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Original Research Article

Acute Toxicity and Behavioural Changes of *Oreochromis Niloticus* Juveniles Exposed To Tamarind (*Tamarindus Indica*) Seed Husk Powder

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Abstract: This study was carried out to determine the toxicity of tamarind seed husk powder to Nile tilapia (*Oreochromis niloticus*). The range – finding test was conducted twice in order to get reliable values that could be used for the definitive test. The toxicant (tamarind seed husk powder) was introduced at varying concentrations of 0mg/l, 2mg/l, 4mg/l, 6mg/l , 8mg/l and 10mg/l in the first range- finding test, while it was introduced at concentrations of 1mg/l, 2mg/l,3mg/l, 4mg/l and 5mg/l in the second range- finding test. However, in the definitive test, the toxicant was introduced at varying concentrations of 1.2mg/l, 1.4mg/l, 1.6mg/l, 1.8mg/l and 2.0mg/l. From the definitive test, the Median Lethal Concentration of tamarind seed husk powder to *Oreochromis niloticus* was found to be 1.52mg/l. The study showed that *Oreochromis niloticus* juveniles exhibited initial erratic movement, rapid opercular movement, skin discoloration and loss of reflex. The water quality data were analyzed by subjecting them to one way Analysis of Variance (ANOVA).Thus, the study indicates that tamarind seed husk powder is toxic to *Oreochromis niloticus* juveniles.

Keywords: Toxicity, tamarind seed husk powder, *Oreochromis niloticus* and behavioral changes.

INTRODUCTION

Pollution is a major problem in the aquatic environment. The fish in this environment is greatly affected as their immune system is continuously affected by periodic or unexpected changes of their environment. Adverse environment situations may acutely or chronically stress the health of fish, altering some of their biological parameters and suppressing their innate and adaptive immune responses (Giron-peres et al., 2007).

Plants are chemical factories, unlike animals having the luxury of teeth and claws and legs to help them get out of a tight spot, most plants spend their lives in one place and have evolved to rely upon elaborate chemical defenses to ward off unwanted predators. For this reason, plants have in their arsenal an amazing array of thousands of chemicals noxious or toxic to bacteria, fungi, insects, herbivores and humans. Fortunately, this chemical diversity also includes many compounds that are beneficial to humans: vitamins, nutrients, anti-oxidants, anti-carcinogens, and other compounds with medicinal value (Novak and Haslberger, 2000). Most plant species in the world are not edible, many because of the toxins they produce. The process of domestication has gradually reduced the levels of these compounds over the millennia so that plant foods are far less toxic than their wild relatives. Many of these toxins evolved as a way to fight off predators, not surprisingly, modern food plants are much more susceptible to disease. Toxin concentrations in a plant can vary tremendously, often by 100 times or more, and can be dramatically affected by environmental stress on the plant (drought, heat, cold and mineral deficiencies) and disease. Different varieties of the same plant species can also have different levels of toxins and nutritional value (D'Mello, 2000).

Tamarind indica is a multipurpose tropical tree used primarily for its fruits, which are eaten fresh or processed, it is also used as seasoning or spice, the fruits and seeds are processed for non-food use. The species has a wide geographical distribution in the subtropics and semi-arid tropics and is cultivated in numerous regions of the world (El-Siddig et al., 2006). Virtually every part of the tree (wood, roots, leaves, bark, seed husk and fruits) is of a significant value in the subsistence of rural people as well as a number of commercial applications. The unique sweet or sour flavour of the pulp is popular in cooking and flavouring (El-Siddig et al., 2006). It is often made into juice, brine/infusion. In Ghana bitter infusion of the pods is usually used for cooking cereals and is often added to the water in which poisonous yams are soaked in order to detoxify them whereas, in India the pulp is used to preserve fish (up to 6 months), when mixed with acetic acid (Paul Das and Banerjee, 2014).

Tamarind is widely grown as a subsistence crop for meeting local demands. It is also grown commercially. Numerous national programmes have recognized tamarind as an underutilized crop with wider potential since demand for products is substantial and the species can be incorporated into agroforestry systems (El-Siddig et al., 2006). There are also well established international trade channels. Further exploitation of tamarind can therefore provide added incomes for poor rural people thereby improving their well-being (El-Siddig et al., 2006).

Aquatic bioassays are necessary in water pollution to determine when the potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effects on aquatic animal (Olaifa et al., 2003). The toxicity of a chemical is totally dependent on the concentration of the chemical in organism or even the concentration at the target receptor in the organism (Ayoola, 2008).Their roles in degradation of the aquatic ecosystem cannot be ignored (Omitoyin et al., 2006).

The Nile tilapia Oreochromis niloticus is a deep-bodied fish with cycloid scales, silver in colour with olive, grey or black body bars, the Nile tilapia often flushes red during the breeding season (Picker and Griffiths, 2011). It grows to a maximum length of 62 cm, weighing 3.65 kg (at an estimated 9 years of age) (FAO 2012) and its average size (total length) is 20 cm (Bwanika et al. 2004). O. niloticus is surface-feeding omnivore fish belong to the family Cichlidae. It is the most widely cultured fish in the tropics (Offem et al., 2010). It is commonly available and easy to manipulate both in capture and culture fisheries due to its qualities such as good taste, hardy nature, fast growth, resistance to diseases, ease with which to reproduce in captivity and switching of diet. Apart from being a good candidate for aquaculture, it serves as an important source of high level of animal protein especially in the developing countries where there are high levels of animal protein deficiencies (Fagbenro and Adebayo, 2002; Uchida et al., 2003; Ogunji et al.,2008).

The objectives of the study were to determine the: active toxic constituents of TSH powder using phytochemical analyses and 96-hour LC_{50} (acute toxicity) of O. niloticus juveniles exposed to increasing concentrations of TSH powder.

MATERIALS AND METHODS

Experimental Site

The study was carried out in the Department of Fisheries and Aquaculture, Bayero University Kano, Kano State

Experimental Fish

Juveniles of Oreochromis niloticus of a mean weight of 26.07±1.23g and mean length of 17.50 + 0.50cm were obtained from Rumbin kifi fish farm, Kano, Kano State. The fish were acclimatized for 14 days in the fish hatchery Department of Fisheries and Aquaculture, Bayero University Kano. The fish were fed twice daily at 0800 and 1600 hours at 5% of their body weight. Prior to and during exposure period fish were starved for 24hrs, to reduce faeces and ammonia in the experimental containers that could act as contaminants to the experiment.

Source and Processing Of Tamarind Seed Husk

The tamarind seed husks were collected within the premises of Bayero University Kano. The plant was authenticated in the Department of Forestry and Wildlife Management, Bayero University Kano where a specimen was maintained for the plant (NO. 1387). The seed husk was removed from the pod and air-dried to a constant weight at (27^oC). The dried samples was grounded into fine powdered form and sieved through 0.25mm sieve.

Physicochemical Parameters of Test Solution

Water quality parameters such as temperature, pH, conductivity, total dissolved solids (TDS) and dissolved oxygen (DO) concentration were monitored in the tanks using digital multi-parameter checker (HI 98126).

Experimental Design

A completely randomized design was used for the experiment. A total of one hundred and eighty (180) juvenile of Oreochromis niloticus were randomly distributed into the plastic containers (60x40x40) size and 70 litres capacity at a stocking rate of 10 fish. The eighteen (18) tanks were assigned to 5 treatments with (control inclusive). In order to determine the LC_{50}, the Oreochromis niloticus were exposed to five different concentrations of TSH powder for 96hrs.

Range Finding Test

Preliminary 96 hours range finding test was conducted for O. niloticus juvenile following static bioassay to determine the toxic range of tamarind seed husk powder to O. niloticus juveniles as described by Parrish (1985). For the range finding test 1.0mg, 1.5mg, 2.0mg, 2.5mg and 3.0mg tamarind seed husk powder per litre of water were used. Ten O. niloticus juveniles were separately weighed with a sensitive electronic weighing scale (mettler Toledo FB602) and stocked each into the 18 tanks filled with 20 litres of tap water. The seed husk powder was introduced directly into the tank containing the fish. The range finding test lasted for 96hours and observations were made at four-hour interval. The behavioural activities of the fish were properly monitored and recorded. The response of the fish to slight stimuli was used as an index of toxicity while the failure to respond to touch was used as an index of death. The LC_{50} of tamarind seed husk powder was determined after 96hours of exposure of test fish to tamarind seed husk powder.

Definitive Test

Results from range finding tests provided guide for the concentration level to be used in definitive test. The definitive test was carried out by filling eighteen (18) plastic tanks with 20 litres of water each. The test was carried out using concentration of tamarind seed husk powder earlier determined from range finding test. The concentrations used were 2.0mg, 4.0mg, 6.0mg, 8.0mg and 10mg of tamarind seed husk powder per litre of water (mg/l). The response of the fish to slight stimuli was used as an index of toxicity while non-response of the fish to tamarind seed husk powder estimated to be lethal to 50% of the test organism after 96 hour of exposure.

Fish Behavioural Pattern and Mortality

The behavioral pattern of the test fishes in each tank such as erratic swimming, gulping for air, loss of reflex were monitored and recorded. Fish mortality was monitored and recorded hourly, 4 hours for the next 24 hours and subsequently every 24 hours for the next 96 hours. The inability of fish to respond to external stimuli was used as an index of death. Dead fish were removed immediately with a scoop net to avoid contamination.

Statistical Analysis

All results were collated and analyzed using computerized, Probit analysis. The mean lethal concentration LC_{50} at selected periods of exposure and an associated 95% confidence interval for each triplicate toxicity test were subjected to probit analysis (Finney, 1971) using statistical package (SPSS, version 22) to determine (LC_{50}).

RESULTS AND DISCUSSION

The mortality rates of Oreochromis niloticus exposed to various acute concentration of TSH powder are shown on Table-1.

Table-1: Mortality of Oreochromis niloticus juveniles exposed to Tamarind Seed Husk Powder over the 96-hr period (Acute test)

Treatments Concentration (mg/L)	Log Concentration	Number Stocked	Mortality							Probit
			12 hrs	24 hrs	48 hrs	72 hrs	96 hrs	Total	%	
T0 (Control, 0)	0	30	0	0	0	0	0	0	0	0
T1 (1.2)	0.07	30	0	0	3	2	1	6	20	4.10
T2 (1.4)	0.14	30	0	0	4	3	3	10	33	4.56
T3 (1.6)	0.20	30	2	4	5	3	2	16	53	5.08
T4 (1.8)	0.25	30	9	5	4	4	3	25	83	5.95
T5 (2.0)	0.30	30	12	6	5	3	2	28	93	6.48

Mortality was directly proportional to the concentration of the toxicant and the length of exposure of the fish to the toxicant. In the definitive test, the toxicity of the toxicant increased with its increasing concentration and length of exposure of the test organism to it. The mortality was observed to be concentration and time dependent in this study. This shows that increase in concentration of TSH powder resulted in higher mortalities. In this study the highest mortality rate (93%) was recorded in T5 (2.0 mg/L) while the least mortality rate (20.0%) as recorded in T1 (1.2 mg/L) Omitoyin et al., (2006), Ateeq et al., (2005), Olurin et al., (2006) and Isiyaku et al., (2019) report similar trend in exposure of Oreochromis niloticus to malachite green, 2, 4-D butachlor, glyphosphate herbicides and mercuric chloride respectively. It has also been stated that substances could be poisonous when exposed to organisms beyond certain concentrations and time limit according to Ogundele et al., (2004). It was observed that the mortality recorded in this investigation increased with increase in the concentration of TSH powder. The first death was noticed 90 minutes after the introduction of toxicant in the bowl with the highest concentration of TSH powder (2mg/l). This is in conformity with Guedenon et al., (2012) who reported the first death in 30 minutes after introduction of toxicant to Clarias gariepinus in acute concentration of mercuric chloride. Olaifa et al (2004) reported the first death three hours after the introduction of toxicant in the exposure of Clarias gariepinus to lethal concentration of copper. Datta and Kaviraj (2002), Fafioye et al., (2004) and Okomoda et al., (2010) recorded the first death 36 hours after the exposure to acute toxicity treatment of Clarias gariepinus with synthetic pyrethyroid Deltamethrin, Raphia vinifera extracts and formalin respectively. Guedenon et al., (2012) recorded the first death after 30 hours while treating Clarias gariepinus with 120mg/l of cadmium sulphate. The duration of resistance of Oreochromis niloticus in the present study appeared to be lowest compared to those in the aforementioned studies.

The LC_{50} found in this investigation is 1.52mg/l similar to that of Hirt and Domitrovic, (1999) when Aequidens portalegneris was exposed to acute concentration of mercuric chloride. Slabbert and Venter (1999) reported LC_{50} of 0.20mg/l when Poecillia reticulate was exposed to mercuric chloride. Shyong and Chen (2000) reported LC_{50} value of 0.168mg/l and 0.161mg/l in the exposure of Variocorhinus barbatalus and Zacco Barbata respectively. Isiyaku et al., (2019), recorded 0.22mg/l as LC_{50} in an acute mercury toxicity treatment to Oreochromis niloticus. The median lethal concentration in this study was the highest recorded compared to those reported by previous workers. The chemical product being the same; the difference in the results could be attributable to the variety in the species used.

Behavioural changes such as loss of reflex and skin discoloration were more noticeable in the treatment that had the highest concentration of toxicant.

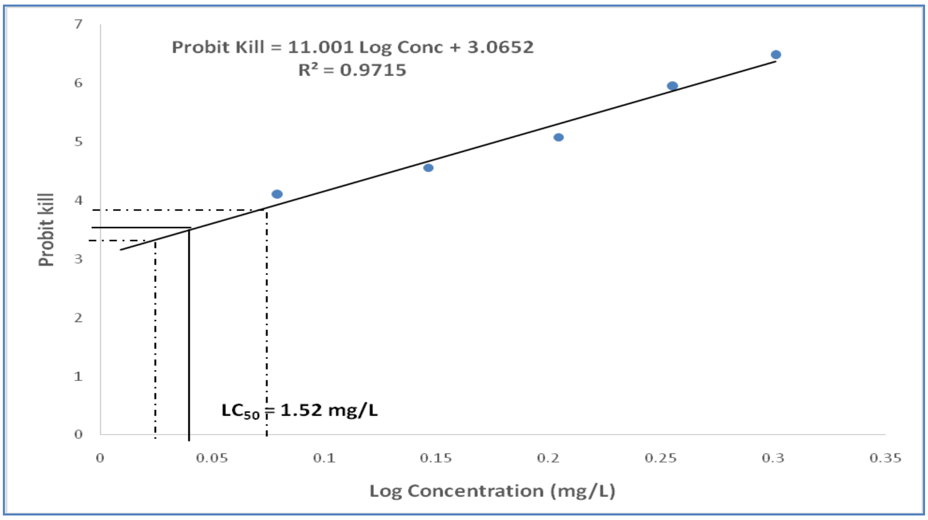

Fig-1: Linear relationship between mean probit mortality and log concentration of Oreochromis niloticus exposed to various concentrations of Tamarind Seed Husk Powder for 96 hrs

Table-2: Behavioral changes of Oreochromis niloticus juveniles in aqueous tamarind seed husk powder during 96-hour exposure at different concentrations

Behaviors	Concentration (mg/L)					
	0.00	1.2	1.4	1.6	1.8	2.00
Air gulping	-	-	+	++	+++	+++
Barbel deformation	-	-	-	-	-	-
Discoloration	-	-	+	++	++	++
Erratic swimming	-	-	+	++	+++	+++
Scratching on plastic tank	-	-	-	+++	+++	+++
Jumping	-	-	+	++	++	++
Resting at bottom	-	+	++	++	+++	+++
Hanging vertically in water column	-	+	++	++	++	+++
Fin deformation	-	-	+	++	++	++

\+ = slightly present
++ = moderately present
+++ = highly present
\- = absent

The results presented in Table-2 summarized the behavioural changes observed in Oreochromis niloticus juveniles exposed to different concentrations of TSH powder. Abnormal response observed with O. niloticus exposed to various concentration of TSH powder in this study were excessive gulping for air, erratic swimming, restlessness, loss of equilibrium, fin and barbell deformation, skin haemorrhage, discolouration and finally death. These behaviour were also reported by Omitoyin et al., (2006), Ladipo et al., (2011); Aderolu et al., (2010), Okomoda and Ataguba (2011) and Isiyaku et al., (2019) to varying toxicants. Bobmanuel et al., (2006) stated that behavioural response of fish to toxicants and different reaction time are due to the effect of chemicals, their concentrations, species, size and specific environmental conditions.

Water quality parameter is very important in determining fish health and safety within their environments. This is because a good water quality influences fish survival, growth and reproduction.Table-3 Shows physico-chemical parameters of the toxicant used in the acute bioassay test on Oreochromis niloticus.

Table-3: Physico-chemical parameters of the toxicant solution used in the acute bioassay test of Oreochromis niloticus juveniles exposed to TSH powder.

Concentrations (mg/L)	Parameters				
	Temp (^oC)	pH	Do (mg/L)	TDS (mg/L)	EC (mg/L)
Control (0)	27.55±0.05 [a]	7.64±0.01 [a]	5.53±0.04[a]	293.50±2.50[a]	592.50±5.50[c]
1.2	27.45±0.05 [a]	7.59±0.03 [a]	5.36±0.01[b]	301.00±1.00[a]	608.00±2.00[c]
1.4	27.55±0.05 [a]	7.56±0.04 [a]	5.32±0.05[b]	311.50±1.50[b]	626.00±3.00[b]
1.6	27.56±0.06 [a]	7.61±0.04 [a]	5.20±0.02[c]	327.00±2.00[c]	635.00±1.00[b]
1.8	27.69±0.01 [a]	7.57±0.05 [a]	4.09±0.02[d]	346.00±2.00[d]	683.50±1.50[a]
2.0	27.48±0.03 [a]	7.51±0.04 [a]	3.58±0.03[e]	349.50±2.50[d]	692.00±0.00[a]

Means across the column with different alphabet are significantly different ($p<0.05$)

DO: Dissolved oxygen; pH: Hydrogen Ion concentration; Temp: Temperature; EC: Electrical conductivity; TDS: Total dissolved solids.

Water quality affects the general condition of cultured organism as it determines the health and growth conditions of cultured organism. The mean temperature and pH values of the control and treatments recorded were within the recommended range of (270C - 320C) and (6.5 – 9.0) for fish culture (Craig, 1991).

CONCLUSION

Contaminations of aquatic environment by toxic plants whether as a consequence of acute o events constitute additional stress for aquatic organisms. Plants products - primarily tamarind seed husk is quickly accumulated by aquatic biota and causes adverse effects. Tamarind seed husk accumulation by organisms has resulted in adverse effects ranging from reduced growth, impaired reproduction and death.

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