secondary lamellae

The gill histology of the **control group (group 1)** revealed normal gill filaments (primary lamella) characterized by a well-organized structure. Several parallel threadlike secondary lamellae were observed, oriented perpendicular to the primary lamellae. The apical part of the filament exhibited a highly cellular epithelium, indicating a healthy state. Additionally, supporting cartilaginous tissue and brachial muscle were observed between the gill filaments, contributing to the overall structural integrity of the gills. **Group 2:** In this group, there was a mild reduction in the number of epithelial cells present in both the primary and secondary lamellae. This reduction in cell density indicates an alteration in the cellular composition of the gill tissue. Specifically, the primary and secondary lamellae exhibited decreased epithelial cell populations compared to the control group. **Groups 3, 4, and 5:** In these groups, a significant and severe reduction in epithelial cells was observed throughout the gill structure. This reduction was evident in the form of multifocal epithelial degeneration along the length of both primary and secondary lamellae. The affected areas exhibited a considerable loss of epithelial cells, indicating a compromised state of the gill tissue. The multifocal degeneration observed suggests widespread damage to the epithelial layer.

4.0 Discussion

Prior to commencement of the toxicity studies, three basic physico-chemical parameters were taken in line with standard practice in toxicological studies before the introduction of the extract into the test water media. A value of 5.2 mg/l was recorded for dissolved oxygen with a value of 29.8°C recorded for temperature and a value of 6.77 was recorded for pH.

Standard threshold values for these parameters are known in aquaculture operations. 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 initial values of the physico-chemical parameters of the test water were found to fall within the recommended threshold limits prior to the commencement of the experiment as previously reported by the authors under reference. The unperturbed values of physico-chemical parameters observed prior to the commencement of the experiment might be link to the absence of impurities or the toxicant and the organisms themselves (Vogels, 2000, Samabaswa and Rao, 1985; WHO, 1998). 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).

Variations in physico-chemical parameters were observed in the experimental aquaria in both batches during the experimental period. The values of physico-chemical parameters recorded varied depending on time and concentration of the extract. As the concentration of the toxicant increased with time, the values of the physico-chemical parameters were observed to sway when compared with the control.

Variation in physico-chemical parameters of the test media is an expected occurrence which has been previously reported by several authors in related studies.

Ayotunde and Ofem (2005) when reporting on the acute and chronic toxicity of *Carica papaya* seed powder to Nile tilapia (*Oreochromis niloticus*), Ayotunde *et. al.*, (2010) when investigating toxicity of *Carica papaya* seed powder to *Clarias gariepinus* fingerlings and effects on haematological parameters, Ayotunde *et. al.*, (2011) when investigating the toxicity of *Carica papaya* on adult catfish (*Clarias gariepinus*), and Cagauan *et.al.*, (2004) when evaluating botanical piscicides on *Oreochromis niloticus* and mosquito fish *Gambusia affinis*.

It is a commonly acceptable scientific finding that concentration stimulates the elevation and / or reduction in physico-chemical parameters of test water during an experiment (Heijerick *et.al.*, 2003; Ayotunde *et.al.*, 2011) couple with the fact that the organisms will also spent their absorbing oxygen in particular for survival (Ogundiran *et.al.*, 2010; Adewoye, 2010).

Results of percentage mortality and survivors of *O. niloticus* exposed to the different concentration of ethanolic extract of *Latana montavidensis* ranged from 0 – 100 % in both batches A and B at the end of the 96-hours bioassay.

In the 0 mg/l concentration, no mortality was recorded in both batches A and B. In the 5 mg/l concentration of the toxicant, 40 % mortality and 60 % survivors were recorded during the 96-hour bioassay. However, 100 % mortality was recorded in the 10, 15 and 20 mg/l concentration in each of the batches with no survivors recorded. The results of the present findings is consistent with earlier assertion 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 of 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 George *et.al.*, (2014) when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *Clarias gariepinus* juveniles.

The present findings align favourably with ecological law of tolerance which states that every organism as a range of survival, which when either the concentration is so high or low may affect the existence or survival of the organism. This was demonstrated in this study as higher percentage mortalities of the test organism were recorded at higher concentration of the extract. The result of mortalities observed in this study conforms with earlier assertion by Ayuba *et. al.*, (2002), Calta, *et.al.*, (2004), Adedeji *et.al.*, (2008) and Ogundiran *et.al.*, (2010) who also observed in their study that mortalities were concentration dependent.

The 96 hours LC₅₀ of any toxicant is a function of the concentration of toxicant, species, time of exposure and the nature of the toxicant. In the present study the 96 hours LC₅₀ was 7.346 mg/l representing a log concentration of 0.866 for both batches (A and B). The 96 hours LC₅₀ of toxicants are known to vary depending on some factors which include the species, nature of toxicant, concentration of toxicant used for the bioassay and time of exposure. In a related study, 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, (2010) when working on the effects of soap and detergent effluents on *Clarias gariepinus* fingerlings. Similarly, 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 0.866 mg/l obtained for both batch A and B were lower than those previously reported by the authors under reference exception of Ekanem *et. al.*, (2011) who reported a higher LC₅₀ value. The differential LC₅₀ value recorded may be attributed to the concentration of toxicant, species used for the bioassay, time of exposure and the nature of the toxicant.

The effects of the ethanolic extract of *Lantana camara* showed pathological effects on the gill lamellae of *Oreochromis niloticus* juveniles. However, the gill lamellae in the control (0 mg/l) concentration of the toxicant were not affected. In the 5 mg/l, 10 mg/l, 15 mg/l and 20 mg/l concentration both primary and secondary lamellae of the gills especially the apical part showed epithelial degeneration as evident in the photomicrograph presented in the results.

The morphological functionalities of the gill of fish place it as an essential organ for biological homeostasis in its aquatic environment. Their roles, especially in respiration, remain highly critical to the fish due to its close association with its immediate aquatic environment and the presence of an extensive respiratory epithelia surface area. This makes it the major organ that is usually vulnerable to unbalanced water quality that may arise from the presence of toxicants in the aquatic environment (Pratheepa and Sukumaran, 2014; Mazon, 2002; Fernandes and Mazon 2003 and Au, 2004).

Histomorphological changes and degeneration of gill lamellae has been reported by several authors. Adu *et al.* (2021) reported on the histopathological alterations in the gills and liver of *Clarias gariepinus* juveniles exposed to acute concentrations of *Anogeissus leiocarpus*, Diana *et.al.*, (2007) when investigating on the biochemical and histological effects of deltamethrin on *Carassius auratus gibelio* with different effects such as 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, histopathological report of Nasiruddin *et al.* (2012) on *Heteropneustes fossilis* exposed to three dried leaves extracts. Studies have been conducted on histopathological changes in the gills, liver and kidney of fish exposed to various substances (Drishya *et al.*, 2016; Abubakar, 2013, Adeshina, *et. al.*, 2019)

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.*, (2015b) 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*.

4.1 Conclusion

Dose-response relationship and histo-morphological alterations on *Oreochromis niloticus* juveniles following exposure to ethanolic extract of *Latana camara* were investigated using static bioassay under laboratory condition. Prior to the toxicity test of the extract on the test organism physico-chemical parameters were taken before stocking of the experimental fish. Variations were observed in the physico-chemical parameters of the test media during the experimental period. The physico-chemical parameters (DO, temperature and pH) were observed to fluctuate with increased concentration of the extract with time when compared to the control. The variation observed in the test media during the 96 hours' bioassay is attributed to the introduction of the toxicant into the experimental aquaria which resulted in the mark shift or variation of these parameters as recorded within the period under study. Percentage mortalities recorded in this study was observed to obey the ecological law of tolerance with higher mortality recorded at higher concentrations in both batches. From the mortalities ratio the 96 hour LC₅₀ was 0.866 mg/l for both batches.

The results of histopathology showed pathological changes in the gills of the test organisms in the 5, 10, 15 and 20 mg/l concentration of the toxicant exception of 0 mg/l concentration of the toxicant which no pathological changes were observed depicts the mortality results. Also, histopathological results confirm the toxic effect of the extract at higher concentration which was evidence in the degeneration of the primary and secondary gill lamellae observed during the period of the study. Based on the results of the study which showed high percentage mortalities when exposed to the ethanolic extract of *Latana 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 to ascertain their potentials as fish feed additives. This recommendation stems from the fact that the toxicity level observed during the study was very high even at lower concentrations. Based on the result of findings from the present studies, *Latana camara* cannot be used as s raw material in the formulation of fish feed.

5.0 References

- Abubakar, M.I. (2013). Toxicity of 2, 3-dichlorovinyl dimethyl phosphate (Sniper 1000EC) on *Clarias gariepinus* (Burchell, 1822) and *Oreochromis niloticus* (Trewavas, 1983) under laboratory conditions. Unpublished Ph.D. Thesis, Department of Aquaculture and Fisheries Management. Federal University of Agriculture, Abeokuta, Nigeria.
- Adedeji, B. A., Adedeji, A. O., Adeyemo, O. K. and Agbede, S. A. (2008). Acute toxicity of diaziam to the African catfish *Clarias gariepinus*. *African Journal of Biotechnology*, 7(5): 651-654.
- Adeshina, I., Jenyo-Oni, A., Emikpe, B.O., Ajani, E.K. and Abdel-Tawwab, M. (2019). Stimulatory effect of dietary clove, *Eugenia caryophyllata*, bud extract on growth performance, nutrient utilization, antioxidant capacity, and tolerance of African catfish, *Clarias gariepinus* (B.), to *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, 1-16.

- 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.
- Akpan, E. R., Ekanem, S. B., and Odaro, J. A. (1999). Toxicity of crude oil to freshwater algae. *African Journal of Fisheries and Aquaculture*, 1:56-61.
- 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) (1998). Standard Methods for the examination of water and waste waters. 20th Edn. New York, 314 Pp.
- Au, D.W.T. (2004). The application of histocytopathological biomarkers in marine pollution monitoring: A review. *Marine Pollution Bulletin* 48: 821.
- Audu, B. S., Wakawa, I. A., Oyewole, O.J. and Changdaya, P.Z. (2021). Histopathological Alterations in the Gills and Liver of *Clarias Gariepinus* Juveniles Exposed to Acute Concentrations of *Anogeissus Leiocarpus*. *Jordan Journal of Biological Sciences*, 4:437-543
- 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). Toxicity 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 (*Datura innoxia*) to the African catfish (*Clarias gariepinus*) fingerlings. *Journal of Aquatic science*; 17:131-133.
- Begum, S., Ayub, A., Zehra, S. Q., Siddiqui, B. S. and Choudhary, M. I. S. (2014). Leishmanicidal triterpenes from *Lantana camara*. *Chem. Biodiver.*; 11:709–718.
- Cagauan, A.G., Galaites, M.C. and Fajardo, L.J. (2004). Evaluation of botanical piscicides on nile tilapia (*Oreochromis niloticus*) and mosquito fish (*Gambusia affinis*, Baird and Girard). Proceedings of the 6th 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 oil spills. Pp 187-192. In: Procedures of oil spill Conference. Api publication, Washington DC.
- Day, M., Wiley, C. J., Playford, J. and Zalucki, M. P. (2003). *Lantana*: current management status and future prospects. ACIAR Monograph 102, 14.
- 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 deltamethrin exposure on the gills of *Carassius auratus gibelio* (Pisces: Cyprinidae) *Biotechnology*, 40(1):65-72.
- Drishya, M.K., Kumari, B. Mohan, K.M., Ambikadevi, A.P. and Aswin, B. (2016). Histopathological changes in the gills of fresh water fish, *Catla catla* exposed to electroplating effluent. *Int. J. Fish. Aquat.*, 4 (2016), pp. 13-16
- Ekanem, A. P and Ekpo, I. A. (2008). Effects of commercial detergents on the juvenile of *Herterobranchus longifilis* (Curvier and Valenciennes). *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.

- Fernandes, M.N. and Mazon, A. F. (2003). "Environmental Pollution and fish gill morphology. In: Val, A.L. Kapoor, B.G. (Eds.). Fish adaptations science publishers, Enfield, USA, pp 203-231.
- 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. (2015b). 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. (2014). 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.
- Ghisalberti, E. L. (2000). *Lantana camara* L. (Verbenaceae). *Fitoterapia*; 71:467-486.
- 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 *Daphnia 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
- Lenika, S., Rajesh, S. and Sudarshan, O. (2005). Evaluation of antimotility effect of *Lantana camara* L. var. aculeate constituents on neostigmine induced gastrointestinal transit in mice. *BMC Complement Altern. Med.*; 5:1-6.
- Mazon, A.F., Pinheiro, G.H.D. and Fernandes, M.N. (2002). Hematological and physiological changes induced by short term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Brazilian Journal of Biology* 62(4A): 621-631. 25.
- Nasiruddin, M., Azadi, M.A. and Jahan, A. (2012). Histopathological changes in gill, liver and intestine of *Heteropneustes fossilis* (bloch) treated with three dry seed extracts. *J Asia Soc Bangladdash Sci.*, 38: 217-226
- 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.
- Omoriege, 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.
- Omoriege, 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.
- Pratheepa, V. and Sukumaran, N. (2014). Effect of *Euphorbia hirta* plant leaf extract on immunostimulant response of *Aeromonas hydrophila* infected *Cyprinus carpio*. *Peer. J*; 2:1-17
- Samabaswa, K. S. and Rao, K. R. (1985). Toxicity of Elsan to the Indian Snakehead (*Channa punctatus*). *Indian Journal of Fisheries*, 3: 153 - 159.

- Sharma, O. P. (1981). A review of the toxicity of *Lantana camara* (Linn) in animals. *Clin. Toxicol.*; 18:1077-1094.
- Sharma, O. P., Singh, U. A. and Sharma, S. (2000). Levels of lantadenes, bioactive pentacyclic triterpenoids, in young and mature leaves of *Lantana camara* var. aculeate. *Fitoterapia*; 71:487-491.
- Smith, L. S. (1982). Introduction to Fish Physiology. T. F. H. Publications Incorporated, 211 West Sylvania Avenue, Neptune. New Jersey, 352 Pp.
- Sousa, E. O., Almeida, T. S., Menezes, I. R. A., Rodrigues, F. F.G. Campos, A.R., Lima, S. G. and da-Costa, J. G. M., (2012). Chemical composition of essential oil of *Lantana camara* L. (Verbenaceae) and synergistic effect of the aminoglycosides Gentamicin and Amikacin. *Rec. Nat. Prod.*; 6:144-150.
- Sousa, E. O., Rocha, J. B. T., Barros, L. M., Barros, A. R. C. and Cost, J. G. M. (2013). Phytochemical characterization and in vitro antioxidant properties of *Lantana camara* L. and *Lantana montevidensis* Briq. *Ind. Crop. Prod.*; 43:517-522.
- Srivastava, S. K., Khan, M., Khanuja, S. P. S. (2005). Process for isolation of hepatoprotective agent "oleanolic acid" from *Lantana camara*. United State Patent.
- 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 Valenciennes) Pisces: Bagridae). *Tropical Environmental Research*, 1:450-459.
- Ural, M. S. and Saglam, N. (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry of rainbow trout *Oncorhynchus mykiss*, Walbaum, 1972. *Pesticide Biochemistry and Physiology*, 83:124- 131.
- Vogels, S. (2000). Quantitative chemical analysis. In Mentham, J., Denney, RC., Barnes, JD and Thomas, MSK (Eds.), 806 Pp.
- World Health Organization (WHO) (1984). Guidelines for drinking water quality. Vol. 1 Recommendations. WHO Geneva, 130 Pp.
- World Health Organization (WHO) (1998). Guidelines for drinking water quality. Vol. 1 Recommendations. WHO Geneva, 130 Pp.