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Acute Toxicity of Graded Palm Oil Mill Effluents on Nile Tilapia (*Oreochromis niloticus* Linnaeus 1758) and African Sharptooth Catfish (*Clarias gariepinus* Burchell, 1822) Fingerlings

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Abstract

This study evaluated the acute toxicity of graded palm oil mill effluents on Nile tilapia(*Oreochromis niloticus* and *Clarias gariepinus*) fingerlings. The fish were obtained from an earthen fish ponds in the Niger Delta University fish farm. The fish were allowed to adapt in a concrete aquarium for 2 weeks. Range findings tests of the palm oil mill effluents were performed. Based on the preliminary results, two series of test concentrations were defined, for *Oreochromis niloticus* ranging between 0.45 and 1.65 mL/L, and for *Clarias gariepinus* ranging between 2.25 and 4.25 mL/L. The experimental set-up was a 24hour renewal bioassay with three replicates each. The results showed behavioural responses including air gulping, surfacing, aberrant swimming, changed opercular movements and changed body pigmentation at varying levels. Mortality rates increased significantly ($p<0.05$) with increasing concentrations of the palm oil mill effluents. The LC_{50} values of *Oreochromis niloticus* fingerlings exposed to palm oil mill effluents at 24 hours, 48 hours, 72 hours and 96 hours were 2.981 mL/L, 1.437 mL/L, 0.963 mL/L and 0.935 mL/L, respectively. The corresponding LC_{50} values for *Clarias gariepinus* at 24 hours, 48 hours, 72 hours and 96 hours were 7.501 mL/L, 5.070 mL/L, 3.602 mL/L and 2.919 mL/L, respectively. The LC_{50} values showed that *Clarias gariepinus*can tolerate higher exposure concentrations of palm oil mill effluents than *Oreochromis niloticus*. The study found that palm oil mill effluents are toxic to fish already at low concentrations. To prevent exposure of fish via runoff, palm oil mills should not be located close to slow flowing surface water. Also direct discharge of effluents into surface water bodies should be prevented.

Keywords: Aquatic Ecosystem; Aquatic Pollution; Fish; Palm Oil Mill Effluents; Water Quality

Introduction

The oil palm is the most productive of all oil bearing crops and has by far exceeded the production of other oil-generating plants such as cotton seed, rapeseed, soybean, groundnut etc. Malaysia, Indonesia, Thailand, Colombia and Nigeria are the leading producing countries accounting for over 90% of total production output. Of these leading countries, Nigeria contributes the least (1 015 000 metric tonnes), about 1.5% of the global output. Oil palm industry in Nigeria is dominated by smallholders. Palm oil processing is a major source of livelihood to several families, especially in southern Nigeria, where oil palm trees are found in the wild and in plantations [1,2,3]. Oil palm cultivation and processing is dominated by smallholders that have only basic equipment for processing available [4].

During palm oil processing three major wastes are produced, including gaseous emissions (air pollutants), solid wastes (empty fruit bunch, palm press fiber, chaff) and liquid wastes (palm oil mill effluents). These wastes are poorly managed in Nigeria. The solid

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wastes and palm oil mill effluents are produced in large quantities in the production mills. A significant amount of the palm oil mill effluents is discharged directly into the environment while a small quantity is reused during boiling processes in the palm oil mills [5]. These palm oil mill effluents have been reported to be causing environmental pollution (soil, air and water) [6].

In the surface water, palm oil mill effluents turn the water brown, smelly and slimy [7]. This could impact on the downstream application of the water and affect aquatic organisms, especially fish. Authors have reported that palm oil mill effluents alter the physicochemical characteristics (temperature, pH, total alkalinity, total solid, total dissolved solid, total suspended solid, magnesium, calcium, sodium, potassium, chloride, sulphate, nitrate, phosphate, zinc, iron, manganese, dissolved oxygen, biological oxygen demand and oil and grease) of receiving water system [7,8]. Increased nutrient concentrations in the water such as nitrate, sulphate, phosphate could cause eutrophication, while the acid pH could promote possible acidification of the water [6]. Changes in water quality such as pH, temperature, salinity, conductivity, hardness affects the survival of fish and other aquatic organisms in the aquatic ecosystem. Specifically, a decrease in dissolved oxygen content could influence the survival of fish in aquatic ecosystem. Considering the potential of palm oil mill effluents to affect aquatic ecosystems, there is a need for this to be studied. Behavioural response in fish often provides preliminary information about the toxicity to an aquatic ecosystem. Acute toxicity of fish provides vital information about the short term basis of a toxicant on the aquatic ecosystem. Several aquatic organisms such as macrophytes, plankton and fish have been widely used to assess pollution in aquatic ecosystem. Among the fish, *Clarias gariepinus* and *Oreochromis niloticus* are among the common fish species that have been widely used in ecotoxicological studies. Hence this study aimed to assess the acute toxicity of palm oil mill effluents on Nile tilapia (*Oreochromis niloticus*) and African sharp tooth catfish (*Clarias gariepinus*) fingerlings.

Materials and Methods

Source of Fish, Transportation and Acclimation

A total of two hundred fingerlings of *Clarias gariepinus* and *Oreochromis niloticus* with a mean weight of 6.5 g (±1.22) and a length of 6 cm (±1.33) were obtained from the wild in the Niger Delta University earthen fish farm using a drag net. The fish were allowed to adapted to the environment for 14 days. During the adaptation period the fish were fed with fish feed. The palm oil mill effluents used in this study were obtained from a smallholder oil palm processor in Ozoro, Delta state, Nigeria.

Range Finding Test (Trial Test)

The range finding test was carried out following a renewal bioassay approach. During experimentation, the test toxicant (palm oil mill effluents) and test solution (borehole water) were renewed daily. A range finding test (trial test) was carried out using the palm oil mill effluents in the following concentrations (2.5 mL/L, 5.00 mL/L, 7.50 mL/Land 10.00 mL/L). Each concentrations of the range finding test contained 4 fish individuals.

Main Experiment

Approximately 0.00 mL, 22.5 mL, 27.5 mL, 32.5 mL, 37.5 mL and 42.5 mL of palm oil mill effluents were pipetted into rectangular aquarium containing 10 litres of water. For *Clarias gariepinus* this resulted in test concentrations of 0.0 (control), 2.25, 2.75, 3.25, 3.75 and 4.25 mL/L. For *Oreochromis niloticus* 0.0 mL, 4.5 mL, 7.5 mL, 10.5 mL, 13.5 mL and 16.5 mL were transferred into 10.0 L of aquarium water each, resulting in test concentrations of 0.0 (control), 0.45, 0.75, 1.05, 1.35 and 1.65 mL/L. The experimental groups (containing 10 fish each) were carried out in triplicate and dispense into (10 L aquarium water and the palm oil mill effluents were renewed every 24 hours Fish were not fed during the 96 hours of exposure. Mortality was determined if a fish did not respond to repeated prodding [9].

Behavioural Response

Behavioural changes were assessed by observing the movements of the fish based on the characteristics previously reported by Oyoroko and Ogamba [10]. The water quality parameters such as pH, temperature, conductivity and salinity were measured using an in–situ portable meter. The results for pH, temperature, salinity and conductivity ranged from 5.7 – 6.6, 25 - 27 ^{o}C, 0.02 – 0.03 mg/l and 63.2 – 74.4 µS/cm., respectively, across the various concentrations of the palm oil mill effluents.

Statistical Analysis

SPSS version 20 was used for the statistical analysis. The data were expressed as mean ± standard error. One-way analysis of variance was carried out $p<0.05$, and where significant difference occurred Waller Duncan statistics was used for mean separation. The percentage mortality was converted to Probit using Finney's Table [11], and then a linear regression of the probit values against the logarithm of the concentrations was carried out using Microsoft Excel. The LC50 values were determined by substituting the 50 probit values into the equation derived from the regression analysis and obtaining the anti-logarithm values [12,13].

Results and Discussion

The behavioural response of *Oreochromis niloticus* and *Clarias gariepinus* exposed to palm oil mill effluents included slight body pigmentation, intermittent swarming and jerky movements that were not observed in the control. Their rate increased as the concentration of the palm oil mill effluents increased. Surfacing and air gulping were low at lower concentration but increased with increasing concentrations of the toxicant. The fish showed moderate swimming and opercular movement at lower concentrations, movements increased with an increasing concentration. These behavioural responses have been reported before when fingerlings are exposed to toxicants [9,14]. The variation in the response suggest the effect of stress. The increased air gulping and opercular movement suggests respiratory distress, probably due to the effect of oil in the effluents. Oil has the tendency to deprive oxygen in aquatic ecosystem. The various behavioural responses that deviate from the control suggest the effect of stress on the test organisms.

Table 1: Mortality rate of *Oreochromis niloticus* fingerlings exposed to graded concentration of palm oil mill effluents

Concentration, mL/L	0 hours	24 hours	48 hours	72 hours	96 hours
0.00	0.00±0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
0.45	0.00±0.00	6.67±3.33ab	13.33±3.33a	23.33±3.33b	30.00±5.77b
0.75	0.00±0.00	10.00±0.00ab	30.00±5.77b	40.00±0.00c	46.67±3.33b
1.05	0.00±0.00	16.67±3.33b	30.00±5.77b	50.00±5.77c	60.00±5.77b
1.35	0.00±0.00	30.00±5.77c	50.00±5.77c	63.33±6.67d	80.00±5.77d
1.65	0.00±0.00	33.33±3.33c	56.67±3.33c	73.33±3.33d	90.00±5.77d
LC_{50}[mL/L]	n.d.	2.981	1.437	0.963	0.935

Data is expressed as mean± standard error (n=3). Different letters along the column indicate significant variations at $p<0.05$ according to Duncan statistics

Table 2: mortality rate of *Clarias gariepinus* fingerlings exposed to graded concentration of palm oil mill effluents

Concentration, mL/L	0 hours	24 hours	48 hours	72 hours	96 hours
0.00	0.00±0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
2.25	0.00±0.00	13.33±3.33b	20.00±5.77b	26.67±6.67b	33.33±8.82b
2.75	0.00±0.00	13.33±3.33b	23.33±3.33bc	33.33±3.33b	40.00±5.77bc
3.25	0.00±0.00	16.67±3.33b	30.00±5.77bcd	40.00±5.77b	56.67±6.67cd
3.75	0.00±0.00	16.67±3.33b	36.67±3.33cd	53.33±3.33c	73.33±3.33de
4.25	0.00±0.00	36.67±3.33c	43.33±6.67d	63.33±3.33c	80.00±5.77e
LC50 [mL/L]	n.d.	7.501	5.070	3.602	2.919

Data is expressed as mean± standard error (n=3). Different letters along the column indicate significant variations at $p<0.05$ according to Duncan statistics

Data is expressed as mean± standard error (n=3). Different letters along the column indicate significant variations at $p<0.05$ according to Duncan statistics The LC_{50} values of fingerlings of *Oreochromis niloticus* exposed to palm oil mill effluents at 24 hours, 48 hours, 72 hours and 96 hours were 2.981 mL/L, 1.437 mL/L, 0.963 mL/L and 0.935 mL/L, respectively. For Clarias gariepinus for LC_{50} values of 7.501 mL/L, 5.070 mL/L, 3.602 mL/L and 2.919 mL/L were observed at 24 hours, 48 hours, 72 hours and 96 hours, respectively.

The mortality rate showed that as the concentration of the palm oil mill effluents increased the mortality rate increased. In addition, no mortality was observed in the control. The observed pattern with mortality increasing with exposure concentrations is typical for toxic effects and is in line with patterns reported by a multitude of authors [9, 10,12,13,15]. Also the increase of effects over time, resulting in a decline of LC_{50}-values over time is typical for prolonged exposure situations. The LC_{50} values showed a decline in values as the exposure period increased in fingerlings of both fish species. Lower LC_{50} values indicate higher toxicity level. As such, the palm oil mill effluents were more toxic to fingerlings of *Oreochromis niloticus* compare to fingerlings of *Clarias gariepinus*. The findings of this study did not conform with the observation of Akinsorotan [16] reported an LC_{50} value of 9.19 mg/L after exposing juvenile *Oreochromis niloticus* to 96 hour of palm oil mill effluents. The observed differences could be due to the age/ size of the fish, and well as the composition of oil mill effluents, which are neither constant over time nor between different oil mills. Anyway, palm oil mill effluents are known to have a low pH, to increase the chemical and biological oxygen demand, total dissolved and suspended solids, debris, heavy metals and alkaline earth metals [16,17,18,19,20,21,22]. Akinsorotan [16] reported that palm oil mill

effluents is toxic to *Oreochromis niloticus* and has the tendency to cause erratic movement, air gulping, loss of reflex, molting, barbell, haemorrhage and excessive mucus secretion. The author also reported that as the exposure period increases it could cause cell proliferation, lamellar fusion, lamellar cell hyperplasia, and epithelial lifting in the gills; and in the liver, it causes vacuolation of hepatocytes and necrosis [23,24,25].

Conclusion

This study investigated the behavioural response and mortality rate of fingerlings of *Oreochromis niloticus* and *Clarias gariepinus* exposed to palm oil mill effluents. The fish exhibited body pigmentation, intermittent swarming and jerky movement, surfacing and air gulping at the different concentrations. The mortality rates increased as the concentrations of the palm oil mill effluents increased. The LC_{50} values showed that palm oil mill effluents are more lethal to fingerlings of *Oreochromis niloticus* than to Clarias gariepinus fingerlings. This suggests that *Clarias gariepinus* can tolerate the toxicity of palm oil mill effluents than *Oreochromis niloticus*. Due to the risk that palm oil mill effluents can induce toxic effects in fish, processing mills should not be located close to surface water system that could receive the effluents after rainfall, and also effluents should never be discharged directly into adjacent surface water bodies.

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