

## Behavioral responses of *Oreochromis niloticus* juveniles exposed to *Leptadania hastata*, *Nymphaea lotus* extracts and urea fertilizer under static system

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### Abstract

Behavioral responses are sensitive indicators of environmental and chemical stress in fish. This study evaluated the behavioral effects of *Leptadania hastata*, *Nymphaea lotus* extracts, and urea fertilizer on *Oreochromis niloticus* juveniles under a static system. Four hundred and eighty (480) *Oreochromis niloticus* juvenile fish, averaged weight (17g) were divided into 48 tanks, exposed to five concentration levels of each treatment: *Leptadania hastata* (1–5ml), *Nymphaea lotus* (5–20ml), and urea fertilizer (0.5–2.5ml). Fish were exposed to graded concentrations of each treatment for 96 hours, and behavioral parameters, including swimming patterns, surfacing, erratic movement, feeding activity, and opercular movement, were recorded. The results showed significant dose-dependent alterations in behavior across all treatments, with high concentrations causing increased erratic swimming, gasping at the water surface, reduced feeding, and lethargy. *Leptadania hastata* extract induced more pronounced behavioral changes compared to *Nymphaea lotus* and urea treatments. *Leptadania hastata* was the most toxic, with an LC50 of 1.70 ml, followed by urea (1.62 ml), and *Nymphaea lotus* (14.79 ml). About 90% mortality occurred at 2.29 ml for *Leptadania hastata*, 2.40 ml for urea, and 28.18 ml for *Nymphaea lotus*. Overall, all treatments caused dose-dependent physiological and tissue damage to the fish. It is therefore, recommended that the application of plant extracts and urea fertilizer in aquaculture systems be carefully regulated to prevent sub-lethal and lethal effects on cultured fish species. Safe concentrations thresholds should be established before the use of these substances in aquaculture, particularly during the juvenile stages of fish culture.

**Keywords:** *Oreochromis niloticus*, behavioral toxicity, *leptadania hastata*, *nymphaea lotus*, urea fertilizer

### Introduction

*Oreochromis niloticus* is a major freshwater fish species cultivated worldwide due to its fast growth, adaptability, and high nutritional value. *Oreochromis niloticus* (Nile tilapia) is among the most widely farmed fish species globally due to its fast growth, high fecundity, and adaptability to diverse environments. In Nigeria, its domestication began in the 1970s and it has since become a staple in aquaculture [1]. Its omnivorous feeding habits, tolerance to fertilized waters, and high reproductive potential make it suitable for intensive and semi-intensive systems. However, the species is vulnerable to overpopulation and can be adversely affected by waterborne toxicants. Understanding the toxicity levels of plant extracts and fertilizers on *O. niloticus* juveniles is crucial to establishing safe levels of exposure and developing eco-friendly aquaculture practices. However, aquaculture systems are increasingly exposed to natural plant extracts and chemical fertilizers, which may have toxicological implications. Behavioral responses in fish, such as swimming activity, feeding, and opercula movements, are sensitive indicators of stress and environmental toxicity.

*Leptadania hastata* and *Nymphaea lotus* are medicinal plants with bioactive compounds that may exert toxic effects at high concentrations. Urea fertilizer, commonly used to enhance aquatic productivity, can also negatively impact fish health when applied excessively. Understanding behavioral responses to these substances is crucial for assessing their safety in aquaculture systems.

Aquaculture has become an essential component of food security and economic development in Nigeria. However, its

sustainability is increasingly threatened by environmental contaminants, particularly from agricultural runoff containing fertilizers such as urea. These substances can alter water chemistry, reduce dissolved oxygen, and lead to toxic effects on cultured fish species like *Oreochromis niloticus*, which is widely farmed across the country. Despite its economic importance, there is limited data on the toxicity thresholds of commonly used fertilizers in relation to juvenile tilapia health and survival [2].

At the same time, traditional medicinal plants such as *Leptadania hastata* and *Nymphaea lotus* are gaining interest in aquaculture as potential feed additives or immune boosters due to their bioactive and therapeutic properties [3]. While these plants are known for their antimicrobial and antioxidant effects, their safety profile in aquatic systems remains inadequately studied [4]. The presence of phytochemicals such as alkaloids, saponins, and tannins, while beneficial at low concentrations may exert toxic effects at higher doses, particularly in sensitive aquatic organisms [5]. The excessive growth of these macrophyte are causing deleterious effect in many earthen endosomes results to weed. Testing their lethal effect can provide a baseline information for their positive use in aquaculture. The lack of toxicological data on these plants and fertilizers presents a major knowledge gap, making it difficult to assess their potential risks or benefits in fish farming systems [6]. Without proper evaluation, their application may pose threats to fish health, reduce growth performance, or compromise the quality of aquaculture products intended for human consumption [7].

It is therefore on this basis that there is an urgent need to evaluate the toxicological effects of *Leptadania hastata*, *Nymphaea lotus* extracts, and urea fertilizer on the behavioral response of *O. niloticus* juveniles under static system.

## Materials and Methods

### Experimental Site

The experiment was conducted at the Department of Fisheries and Aquaculture Teaching and Research Farms located at the main campus of Usmanu Danfodiyo University, Sokoto, on latitude 13° 07' 78" N and longitude of 05° 12' 25" E at 275m above sea level (Google, 2011). The site is located in Sudan Savanna agro-ecological vegetation zone of Nigeria. The climate is characterized by a long dry season, cool dry air during the harmattan from November to February and hot season from March to May. Annual rainfall in the area ranged from 500 to 724 mm. The mean relative humidity is 14.9% and 40% between March and June and 41°C maximum [8].

### Experimental Fish

Four hundred and eighty healthy juveniles of *Oreochromis niloticus* of averaged weight 17±0.5g were used for the toxicity test. The *Oreochromis niloticus* juveniles were obtained from the Teaching and Research Farm of the Department of Fisheries and Aquaculture Usmanu Danfodiyo University, Sokoto.

### Preparation of Treatments

The stock solution of the test chemical was prepared by dissolving 300g of Urea fertilizer in 11 litre of test water in a conical flask following the procedures of [9]. Each treatment was tested using concentration of the urea fertilizer. This was done by placed five normal concentration (0.5, 1.0, 1.5, 2.0 and 2.5 ml) of stock solution of urea fertilizer in separate tanks and ten fishes were stocked in each tank. Mortality of the stocked fish was observed at 0, 12, 24, 48, 72 and 96 hours.

### Collection and Processing of plant leaves samples

Fresh leaves of *Leptadania hastata* and *Nymphaeaceae lotus* were collected from earth ponds at the Teaching and Research farms of the Department of Fisheries and Aquaculture at the main campus of Usmanu Danfodiyo University, Sokoto, and the plants were identified and authenticated with the aid of [10]. The fresh leaves were collected, washed to remove any dirt material and air-dried at room temperature (25±2°C) for 2 weeks 1kg was prepared. After drying, the leaves were grinded using Laboratory pestle and mortar and then sieved with 100-micron sieve to obtain a fine powder. Three hundred grams (300g) of the dry fine powder of each were later soaked in 11 liters of extracted (agitated in an orbital shaker at 185r/min for 48 h) with 1000mL of deionized water for 48hrs. The solution was filtered using muslin cloth to separate extract from residue. The aqueous solution was kept in a plastic container at room temperature (25±2°C) and immediately applied in the different treatment concentration

### Experimental Design

The experiment was analyzing by placing the treatments in a

Randomized Complete Block Design (RCBD) consisting of three treatments and five different levels of varying concentrations of *Leptadania hastata* (1ml, 2ml, 3ml 4ml and 5ml), Urea fertilizer (0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml) and *Nymphaeaceae lotus* (5ml, 8ml, 12ml, 15ml, and 20ml). Ten fishes (*O. Niloticus*) were allotted to each test tank in triplicates.

### Bioassay Test

A four-day static toxicity bioassay was conducted in the Department of Fisheries and Aquaculture Teaching and Research Farm I (production section), Usmanu Danfodiyo University, Sokoto.

### Behavioral Observations

During the bioassay test, observations of the behavioral responses such as frequent jumping, erratic swimming, and loss of reflex, hyperventilation and discolouration were made at 0, 12, 24, 48, 72 and 96hours post-exposure following the procedure [11]. Each observation was noted in each treatment.

### Water quality parameters

Water quality parameters monitored during the experimental period included pH, Dissolved Oxygen (DO), Temperature, Nitrate (NO<sub>2</sub>), and Ammonia (NH<sub>3</sub>/NH<sub>4</sub>). Readings were taken four times daily at four-hour intervals: 9:00 AM, 2:00 PM, 6:00 PM, and 10:00 PM, throughout the 96-hour toxicity exposure period. Water samples were collected from each experimental tank (including treatment and control groups) using 500 mL clean plastic flasks, previously rinsed with distilled water and acclimatized with water from the respective tanks to avoid contamination. For each sampling time, a composite sample was taken from three different points (top, middle, and bottom) in the tank and thoroughly mixed to ensure representativeness.

The following equipment and methods were used:

- pH:** Measured using a digital handheld pH meter (Model: Hanna HI 98107), calibrated before use with standard buffer solutions (pH 4.0, 7.0, and 10.0).
- Dissolved Oxygen (DO):** Determined using a portable DO meter (Model: YSI Pro20i).
- Temperature:** Recorded simultaneously using the temperature probe of the DO meter and cross-verified with a mercury-in-glass laboratory thermometer.
- Nitrate (NO<sub>3</sub><sup>-</sup>):** Determined using spectrophotometric method (APHA, 2017) after sample filtration using Whatman No. 42 filter paper (APHA, 2017).
- Ammonia (NH<sub>3</sub>/NH<sub>4</sub>):** Measured using the Nesslerization method, read at 425 nm wavelength using a UV-Vis spectrophotometer (Model: Jenway 7315).

All water quality analyses were carried out at the Agricultural Chemistry Laboratory, Department of Soil Science and Agricultural Engineering, Usmanu Danfodiyo University, Sokoto. Parameters were also measured at 24-hour intervals (i.e., at 0, 24, 48, 72, and 96 hours) to monitor temporal trends across treatments.

### Statistical Analysis

Accumulative mortalities of exposed fish were used to estimate the LC<sub>50</sub> and LC<sub>90</sub>. This was determined from the graph of percentage mortality against concentration using Probit Analysis. All biological data resulting from the experiments were subjected to One-Way Analysis of Variance (ANOVA) using SPSS 18.0 software. Differences were considered significant at  $P < 0.05$

### Results and Discussion

#### Behavioral responses of *O. niloticus* to *Nymphaeaceae lotus*

The behavioral responses of the tested fish to the *Nymphaeaceae lotus* at different concentrations were observed and recorded as presented in Table 1. Observations were carried out for external changes on the fish body during the experiment. The behavioral changes observed were erratic swimming, frequent jumping loss of reflex, hyperventilation and discoloration. No significant behavioral responses were observed in (0ml, 5ml, 8ml, 12ml, 15ml and 20ml) for 48 hours respectively. The fish with 12ml concentration did not show any signs of frequent jumping and discoloration but started showing signs of erratic swimming after 48 hours, loss of reflex after 72 hours. Abnormal behaviors displayed by the fish increased with increasing concentration of the test extract and with the time of exposure.

There were no obvious changes in fish behavior at concentrations less than 15ml for the first 48 hours of exposure. However, the control (0.0ml) fish did not exhibit any abnormal behavior (Table 1). Those with concentration 12ml, 15ml and 20ml were calm for only 48 hours and then started showing signs of frequent jumping at 72 hours, erratic swimming, hyperventilation, loss of reflex and discoloration at 72 hours after which they all died after 72 hours of treatment. The tested fish showed increase in weakness, motionless and gasping for air with slow opercula movement as the concentration level of *Nymphaeaceae lotus* increases with duration of exposure. The fish were confirmed dead when they could not respond to any stimulus.

Recent phytochemical and acute-toxicity screening work [12] for *N. lotus* shows a mixed picture: some studies (rodent models / fraction studies) report limited acute toxicity at traditional doses, while others focusing on aquatic exposure demonstrate behavioral and growth impacts at higher concentrations echoing caution about dose-dependent effects. Literature on *L. hastata* is sparser for fish, but recent sub-chronic studies in mammals and older fish studies suggest potential organ/tissue effects at higher doses; that support emphasis on the knowledge gap for fish, and that more fish-specific toxicology is needed.

#### Behavioral Response of *O. niloticus* to Urea Fertilizer

The behavioral responses of the tested fish to the toxicant at different concentrations were observed and recorded (Table 2). Further observations were carried out for external changes on the fish body during the experiment. The behavioral changes observed were erratic swimming, frequent jumping loss of reflex, hyperventilation and discoloration. No significant behavioral responses were observed in the fish in concentrations (20ml) for 24 hours. The fish with concentrations (0ml, 0.5ml, 1.0ml, 1.5ml and 20ml) respectively did not show any signs of frequent

jumping and discoloration but started showing signs of erratic swimming after 12 hours, loss of reflex after 24 hours. Abnormal behaviors displayed by the fish increased with increasing concentration of the test extract and with the time of exposure. There were no obvious changes in fish behavior at concentrations less than 20ml for the first 24 hours of exposure. However, the control (0.0ml) fish did not exhibit any abnormal behavior (Table 2). Those with concentration 1.0ml, 1.5ml, 2.0ml and 2.5ml were calm for 24 hours and then started showing signs of frequent jumping, erratic swimming, hyperventilation, loss of reflex and discoloration at 48 hours after which they all died after 72 hours of treatment. The tested fish showed increase in weakness, motionless and gasping for air with slow opercula movement as the concentration level of Urea fertilizer increases with duration of exposure. Behavioral responses noted were frequent jumping, restlessness, skin discoloration, opercula movement, hyperactivities, gulping of air and erratic swimming before death (Table 2). At very low concentration (0ml, & 0.5ml), test organisms tolerated the test solution up to 24hours of exposure, while at high doses (1.0ml, 1.5ml, 2.0ml, and 2.5ml), and they lost their swimming patterns. As the experiment progressed, the test organisms were seen to get weaker, with ventral surface turned upward and those that couldn't tolerate the concentrations any longer went into comatose.

Multiple studies corroborate that acute and sub-lethal urea exposures cause behavioral stress in tilapia (gasping, erratic swimming, loss of equilibrium), and that altered DO, conductivity and ammonia are important mediators of those effects. Newer [13] experimental work uses similar bioassay durations (24–96 h) and recommends better guidelines for safe pond urea dosing consistent with call for establishing safe thresholds.

#### Behavioral Response of *O. niloticus* to *Leptadania hastata*

The behavioral responses of the tested fish to the toxicant at different concentrations were observed and recorded (Table 3). Further observations were carried out for external changes on the fish body during the experiment. The behavioral changes observed were erratic swimming, frequent jumping loss of reflex, hyperventilation and discoloration. No significant behavioral responses were observed in the fish in concentrations (0ml) for 96 hours. The fish with concentrations (0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml) respectively shows signs of erratic swimming, frequent jumping, hyperventilation, loss of reflex and discoloration before 12 hours of exposure. Abnormal behaviors displayed by the fish increased with increasing concentration of the test extract and with the time of exposure. The test organism showed distress in behavior immediately they were introduced into the bioassay tanks. Abnormal behaviors displayed by the fish increased with increasing concentration of the test extract and with the time of exposure. There were obvious changes in fish behaviour at concentrations less than 1ml for the first 12 hours of exposure. However, the control (0.0ml) fish did not exhibit any abnormal behavior (Table 3). Behavioral responses noted were frequent jumping, restlessness, skin discoloration, opercula movement, hyperactivities, gulping of air and erratic swimming before death. At very low concentration (1ml), test organisms don't tolerate the test solution up to 24 hours of exposure, while at high doses (2ml, 3ml, 4ml and 5ml) they lost their swimming patterns.

As the experiment progressed, the test organisms were seen to get weaker, with ventral surface turned upward and those that couldn't tolerate the concentrations any longer went into comatose. The tested fish showed increase in weakness, motionless and gasping for air with slow opercula movement as the concentration level of *Leptadania hastata* increases with duration of exposure.

Fish toxicity studies for *L. hastata* are relatively limited

compared with other macrophytes, but older and recent reports on *Leptadania* extracts (including mammalian subchronic studies) indicate bioactive compounds that can induce organ/tissue and behavioral effects at moderate to high doses. Finding showed that *L. hastata* shows stronger acute behavioral toxicity than *N. lotus* is consistent with previous reports in non-fish models and an aquatic toxicity study [5].

**Table 1:** Behavioral responses of *O. niloticus* to *Nymphaeaceae lotus*

Concentration (ml)	24 Hours						48 Hours						72 Hours						96 Hours					
	0.0	5	8	12	15	20	0.0	5	8	12	15	20	0	5	8	12	15	20	0.0	5	8	12	15	20
Frequent jumping	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+
Hyper ventilation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+

Note: "+" indicates presence, "-" indicates absence

**Table 2:** Behavioral Response of *O. niloticus* to Urea Fertilizer

Concentration (ml)	24 Hours						48 Hours						72 Hours						96 Hours					
	0	0.5	1.0	1.5	2.0	2.5	0	0.5	1.0	1.5	2.0	2.5	0	0.5	1.0	1.5	2.0	2.5	0	0.5	1.0	1.5	2.0	2.5
Frequent jumping	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Erratic swimming	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Loss of reflex	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Hyper ventilation	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Discoloration	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+

Note: "+" indicates presence, "-" indicates absence

**Table 3:** Behavioral Response of *O. niloticus* to *Leptadania hastata*

Concentration (ml)	24 Hours						48 Hours						72 Hours						96 Hours					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Frequent jumping	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Erratic swimming	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Loss of reflex	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Hyper ventilation	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Discoloration	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+

Note: "+" indicates presence, "-" indicates absence.

**Conclusion and Recommendation**

The vast growth in human population and the proliferation of industries in Nigeria has resulted in the discharged of large amount of toxic waste into water bodies where they pollute and degrade both the flora and fauna of the ecosystem resulting in habitat loss. This study has revealed that *Oreochromis niloticus* juvenile's exposure to extract from *Leptadania hastata*, Urea fertilizer and *Nymphaeaceae lotus* is enough to induce various toxic effect which make the fish vulnerable to diseases and eventually death.

From this study, it is evident that increasing concentrations of *Leptadania hastata*, Urea fertilizer and *Nymphaeaceae lotus* could lead to fish abnormal behavioral responses and dysfunction in the health of the fish. *Leptadania hastata*, *Nymphaea lotus* extracts, and urea fertilizer significantly affect the behavioral responses of *O. niloticus* juveniles. High concentrations induced erratic swimming, reduced feeding, and increased surfacing. These results underscore the need for cautious use of plant extracts and fertilizers in aquaculture systems.

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