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Dose-Response Relationship and Histo-morphological Alterations on *Oreochromis niloticus* Juveniles Following Exposure to Ethanolic Extract of *Latana camara*

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Authors' contributions

This work was carried out in collaboration among all authors. Author UUG conception, design and development of the topic, data collection and analysis, initial drafting and reviewing the manuscript and final approval of the prepared manuscript. Author AJO conception, design and development of the protocol, supervision of the experiments, data analysis and reviewing the manuscript. Author OOA section the gill tissues for histopathological examination and interpretation of the results. Author IEG student who actually played the supporting role in the experiments and reviewing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research intends to evaluate the toxicity of *latana camara* and ascertain if it can be useful as a plant-based additive in the formulation of fish feed.
Study Design: The study was conducted using two replicate (Batch A and B) for 96 hours under controlled laboratory conditions. 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.
Place and Duration of Study: Experiment was conducted in Akwa Ibom State University, Obio Akpa Campus, Akwa Ibom State between February, 2023 and June, 2023.
Methodology: Total of two hundred (200) juveniles were collected for the studies. One hundred (100) juveniles were used during range findings test and the left over for the actual toxicity test. Prior to commencement of the toxicity test, experimental fish were allowed to acclimatize. Each of the ten (10) plastic aquaria was stocked with ten (10) O. niloticus juveniles. The prepared plant extract at varying concentration was added to each stock aquaria and allowed to stand for 96 hours for mortality examination. Gills tissues were isolated from the fish samples in each of the concentration after 96 hours for histopathological examinations.
Results: The experimental animals showed differential percentage mortalities with toxicant concentrations. The 96 hours LC_{50} 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 = .05) had no significant difference in mortality. 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.
Conclusion: From the findings, it is observed that extract obtained from *L. camara* is toxic to aquatic life and cannot be recommended as a plant-based additive in the formulation of fish feed.

Keywords: Dose-response; relationship; histomorphological alterations; exposure; ethanolic extract; Latana camara.

1. INTRODUCTION

"Lantana is a genus of both herbaceous plants and shrubs comprising of about 150 species and belongs to the family Verbenaceae" [1]. "*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" [2,3]. 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 [4]. "*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" [2].

"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" [4,1,5]. "It is an excellent plant that contained several classes of bioactive natural products including triterpenoids, flavonoids, steroids, glycosides, oligosaccharides, phenylpropanoid glycosides, and naphthoquinones" [6,7].

"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" [1].

Although, *L. camara* is well studied for its pharmacological properties [1], it is recognized so far among the most toxic plants [1]. "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" [1]. In addition, methanolic extract of the leaves showed anti-inflammatory, anti-pyretic, analgesic, antioxidant and antibacterial activities [8].

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 *L. camara* and ascertain if it can be useful as a plant-based additive in the formulation of fish feed. Also, as an invasive species its pertinent to understand its toxicity and make accurate recommendations based on the results that will be obtained from the study.

2. MATERIALS AND METHODS

2.1 Collection of Test Organism

Juveniles of *Oreochromis niloticus* 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 juveniles were acclimatized in a re-circulatory glass aquaria measuring 96 x 50 x 29 cm containing clean tap water of similar salinity as 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 [9,10].

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 and Ecological studies Herbarium, University of Uyo, Uyo, Akwa Ibom State Nigeria.

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 [11], 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 [12].

2.7 Stocking of Specimen

Each of the Ten (10) plastic aquaria was filled with two liters of fresh water and 10 *Oreochromis niloticus* juveniles was stocked in each aquarium. The ethanolic extract 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.

2.8 Monitoring of Water Quality

Water Quality Parameters was monitored prior to commencement of the experiment and also periodically according to Standard Method [13,14]. Parameters that were monitored include dissolve Oxygen (DO), pH, And Temperature (^{0}C). Temperature and pH were 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 (*L. camara*) on the juveniles was monitored on a 24 hours' basis for 96 hours as recommended by [10,15].

2.9.1 Determination of mortality and survival rates of juveniles

The percentage mortality and survival rates of the juveniles in the different concentrations of the ethanolic extract of *Lantana camara* during the period of study was determine using the formula:

$$\% \text{ mortality} = n/N \times 100 \text{ [16].}$$

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 Juveniles at the end of the experiment (96 hours) [10].

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 *Oreochromis niloticus* juveniles was determined by graphical method (Probit Level Determination as recommended by [10,15,17,18]. At Lethal Median Concentration LC_{50}, after 96 hours of test, the number of juveniles that are expected to die was determined from the graph. Similarly, the concentration that will kill 5% of the stocked juveniles at the end of the test (96 hours) was determined at the probit level [10,15,17,18].

2.10 Collection of Samples for Histopathology Examination

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 *P. capitata* was presented in tables. Two-way analysis of variance (ANOVA) was used to test for significant (P = .05) difference considering the extract concentration and mortality time. Also, the LD_{50} was determined using Probit analysis. All statistics were carried out using SPSS version 20.0.

3. 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.

3.2 Variation in Water Quality (Physico-chemical parameters) in the test Media with *O. 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 *O. niloticus* (Batches A and B) in the different concentration of the extract and time.

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 (^{o}C)	pH
Oreochromis niloticus	5.2	29.8	6.77

Dissolved oxygen concentrations ranged between 4.00 – 5.2 mg/l in the Omg/l concentration of the extract. The highest DO value was recorded at the 24 hours 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 hours of test while the highest value was recorded during the 24 hours 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 hours of test.

Temperature value were observed to range between 27.1 – 27.6^{o}C during the 96 hours' bioassay. The least value of 27.1^{o}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^{o}C was recorded during the 24 hour and 96 hours 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 6.31was recorded during the 24 hour of test in the 0 mg/l concentration of toxicant (control).

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 bathes. 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 = .05) in mortality between the two batches in the different concentration and time.

3.4 96 Hours LC_{50} Determination

The 96 hours LC_{50} for *O. niloticus* exposed to the different concentrations of the ethanoic extract of *L. camara* is shown in Table 4 for both batches. The 96 hours LC_{50} 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.

3.5 Histopathology of the Gill of *O. niloticus* Exposed to the Different Concentrations of the Ethanoic Extract of *L. camara*

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.

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 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Dissolved Oxygen (mg/l)	O	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
Initial: 5.2 mg/l	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
	5	4.2	1..5	1.4	1.3	4.2	1..5	1.4	1.3
Temperature (^{o}C)	O	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
Initial: 29.8 ^{o}C	4	27.4	27.4	27.2	27.3	27.4	27.4	27.2	27.3
	5	27.4	27.3	27.1	27.1	27.4	27.3	27.1	27.1
pH	O	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

Table 3. Summary of the percentage mortality and survivors of *O. niloticus* in the different concentrations of the ethanoic extract 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
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

Table 4. LC_{50} 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)
Latana camara	*Oreochromis niloticus*	P= 2.295 - 2.650X	2.006	7.346	0.866

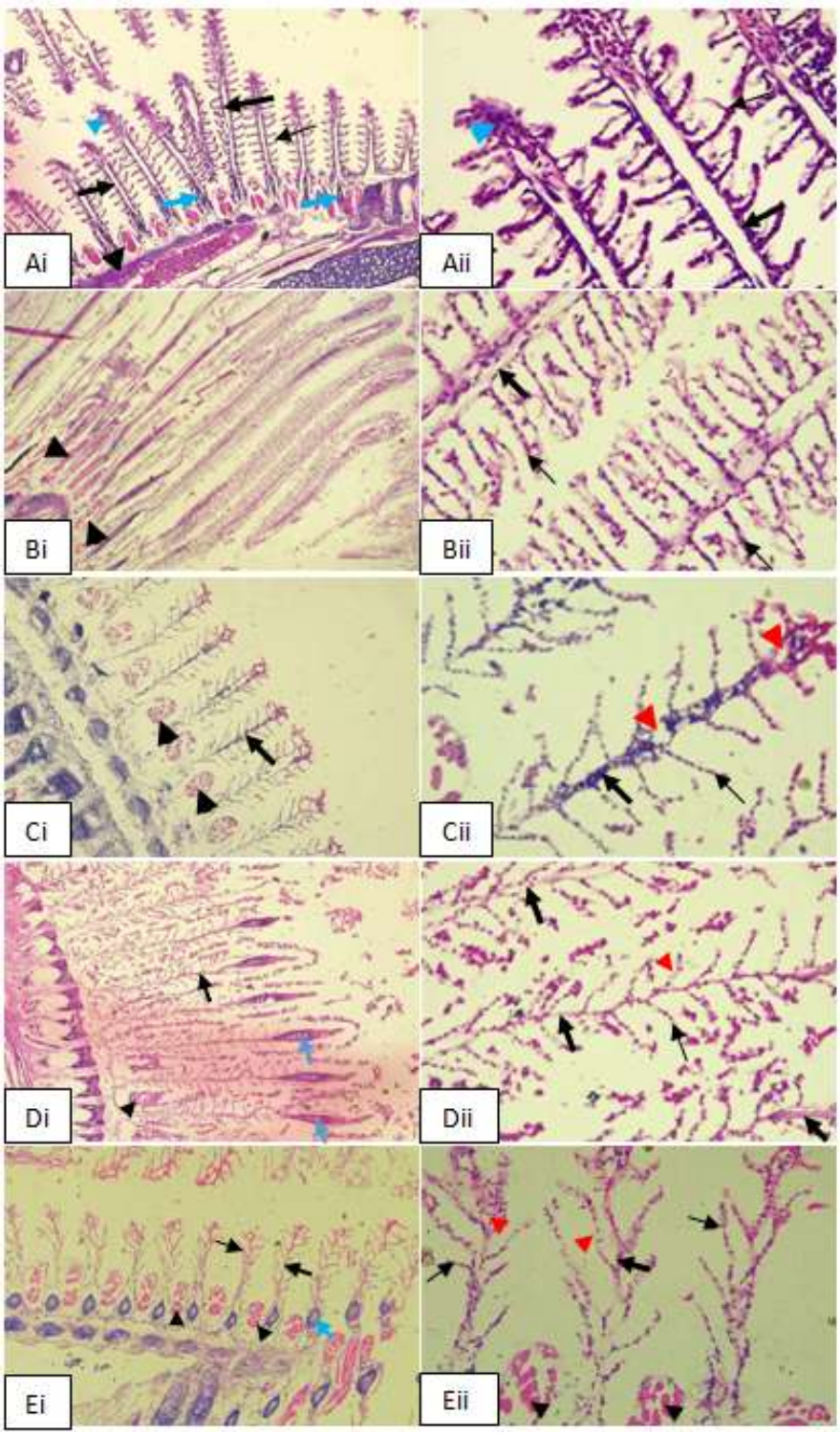

Fig. 1. Photomicrograph of Gill arch tissue sections of Group 1-5. Haematoxylin and Eosin (H&E) stain. Each group was shown x100 and x400 magnification

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-riched 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

4. 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^{o}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 ^{o}C for temperature are recommended values for standard operation of aquaculture [19-24]. 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 [25-27]. Impurities, pollutants and toxicants are known to play a role either to elevate or reduce the different physico-chemical parameters in aquatic environment [28-30].

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 [31] when reporting on the acute and chronic toxicity of *Carica papaya* seed powder to Nile tilapia (*Oreochromis niloticus*), [32] when investigating toxicity of *Carica papaya* seed powder to *Clarias gariepinus* fingerlings and effects on haematological parameters, [33] when investigating the toxicity of *Carica papaya* on adult catfish (*Clarias gariepinus*), and [34] when evaluating botanical piscides 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 [33,35] couple with the fact that the organisms will also spend their absorbing oxygen in particular for survival [36, 37].

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 [21] when reporting on the laboratory bioassay of the potential effect of rubber extract (*Hevea Brasiliensis*) on the Survival of fingerlings of *Oreochromis niloticus*; [22] 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; [38] when working on the toxic effect of crude oil on hatchery reared *Oreochromis niloticus* fingerlings and [23] 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 [36,39-41] who also observed in their study that mortalities were concentration dependent.

The 96 hours LC_{50} 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 LC_{50} was 7.346 mg/l representing a log concentration of 0.866 for both batches (A and B). The 96 hours LC_{50} 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, [36] reported 96 hours LC_{50} 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 LC_{50} of 0.1 mg/l and 0.03 mg/l was reported by [37] when working on the effects of soap and detergent effluents on *Clarias gariepinus* fingerlings. Similarly, [33] reported the 96 hours LC_{50} of 0.033 – 0.33 mg/l on Clarias 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 [42] 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 0.866 mg/l obtained for both batch A and B were lower than those previously reported by the authors under reference exception of [42] who reported a higher LC_{50} value. The differential LC_{50} 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 [43-46].

Histomorphological changes and degeneration of gill lamellae has been reported by several authors. [47] reported on the histopathological alterations in the gills and liver of *Clarias gariepinus* juveniles exposed to acute concentrations of *Anogeissus leiocarpus*, [48] when investigating on the biochemical and histological effects of deltamethrim on *Carassius auratus gibelio* with different effects such as lamellae cells hypertrophy and nuclear pycnosis in the basal cells, [49] reported histopathological changes in the gills of *Clarias gariepinus* exposed to refined petroleum oil and kerosene under laboratory conditions, histopathological report of [50] 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 [51-53].

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 [38] 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, [54] when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *Clarias gariepinus* juveniles; [55] when studying the effect of *Euphorbia hirta* leaf extract on histopathology of juveniles *Clarias gariepinus* and [23] when reporting on the histopathological alterations in gills of fingerlings of *Clarias gariepinus* following sub-lethal acute exposure to *Hevea brasiliensis.*

5. CONCLUSION

Dose-response relationship and histo-morphological alterations on *Orechromis 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 LC_{50} 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 *L. 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, *L. camara* cannot be used as s raw material in the formulation of fish feed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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