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Piscicidal effect of *Luffa cylindrica* fruit extract on tilapia fingerlings, *Oreochromis mossambicus* in captive condition

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Abstract
Determination of lethal concentration (LC_{50}) value of *Luffa cylindrica* fruit extract on tilapia, *Oreochromis mossambicus* in captive condition has been carried out in the present study. Five different concentrations of the extract 8 g l^{-1}, 10 g l^{-1}, 12 g l^{-1}, 14 g l^{-1} and 16 g l^{-1} were made by adding the extracts proportionately to the water of the aquarium. The LC_{50} value of *L. cylindrica* on *O. mossambicus* was found as 10.54 g l^{-1} for 96 hours exposure periods. The dose response mortality and behavioral changes were studied against the fruit extract of *L. cylindrica.* The analysis of variance between the mortality of the treated fish and the concentration of fruit extract of *L. cylindrica* extract was found to have a significant ($P<0.05$) relationship. The correlation coefficient between concentration and mortality of fish was calculated ($R = 0.950$) and showed a strong positive correlation between different concentrations of the fruit extracts. The present finding established that *L. cylindrica* has potential pisicidal effect on fish and could be use widely to control unwanted fishes in the aquaculture system.

Keywords: Acute toxicity, LC_{50} values, *Luffa cylindrica, Oreochromis mossambicus*

1. Introduction
Plant based piscicide have been address as best alternative of chemical piscicide in aquaculture to control fish fry predators and unwanted fishes from aquaculture ponds. Plant extract are consider as desirable due to their properties of eco-friendliness, ease of availability, high efficiency, reduce toxicity to non-targeted animals and rapid biodegradability. Different parts of this piscicidial plants are used in various way, it can be applied directly in the form of extract, aqueous or dissolved in alcohol [1]. Plants extracts are called botanicals and when toxic to fish are called piscicides [2]. Production of plant piscicide in long run may also become an important industry using biotechnological methods. Plant extracts are considered promising agents because of their eco-friendliness, ease of availability, high efficiency, rapid biodegradability and reduced toxicity to non-targeted animals [3].
Several plants from the world have been applied for catching fish. The toxic parts of plants as fish poisons include roots, seeds, fruits, bark, leaves etc. [4, 5] reported that *Luffa aegyptiaca* and *Luffa cylindrica* respectively have piscicidal effects. The widely used piscicide plant *L. cylindrica* is commonly called vegetable sponge gourd belong to Cucubitaceace family. The local farmers in Nigeria use the fruits of this plant to harvest fish from water bodies for human consumption.
Oreochromis mossambicus a native fish to southern Africa is a very popular fish in commercial aquaculture in worldwide being hardy in nature. Due to wide spectrum of diet habits, making them thrives in adverse aquatic conditions. The species is resistance to wide varieties of water quality issues and have been widely used as bioassay organisms. There are no works have been reported on the piscicidal effect of *L. cylindrica* of fishes in Indian condition. In this context the present study being carried out to observe the toxic effects of this plant on *O. mossambicus* in control condition.

2. Materials and methods
2.1 Experimental site and collection of *Luffa cylindrical* fruits
The study was carried out in the Laboratory of Department of Fisheries Resources Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal between May to June, 2016**.** The fresh fruit of *Luffa cylindrica* was collected from nearby local market and it was sliced into smaller size using a knife and homogenized using electric grinder.

The pure extract of this aqueous content was collected into bottle, covered and put in a refrigerator at 4 ˚C to keep it fresh until the time of administration. In experimental tanks the aqueous extract was allowed to stand for 30 min after proper mixing before introducing the fish. In control no extract was added and fishes were maintained in water without extract concentration.

2.2 Phytochemical analysis

The phytochemical analysis of the aqueous extract of this fruits, *L. cylindrica* was done by following the methodology of [6, 7].

2.3 Collection and acclimatization of experimental fish sample

Healthy fingerlings of *Oreochromis mossambicus* (Peters 1852) with average length of 10 ± 0.38 cm and average weight of 23 ± 0.07 gm were purchased from the local fish market and maintained in wet laboratory for 3 weeks, prior to the experiment. Fishes were fed pelleted feed and maintain optimum level of water quality. Fish aquaria (60 cm × 30 cm × 30 cm) were well aerated and the water was exchanged with fresh water as and when required.

2.4 Toxicity test of on *Oreochromis mossambicus*

For 96 hour median lethal concentration value (LC_{50}), the experiments were conducted in glass aquariums filled with 15 litre of chlorine free tap water. Five different concentrations were made *i.e*. 8 gm l^{-1}, 10 gm l^{-1}, 12 gm l^{-1}, 14 gm l^{-1} and 16 gm l^{-1} with one kept as control. In each aquarium, 20 fishes were kept and exposed to different concentrations as above with replications. In control, no extract was added and the fishes were maintained in the water without extract concentration. The fishes were exposed to aqueous extract of fruit of *Luffa cylindrica* for 96 hours by following standard procedure used for toxicity test [8]. Feeding of fishes was stopped during the experiment period. Hypoxic condition of water was avoided by adequate aeration. The tested fishes were kept under continuous observation during the experimental period. The behavior of the fish were observed and recorded from time to time. The mortality rate was recorded periodically in each aquarium. The dead fishes were removed and preserved for further investigation. The LC_{50} value of the fish species was calculated by using Probit analysis method (Finney, 1971).

2. 5 Analysis of water quality parameter

Some important physico-chemical parameter of water such as Dissolved Oxygen (DO), free carbon dioxide (CO_2), total alkalinity and the ammonia content were studied. Water quality parameter during median lethal test for 96 hour experiment was analyzed at the beginning and end of the experiment by using the methods described in APHA [8].

2.6 Statistical analysis

The LC_{50} value of *L. cylindrica* for *O. mossambicus* was calculated using Probit analysis method (Finney's, 1971). One way ANOVA were performed using SPSS software to assess the effect on concentration on the mortality of fish. The regression analysis were done by Microsoft excel to assess the relation between mortality and exposure period in different concentration of aqueous extract of *L. cylindrica* fruit.

3. Results and discussion

3. 1 Toxicity test of on *Oreochromis mossambicus*

The median lethal concentration or 96 hour LC_{50} value of *L. cylindrica* was found to be 10.54 gm l^{-1} for *O. mossambicus* fingerlings. The relationship between aqueous extract of *L. cylindrica* concentrations and the mortality rate of the tested fish is presented in Table 1. The percentage of mortality of fishes being exposed to fruit extract increased with the increase in concentration of extract and also time exposure (Fig. 1). Analysis of variance (ANOVA) for effect of concentration of extract on percentage of mortality of fish was found significant ($P<0.05$) difference between the concentration of the extract and the mortality of fish. The correlation coefficient (R) value between concentration of the extract and the fish mortality was 0.950 (Fig. 1). During this medial lethal concentration (LC_{50}) test behavioral changes in *O. mossambicus* also observed and mentioned in the Table 1. No mortality was recorded in control.

[8] Reported that LC_{50} value of *Zanthoxylum rhetsa* in *Heteropneustes fossilis* was found to be 70.1 mg l^{-1} for 96 hours exposure periods. [5] Reported that the LC_{50} value of *Luffa cylindrica* fruit extract on African catfish *Clarias gariepinus* (Buchell 1822) juveniles was 14 gm l^{-1}. [10] Reported LC_{50} values of Mancozeb a synthetic chemicals to *Oreochromis mossambicus* was 14.40, 13.40, 12.34 and 11.68 mg $^{-1}$ at 24, 48, 72 and 96 hours respectively. [11] Was observed a sub-lethal dose of *Mohua* extract was 100 mg l^{-1} against in walking catfish *Clarias batrachus*. [12] Studied evaluation of botanical piscicides in nile tilapia *Oreochromis niloticus* L. with ten locally available plants of Philippines and 96-hour lethal concentration (LC_{50}) was found as adelfa (0.083 ml l^{-1}), makabuhai (0.44 ml l^{-1}), ampalaya (0.45 ml l^{-1}), neem (2.57 ml l^{-1}), lagundi (2.93 ml l^{-1}), kalamansi (3.12 ml l^{-1}), sambong (5.11 ml l^{-1}), physic nut (12.8 ml l^{-1}), agave (30 ml l^{-1}), madre de cacao (52 ml l^{-1}).

Dimethoate an organophosphate insecticide and acaricid while used as fish toxicant the 96-h LC_{50} value was reported 65 mg l^{-1} for *Clarias batrachus* [13], 47 mg l^{-1} (96 hr) for *Channa punctatus* [14] and 17.9 mg l^{-1} (24 hrs) for *C. punctatus* [15, 16] Reported different pisicidal plant from Nepal against catfish like *Ophiocephalus punctatus*, *Clarias batrachus* and *Heteropneustes fossilis* with LC_{50} value of 90 mg l^{-1}, 102.4 mg l^{-1} and 109.1 mg l^{-1} respectively.

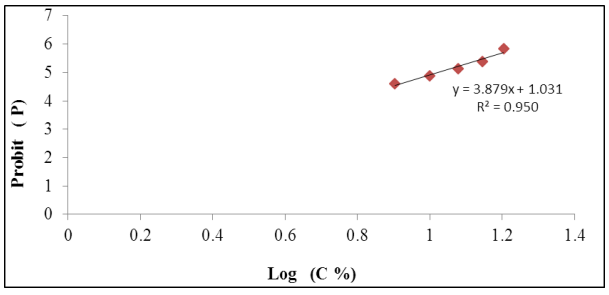

Fig 1: Percentage mortality of *O. mossambicus* against log concentration of *L. cylindrica* fruit extract at 96 hour exposure periods.

3.2. Behavioural changes observed in *Oreochromis mossambicus*

The present study showed that *L. cylindrica* has great affects to changes the behavioral activity of *O. mossambicus* in control condition. Morphological changes also been observed in *O. mossambicus* exposed to higher concentration of *L. cylindrica* extract and which was observed by seeing the colour changes of the skin with swollen and reddish eyes, heavy secretion of mucus from all over the body. The experimental specimens showed excessive mucus secretion and hyper-excitability at the higher concentrations during 96 hours of exposure time. The loss of equilibrium of fish was

more in higher concentrations.

Table 1: Behavioral changes in *O. mossambicus* during medial lethal concentration (LC_{50}) test in aqueous extract of *Luffa cylindrica* fruit.

Exposure Period	Behavior Changes
1-12 hour	Fish become alert; disrupt schooling behavior, vertical movement, gasping for air, excess mucus secretion, changes in skin coloration.
13-24 hour	Lips and eye became swollen and reddish, loss of equilibrium, involved in jumping, increase air gulping.
25-36 hour	Fishes went to side of the aquarium, fish became sluggish and operculum beats decreased, excessive mucus secretion.
37-48 hour	Reduced caudal fin beat frequency, operculum movement restricted indicating lethargies.
49-96 hour	Mucus secretion in gill was disrupted with red patches, operculum beat decreases. At the end of the period fish gathered at aquarium corner and became motion less with taking a vertical position and died.

[17] Mentioned that the excess mucous secretion all over the body surface of fish exposed to synthetic chemical Mancozeb was probably due to the dysfunction of regulatory mechanism. According to [18] excess mucus secretion occurred due to the dysfunction of the central nervous system in fishes. Higher opercular movement was observed with increase in the toxic concentration during the study. [19] Mentioned that, the toxins exposure in fishes increases the operculum movement and was well established.

[5] Also observed similar behaviour changes when exposed *Clarias gariepinus* in *Luffa cylindrica* fruit extracts. The present findings also agreed with the works of [10] in *O. mossambicus*; [20] in *Clarias gariepinus*; [21] in *Poecilia reticulata*, when they exposed experimental fishes to acute concentrations of different plant extracts.

4. Conclusion

The study showed that the fruit extract of *L. cylindrica* toxic to tilapia *(Oreochromis mossambicus)* fingerlings. For commercial aquaculture, control and eradication of unwanted fishes form the water bodies is prerequisite. Synthetic chemicals are very effective to killing fishes in shorter period of time but not environmental safety to use. On other cases plant piscicides not hazardous to the environment and have duel effects, killing fishes as well as act as manure after certain period of time interval. The present finding established that locally available plant materials like *L. cylindrica* have the potential to eradicate unwanted fishes and can be used as piscicide in large scale which is biodegradable and environmental safety. The study might contribute largely to introduction of new plant based piscicide in aquaculture management system for fish farmer.

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