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Acute-lethal toxicity (LC_{50}) effect of Moringa oleifera (Lam.) Fresh Root Bark Extract on Oreochromis niloticus Juveniles Under Renewal Toxicity Exposure

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Abstract

Acute-lethal toxicity is a tool used in piscicide bio-safety assessment in fish farming prior to its proper application in sustainable aquaculture. Piscicides of plant origin are usually considered for bio-safety assessment because of their effects on non-target aquatic species in fish pond. Acute-lethal toxicity is an indication of baseline data assessment before any piscicide of plant origin could be used in sustainable aquaculture. This study assessed the acute-lethal toxicity (LC_{50}) effect of Moringa oleifera fresh root-bark extract on fresh water fish, Oreochromis niloticus juveniles for 96-h under renewal toxicity exposure. Median Lethal Concentration (LC_{50}) for O. niloticus juvenile was 97.61 mgl^{-1} and high mortality was obtained at 200 mgl^{-1} where fish showed abnormal behaviour such as erratic swimming, mucus secretion, loss of scales, haemorrhages, and stiff fin rays prior to death. Fresh root-bark extract of M. oleifera could serve as pond management tool in aquaculture to assess bio-safety level of targeted and non-targeted aquatic organisms in pond.

Keywords: *Moringa oleifera,* Acute Toxicity, Freshwater fish, Piscicide.

Introduction

The purpose of the acute-lethal toxicity is to determine lethal toxic effect of a toxicant within a short duration of usually 96 hours or 4 days on a particular tested organism. The acute-lethal toxicity test with fish species is to help in the assessment of possible risk to similar species in natural environments, as an aid in determination of possible water quality criteria for regulatory purposes, and for use in correlation with acute testing of other species for comparative purposes (USEPA, 2000). Acute-lethal toxicity test can be done in the laboratory using static, semi-static and renewable methods as the case may be. In modern toxicology, it is usually advisable for the toxicologist to use the renewable method whereby test solutions are renewed at 48 hours in a 96-hour acute-lethal toxicity test (Adesina, 2008). Test solutions should be renewed with the original biocide (toxicant) solution prepared at the start of the test (Marshall, 2003). The acute-lethal

toxicity test at times, however, is used alongside other techniques such as histopathology, haematology and bioaccumulation for a better comprehension of the impact of the toxicant on the test organism (Fafioye, 2001). Bio-safety of food fish from aquaculture for human consumption is of great public health concern in Nigeria and other developing countries(Adesina, 2008 ; Adeyemo, 2012).

The food fish from aquaculture systems are quite acceptable and the farming processes must be acceptable to meet general, cultural, gender and social norms, especially when the output is intended for domestic consumption (FAO, 2000; Faturoti, 2000; Adesina, 2008). Indiscriminate and heavy use of synthetic pesticides in agricultural fields for control of insect pest and vectors of diseases caused serious environmental hazards (Mian and Mulla, 1992). Natural products have gained greater importance, since it is believed that the natural compounds are ecologically safe and culturally more acceptable than synthetic pesticides (Singh and Singh, 2002). Despite all the above-mentioned advantages, interest is on eco-toxic properties of plant origin pesticides (Singh and Singh, 2002). Pesticides of plant origin cannot be used directly in freshwater unless their toxicity has been studied on the non-target animals sharing the habitat with the target animals (Singh and Singh, 2002). However, there is the need to search for aquaculture management practice that will be environmentally acceptable, bio-technically feasible, socio-economically viable, cost-effective and free from water pollution to avoid health risk to the consumers of food fish from aquaculture. *Moringa oleifera* is a tree belonging to the family Moringaceae, usually growing outside the forest areas in south-western Nigeria (Adesina, 2008; Adesina and Omitoyin, 2011).The tree is under-utilized in aquaculture, but is known to contain some piscicidal compounds that could be lethargic to freshwater fishes. The use of biodegradable piscicides of plant origin as pond management tool in sustainable aquaculture can be used to minimize environmental hazards caused on aquatic ecosystems by the non-biodegradable piscicides. *Oreochromis niloticus* is a member of the family cichlidae. *Oreochromis niloticus,* is a highly cultivable species and amenable to laboratory work(Fafioye, 2012).

This study assessed the acute-lethal toxicity (LC_{50}) effect of *Moringa oleifera* fresh root-bark extract on fresh water fish, *Oreochromis niloticus* juveniles for 96-h.

Materials and methods

Collection and processing of plant part for the toxicity test

The fresh root of *M. oleifera* was collected from the Department of Forestry Resources Management field plot, University of Ibadan, Nigeria. A sharp knife was used to peel- off the fresh root-bark, which was ground in a mortal to obtain macerated samples. *Moringa oleifera* was identified and authenticated at the Department of Forestry Resources Management, University of Ibadan, Nigeria. Fifty grammes (50g) of macerated fresh root-bark of *M. oleifera* was weighed into a conical flask and 100mls of distilled water was added and shaken before filtration using a dry Whatman filter paper into a graduated 1L measuring cylinder to obtain cold water extract. This was then stored at 4^0C until used as described by Adewole (2002).

Experimental animals

One hundred and eighty samples of *Oreochromis niloticus* were brought to the laboratory from the University of Ibadan Department of Wildlife and Fisheries Management Farm and acclimatized for 14 days in de-chlorinated water, under the following conditions; water temperature 23 – 25 ^{0}C, 12-h light-dark cycle; pH 7.0 – 7.2 . Fishes were fed twice a day with commercial floating feed containing 30 % of protein and fed at 3 % of their body weight as maintenance ration. Faeces and food debris were siphoned out on a daily basis and water replaced every 24 hours to avoid contamination of the fishes before the beginning of the experiment.

Acute-lethal toxicity study for 96-h

Range finding concentrations of: 0,0.2,2,20,200,and 2000 mgl^{-1} were obtained using five fish as test animals and replicated thrice to determine the acute-lethal concentrations, and the following values were obtained; 0,19,34,62,111,and 200 mgl$^{-1,}$. Ten healthy juveniles of *Oreochromis niloticus* L. of both sexes, were selected for the acute-lethal toxicity test which lasted for 96 hours. The juveniles were of mean length 10.35 $\pm$ 0.20cm and mean weight 10.43 $\pm$ 0.28g, while those used for the range finding toxicity test for 24-h were of mean length 10.25 $\pm$ 0.30cm and mean weight 10.23 $\pm$ 0.26g respectively. Fish mortality and behaviour were monitored and recorded prior to death (Fafioye, 2012). Each solution was renewed daily using the method of Buikema *et al* (1982) as described by Fafioye (2012).

Water quality assessment

Water quality parameters assessed were dissolved oxygen (DO), pH and temperature (APHA, 1998 using 2003 model of Lamotte water quality test kit.

Experimental Design

The experiment was conducted using a complete randomized design (CRD), comprising of six treatments with three replicates.

Statistical analysis

Mortality data were analysed using probit software USEPA (2000), while LC_{50} was calculated logarithmically (Steel *et al.*1997).

Results and discussion

Dissolved water oxygen, pH and temperature during the acute-lethal toxicity test were 3.3-6.2mgl^{-1}, 7.05-7.75, and 23.0 ^{0}C respectively. These values are within acceptable ranges for culturing Tilapia fish in the tropics (Ayoola *et al*., 2011; Fafioye, 2012). Mortality was highest (77%) at 200.0 mgl-1 and lowest, 20% in 19 mgl-1 after 96-h of exposure (Table1). Fish mortality increased with increasing concentration, but later decreased with time. This shows that mortality is dose-dependent. No mortality was recorded in the control experiment during the toxicity test. This is also an indication that toxicity is dose-dependent and varies within the time of exposure of aquatic organisms to toxicants (Akinwande *et al*., 2007; Ayoola *et al*., 2011; Fafioye, 2012). The lower and upper limits of 96-h LC50 obtained were 51.81 mgl-1 and 490.19 mgl-1, while the mean 96-h LC50 was 97.61 mgl-1(Fig.1). The results of this study were similar to those of several workers (Kulakkattolickal, 1987, 1989; Van Andel, 2000; Singh and Singh, 2009) on various aquatic organisms to different pesticides.

The piscicidal potential and phytotoxic properties of plant extracts have been reported by several researchers such as Adewole (2002), Akinwande *et al*. (2007) Ayoola *et al*. (2011) and Fafioye (2012). The estimated 96- h LC_{50} (97.61 mgl-1) of *M.oleifera* fresh root-bark cold water extract for the juvenile *O.niloticus* in the present study is far higher than 2.44 mgl^{-1} reported by Fafioye (2012) when white Tilapia fingerlings were exposed to water extract of Almond *Terminalia catappa*. This implies there is a variation in tolerance limit of fish when exposed to toxicants at different concentrations and ages at the time of exposure to the toxicants, as similarly observed when *Sarotherodon galilaeus* juveniles exposed to ethanolic extracts of cocoa bean shell (Olaifa *et al*., 2008).The abnormal behaviour in relation to fish stress included erratic swimming, mucus secretion, gasping for air, loss of scales, haemorrhages, and stiff fin rays; which are indications of lethargic effect of *M. oleifera* fresh root-bark cold water extract. The abnormality observed could be due to nervous disorder to impaired metabolism, but could in addition be due to nervous disorder as earlier reported by Agbon *et al*. (2002) and Aguigwo (2002).

Table 1: Percentage mortality of *Oreochromis niloticus* juveniles exposed to fresh root bark cold water extract of *Moringa oleifera* (Lam.) for 96-h.

Treatment	Conc. (MgL_{-1})	Number of fish/ tank	Percentage mortality (%)		
			Replicate 1	Replicate 2	Replicate 3
A	0	10	0	0	0
B	19	10	20	20	20
C	34	10	40	30	20
D	62	10	40	30	30
E	111	10	50	40	40
F	200	10	80	60	90

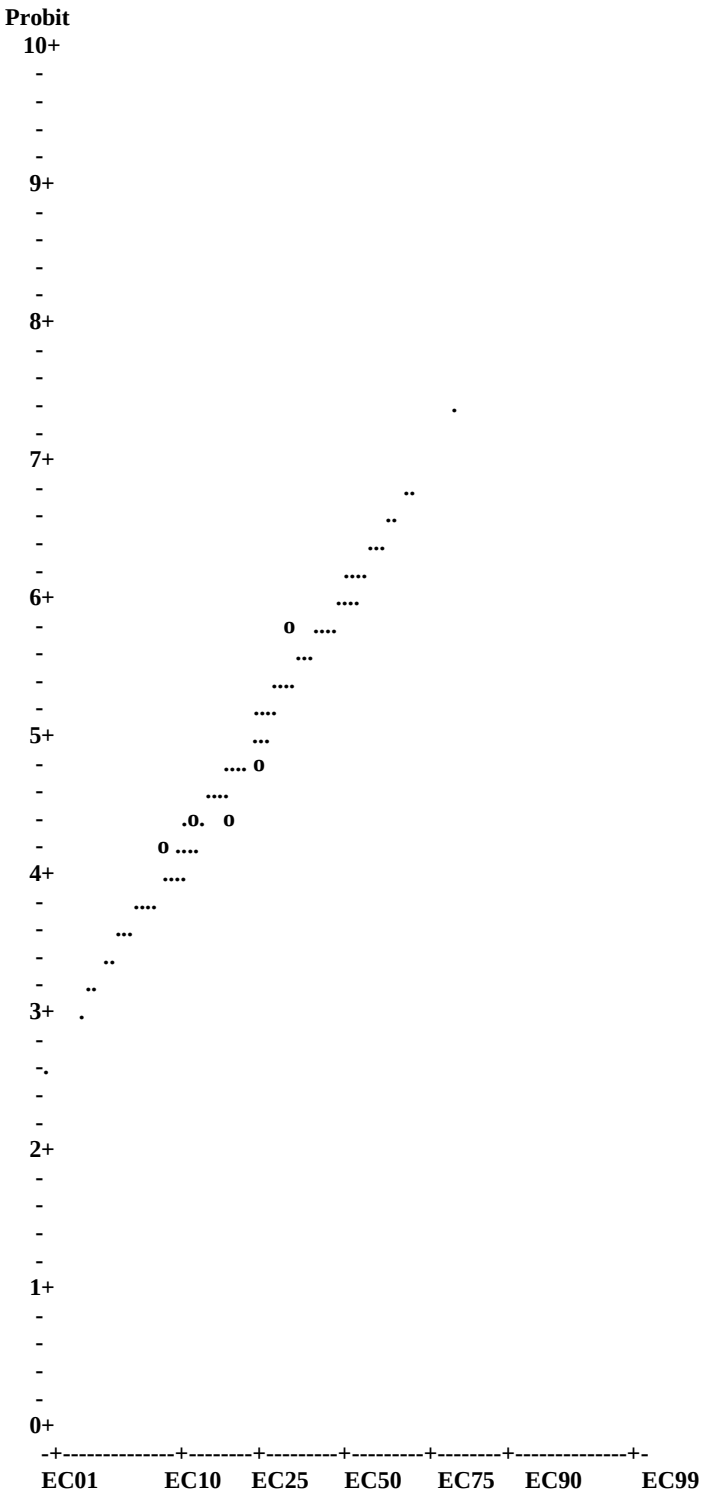

Fig.1: Probit mortality of *Oreochromis niloticus* juveniles exposed to Fresh root-bark cold water extract of *M.oleifera* at 96-h.

Conclusion

Fresh root-bark extract of *M.oleifera* could serve as pond management tool in aquaculture to assess bio-safety level of targeted and non- targeted aquatic organisms in a pond.

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