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Preliminary Toxicological Screening of Ichthyotoxic Compound of *Moringa oleifera* (*LAM.)* Hot Ethanolic Extract to Freshwater Fish, Oreochromis Niloticus (L.) Fingerlings.

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ABSTRACT

Moringa oleifera is an indigenous tree growing in Southwestern Nigeria for food and medicinal property. Therefore this study evaluates the preliminary toxicological screening of ichthyotoxic compounds in *Moringa oleifera* to freshwater fish, *Oreochromis niloticus* fingerlings. The morphological parts such as leaf, seed, stem-bark, pod (fruit), root-wood and root-bark were subjected to standard phytochemical screening method of plant metabolites. Screening done was just only to identify their presences and not quantify as limited to the current research. The Fresh Root-bark Hot Ethanolic Extract (FRBHEE) was toxicologically tested on fish fingerlings for 96hours.Acute toxicity concentrations obtained were 0, 10,17,31,56,100 where 0mgl^{-1} served as control. The Median Lethal Concentration (LC_{50}) for Oreochromis niloticus fingerlings obtained was 43.09 mgl^{-1} with 95% upper and lower limit confidence interval between 74.78-28.65 mgl^{-1} respectively. High mortality was obtained at 100mgl^{-1} of FRBHEE where fish behavioural changes such as rolling upward, backward swimming, sudden mucus secretion, haemorrhages, stiff fin rays and erratic collision on wall of the tank attested to ichthyotoxic property of FRBHEE. Therefore, ichthyotoxic compounds found in *M.oleifera* could further be quantified to know the exact grammes it's contained. FRBHEE could serve as organic piscicides in aquaculture pond management to wipe out predators prior to stocking of fish pond with desirable fish species.

Keywords: Moringa oleifera, ichthyotoxicity, fish, phytochemical, LC_{50}

INTRODUCTION

Piscicidal plants have been widely used by traditional societies all over the world in the past to catch fish (Harada, 1992; Cannon *et al.,* 2004). From these plants, various piscicidal compounds have been phytochemically screened and isolated. Rotenone has been isolated from *Derris elliptica, D. montana and D. pubipetala* (belonging to the family: Leguminosae), the roots of which were widely used in South Asia (Harada, 1992; Finlayson *et al*., 2000 and Agbon *et al.* 2004). Several plants belonging to different families, which possess a number of compounds as saponins, tannins, alkaloids, di- and tri-terpenoids have high pesticicidal activity and used in freshwater bodies to control harmful snails, disease causing insects, such as mosquito larvae and

weed fishes (Tiwari and Singh, 2003). *Moringa oleifera* is a tree belonging to the family Moringaceae, usually growing outside forest area in Southwestern Nigeria (Adesina *et al*., 2008; Adesina and Omitoyin, 2011). There is little information on the toxicological screening of *M.oleifera* ichthyotoxic compounds with ethanolic approach for its potential as piscicide in aquaculture management, hence the need for the present study to evaluate piscicidal activity of ichthyotoxic compound presence in *M.oleifera (Lam.)* Fresh Root-bark Hot Ethanolic to freshwater fish, *Oreochromis niloticus* fingerlings.

MATERIALS AND METHODS

Collection of Plant Materials and Identification

Morphological parts of *M.oleifera* which include the powdered leaf, seed, stem-bark, pod (fruit), root-wood, root-bark samples were collected for the screening of presence or absence of ichythyotoxic compounds. *M.oleifera* was identified by Dr. L. A. Adebisi, Forest taxonomist, Department of Forestry Resources Management, Faculty of Agriculture & Forestry, University of Ibadan, Nigeria.

Plant Parts Extraction for Phytochemical Screening and Preparation for the Toxicity Test

The extraction of different morphological parts of *M.oleifera* was carried out using method of hot extraction with different solvents as described by (Furnis et al 1991; Adewole et al., 2009). The hot ethanolic fresh root-bark extract of *M.oleifera* obtained after screened for the presence of secondary metabolites was used for the acute toxicity test for 96hours to ascertain its toxicological principles using aquatic animal of freshwater origin.

Phytochemical Screening of Ichthyotoxic Compounds

The extracts obtained were screened phytochemically to detect the presence or absence of ichthyotoxic compounds in *M.oleifera* using standard procedures employed by Harbourne (1973), with some modification according to the method described by Sofowora (1993), Adeoye and Oyedapo (2004) on phytochemical screening of toxic substances and their bioactivity on animals. The following compounds were screened: alkaloids, saponins, tannins. Glucosinolates, cyanogenic glycosides, phytate, triterpenes and steroids for their presence only but not quantify as limitation to the current research. The FRBHEE obtained was tested toxicologically on freshwater fish, *Oreochromis niloticus* fingerlings to determine its piscicidal potential in aquaculture pond management.

Determination of LC50 Value from Acute Toxicity Test for 96 hours

Test Animal and Acclimatization

The test fish, healthy tilapia (*Oreochromis niloticus* L.) fingerlings (mean length: 5.41 $\pm$ 0.25cm, Mean weight: 5.42 $\pm$ 0.28g) of both sexes were selected for the acute-lethal toxicity definitive test while fingerlings (mean length: 5.37 $\pm$ 0.25cm and mean weight: 5.82 $\pm$ 0.06g) were respectively used for the range finding toxicity test. The fingerlings were obtained from the Department of Wildlife and Fisheries Management Teaching and Research Fish farm, University of Ibadan,

Nigeria. The fishes were acclimatized for 14 days in the laboratory inside a plastic rearing facility for adaptation prior to the experimental period. Fish were fed twice daily with maintenance conventional feed before toxicants were introduced to the aquatic animals. The water used was dechlorinated water exposed to air for 48hours and renewed every 24hours.

Acute Toxicity Experiment for 96 hours

Range Finding Toxicity Experiment for 24hours

Prior to acute toxicity experiment, range finding toxicity test was carried out for 24 hours to determine the concentrations to use in acute toxicity experiment for 96hours.The range finding toxicity test concentrations were determined with the FRBHEE prepared in the following orders of concentrations for the range finding toxicity test on *O. niloticus* fingerlings . For the *O. niloticus* fingerlings, the following concentrations of FRBHEE were prepared by dissolving 5g of the extracts in 100ml of distilled water and serially diluted to produce 5000mgL^{-1} per 5 litres of water as modified by Odiete (1999) method on range finding toxicity test which resulted into: 0.1, 1, 10, 100, 1000mgL^{-1} and 0mgL^{-1} as the control concentration. Mortality range was observed between 10 100mgL^{-1} concentration. The concentrations of each of the *M. oleifera* extracts were replicated three times and three *O. niloticus* fingerlings were tested per the concentrations of FRBHEE, respectively for 24-h. The fishes were starved for 24-h before the experiment and the number of mortality in each concentration were recorded accordingly.

Acute Toxicity Test, Experimental Design and Bioassays Procedure for 96 hours

The definitive toxicity test was carried out for 96-h based on the results of the range finding test obtained from the *M. oleifera* FRBHEE on *Oreochromis niloticus* fingerlings for 24-h. However, the definitive toxicity test was carried out and each of the *M. oleifera* extracts FRBHEE was exposed to *O. niloticus* fingerlings using a spacing factor of 1.8 to determine the definitive toxicity concentrations that were used during the 96-h bioassays as earlier described by Odiete (1999).

Ten fingerlings of *O. niloticus* were starved for 48h and released into each translucent aquaria (55cm x 36cm x 27cm) of 55 litres capacity and different concentrations of FRBHEE were introduced to the fishes for 96-h. The concentrations were prepared in weight to volume (W/V) of each *M. oleifera* extract and expressed in gram per volume (mgL^{-1}). However, six different concentrations for each separate experimental design were labelled A, B, C, D, E, F where A was the control concentration (0mgL^{-1}). The definitive concentrations were carried out on *O. niloticus* fingerlings and FRBHEE concentrations for the fingerlings determined were: 0, 10, 17, 31, 56, 100mgL^{-1}.In each of the experimental set-up, each has three replicates where aquaria labeled "A" served as the control experiment (0mgL^{-1}) throughout the 96-h exposure period to the piscicides. The experimental procedures followed static renewable bioassays of USEPA 712-C-96-118 of chemical testing (USEPA, 1996) with little modification to suit the tropical condition as similarly observed by Odiete (1999) and Omitoyin (2006), respectively. Behavioural pattern of the exposed fishes were observed and recorded accordingly at each concentration of the piscicide and with the unexposed fishes behaviour as the control experiment as similarly reported by Ayoola

(2011).The mortality of fishes were recorded at three hours interval for the 96-h duration of the toxicity experiment. Dead fishes were promptly removed and mortality was specifically recorded at I-h to 96-h of exposure time as described by Odiete (1999).

Water quality Assessment

Water quality parameters assessed were Dissolved Oxygen (DO) , pH and water temperature following the principles of water and waste water assessment with the Lamottee freshwater quality test kit 2003 model. The three parameters were significant during the acute toxicity exposure.

Behavioural Changes of Fish monitored during the Acute Toxicity Period

Behavioural activities of fish fingerlings exposed during the acute toxicity test was monitored and observed accordingly on each concentration as evidence of piscicidal activity.

Statistical Data Analysis

The mortality data was used to estimate the medial lethal concentrations (LC_{50}) of the piscicide (FRBHEE) using probit method of Finney (1953), but mortality data were analysed with probit software USEPA (2000).

RESULTS AND DISCUSSION

Phytochemical Screening of ichthyotoxic Compounds in *M.oleifera*

The result of phytochemical screening of the various morphological parts of *M.oleifera* for the presence or absence of ichythyotoxic compounds are presented in Table1.

Table 1: Phytochemical description of ichthyotoxic compounds in *M.oleifera* morphological parts.

Morphological parts of *M.oleifera*						
Chemical constituents	Leaf	Seed	Stem-bark	Pod (fruits)	Root-wood	Root-bark
1.Tannins	+	-	+ (Negligible)	+	-	-
2.Saponins	+	+	+	+	-	-
3.Glucosinolates	-	+	+	+	-	-
4.Cyanogenic Glycosides	-	+	+	+	-	-
5.Alkaloids	-	+(Trace)	-	-	+	+
6.Phytates	+	+	+	+	-	-
7.Triterpenes and Steroids	-	-	+	-	-	-

Legend= +: Presence of ichthyotoxic compounds.

\- : Absence of ichthyotoxic compounds.

The presence of ichthyotoxic compounds in appreciable amount such as tannins, alkaloids, saponins, cyanogenic glycosides in *M.oleifera* could produce mortality and abnormal behavior in fish. Their presence could deter the physiological processes which eventually could result in growth deterrent in fish. It is well documented by Al-owafeir (1999): that the presence of tannic acid as plant metabolite reduced growth and feed utilization in African catfish, *Clarias gariepinus*. However, the presence of large amount of metabolites in *M.oleifera* extracts such as tannins, saponins and alkaloids could have resulted in mortality and growth depression in juveniles of *Oreochromis niloticus* at 3mg/l and 6mg/l as observed when the fresh root-bark extract of *M.oleifera* was introduced to fish (Adesina, 2008). Phytochemical screening of *M.oleifera*

metabolites reported to be present in the current study agrees with reports of (Obutor, 2004; Fatoba *et al.,* 2003; Adewole, 2009). Some of these metabolites present in the ichthyotoxic plants have been known to affect fish and other animals' physiological responses during their biological evaluations and interactions (Fafioye, 2004; Adewole, 2009).

Determination of LC50 Value from Acute Toxicity Test for 96 hours

Water quality parameters

The water quality parameters obtained during the toxicity exposure of *O.niloticus* fingerlings to FRBHEE were: Dissolved Oxygen(6.5-11.0),Temperature(23-25^{0}C) and pH(7.0-7.5).The parameters fall within the range values recommended for the rearing of freshwater fish of Chiclidae family: Tilapia group where O. niloticus fingerlings in the current research belongs to. The observation has been supported by several searches on Tilapia fish species. Fafioye (2012), Olufayo and Akinpelumi (2012) cited similar water quality information on Tilapia fish.

Mortality

The result of acute toxicity of fish exposed to FRBHEE for 96-h shows in Table 2 that mortality increases at higher concentration of 100mgl^{-1} with time of exposure.

Table 2: The percentage mortality of *Oreochromis niloticus* fingerlings exposed to fresh root bark hot ethanolic extract of Moringa oleifera Lam.for 96-h

Treatment	Conc.in MgL^{-1}	Number of fish/ tank	Percentage mortality (%)		
			Replicate 1	Replicate 2	Replicate 3
A	0	10	0	0	0 (0%)
B	10	10	20	10	10(10%)
C	17	10	30	30	10(23%)
D	31	10	30	20	40(30%)
E	56	10	60	40	50(50%)
F	100	10	80	90	100(90%)

This is an indication that mortality is dose-dependent. At higher concentration of 100mgl^{-1} FRBHEE exposure the mortality was 90%.and at lowest concentration (10mgl^{-1}) of the FRBHEE; the mortality data was at 10%. No dead was observed and recorded in the control experiment.
However, as observed that mortality is a function of high toxicity exposure of fish to toxicants in

aquatic environment. This agrees with Dahunsi *et al.* (2011) when freshwater fish, *Clarias gariepinus* was exposed to sub-lethal concentrations of chemical additives effluent. The Median Lethal Concentration (LC_{50}) for Oreochromis niloticus fingerlings obtained in the study was 43.09 mgl^{-1} with 95% upper and lower limit confidence interval between 74.78-28.65 mgl^{-1} respectively. Other researchers have reported lower LC_{50} values when freshwater fish were exposed to different processed toxicants (Cannon *et al*., 2004; Agbon *et al*., 2004; Adesina, 2008). The estimated values of 96-h LC_{50} (43.09mgl^{-1}) obtained with FRBHEE of *Moringa oleifera* is lower when compared with what was obtained at 967.61mgl^{-1} when Fresh Root-bark Cold Water Extract (FRBCWE) was introduced to *O.niloticus* juvenile (Adesina *et al*., 2013). This is an indication that mortality and stress of fish to toxicants is a function of age group of fish that was exposed to the toxicants. Fishes have various tolerance limit of toxicant exposure as reported by different researchers on different 96-hLC_{50} values obtained when diferent age group of freshwater fish species exposed to different toxicants (Agbon *et al.,* 2004; Dahunsi *et al*., 2011).

Behavioural Responses of O.niloticus Fingerlings Exposed to FRBHEE

Fish behavioural changes such as rolling upward, backward swimming, sudden mucus secretion, haemorrhages, stiff fin rays and erratic collision on wall of the tank attested to ichthyotoxic property of FRBHEE. Also spirally uncoordinated movement, gasping for air and loss of reflect action were similarly observed at 100mgl^{-1} during the exposure of *O.niloticus* fingerlings to fresh root-bark hot ethanolic extract(FRBHEE) of *Moringa oleifera h*as reported by (Olufayo and Akinpelu, 2012). Abnormalities observed prior to mortality are indications of depleted oxygen content due to higher demand for oxygen (Dahunsi *et al*., 2011). In the current study, abnormal fish behavior occurred more at higher concentrations and shows that fish behavior is dose-depend. Abnormalities observed in fish behavior are an indication that fish undergone stress when exposed to toxicants (Adesina, 2008).

CONCLUSION

Ichthyotoxic compounds found in *M. oleifera* resulted in mortality of exposed fish could serve as organic piscicide in aquaculture pond management to wipe out predators prior to stocking of fish pond with desirable fish fingerlings. Further research on quantification of each of the screened secondary metabolites of *M.oleifera* could be an eye opener as fisheries management tool in aquaculture that could be mixed with feed additives to control the population of unwanted fish and predators in newly stock pond.

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