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Evaluation of piscicidal potential of *Moringa oleifera* Lam.

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ABSTRACT

The sub-lethal concentrations of leaves and barks of *Moringa oleifera* (Drumstick) was evaluated against a weed fish (*Puntius sophore*) of nursery pond in Tripura and other NE States. The study was conducted using glass aquaria following the static bioassay method. The stocking density of fish (avg. weight: 0.2g/3.5cm) was 6/tank. The leaf and bark extracts were prepared by soaking it in water (1:5) overnight. The test concentrations were 0, 10, 20, 40, 80, 160, 320 and 640 ml/L and test duration was 48 hr. Fish mortality and water quality were recorded at 12 hr. interval from the commencement of the trial. Fish showed erratic swimming, piping, ataxia, *etc*. within 15 min. of application of the extracts. Fish mortality was found dose-dependent and it was increased with time. The concentration at which all fish were died (LC_{100}) with both the extracts was 320 ml/L. The dose-response curves were best-fitted into logarithmic equations. The equation with leaf extract was $y = 25.76\ln(x) – 60.50$ ($R^2 = 0.97$) at 24 hr. and $y = 24.04\ln(x) – 43.47$ ($R^2 = 0.95$) at 48 hr. The 24 hr. LC_{50} was 73.6 ml/L and 48 hr. LC_{50} was 50 ml/L for leaves. The equation with bark extract was $y = 23.18\ln(x) – 46.80$, ($R^2 = 0.94$) at 24 hr. and $y = 17.17\ln(x) – 6.23$, ($R^2 = 0.96$) at 48 hr. The 24 hr. LC_{50} was 66.7 ml/L and 48 hr. LC50 was 27.2 ml/L for bark. Overall, bark extract was more lethal than leaf extract on *P. sophore*. From this, it was concluded that, Drumstick plant could be a potential bio-piscicide for nursery pond preparation.

1. Introduction

Fish seed production encounters huge loss due to the presence of weed and predatory fishes in the pond during nursery fish rearing. These unwanted fishes compete with the spawns of the candidate fishes for food and space and hamper its growth and survival. Therefore, it must be removed from the pond before stocking the spawns of the candidate fishes. The most effective piscicides for removing these fish are Mohua oil cake and Derris root powder. But Mohua is not readily available in Tripura and other North Eastern States, and Derris powder being suspected to be associated with Parkinson's disease is not suggested in farming. Thus, synthetic pesticides like aldrin, endrin, endosulfan *etc.* are used by the farmers. These synthetic piscicides are highly persistent in nature and affect the non-target organisms (Fafioye, 2005). Hence, it is discouraged in aquaculture and instead of that, botanicals are suggested. Botanicals are biodegradable and broad-spectrum in activities (Olaifa *et al.,* 1987); it can be extracted in commercial scale. A number of plant materials, having piscicidal property, were identified, and one of it is *Moringa oleifera* (Family: *Moringaceae*). It is commonly known as Drumstick tree or Horseradish tree or Benzoil tree. It contains a number of bioactive compounds such as alkaloids (moringine, and moringinine), phenols, tannins, saponins, glucosinolates, oxalic acid, lectins, pterygospermin, spirochin, benzyisothiocyanate *etc.* (Wise, 2006), which can be utilized for sustaining aquaculture (Kamble *et al.,* 2014). Drumstick is very popular in NE India. In Tripura, it is popularly called '*Sajna*'. It is widely naturalized in the ecosystem of Tripura and used for vegetable purpose and herbal remedies. Its piscicidal property has been assessed against the juvenile of African catfish (Adelakun *et al.,* 2017); however, little is explored on this aspect in other fishes (Wise, 2006). Therefore, this study was conducted to determine the sub-lethal concentration of *M. oleifera* (LC_{50}) on *Puntius sophore* (Sophore barb) which is a potential weed fish in Tripura and other NE States.

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2. Materials and Methods

The study was conducted during 2018 in the ICAR research Complex for NEH Region, Tripura Centre, Lembucherra, Tripura (23054'N Lat., 91019'E Long., 16.2m above MSL). For this, two hundred healthy barbs (*P. sophore*) were collected from the ponds of the Centre and stocked in rectangular glass aquaria (10L) @ 6 fish/aquarium. Aeration was constantly provided and pellet feed was fed to the fish twice daily during the period of acclimatization over a week. Uneaten feed and fecal matters were regularly siphoned-out from the tank and water was daily exchanged to prevent the deterioration of water quality parameters in the tanks. Feeding was terminated 24 hr prior to the initiation of the trial. Three fish were randomly sampled from each aquarium and estimated for weight and length. The average weight (wet) of each barb was 0.2g and length was 3.5 cm. Fresh leaf and bark samples of the Drumstick were collected from the ICAR farm. A kilogram of the leaves and a kilogram of bark were chopped into small pieces and later slightly pounded using a grinder to obtain macerated samples. Then distilled water was added to the macerated samples @ 5:1 and the materials were left for overnight in room condition to simulate the biochemical process. Afterward, the extracts were obtained by filtering through Whatman filter paper. The extracts were used immediately to ensure its freshness and potency.

The test concentrations were- 0, 10, 20, 40, 80, 160, 320 and 640 ml/L of water for both leaf and bark extract (Adelakun *et al.,* 2017). Fish mortality was observed at 12 hr. interval until the time limit of 48 hr. from the start of the experiment (EIFAC, 1983). The water quality parameters were regularly tested and maintained. Fish were considered affected by the extracts when it manifested erratic swimming, piping, loss of reflex, discolouration and pronounced ataxia. The inability of fish to respond to external stimuli and mechanical prodding was used as the determinant of death. Dead fish were continuously removed and counted for the determination of the median lethal concentration (LC_{50}), that is, the concentration at which 50% of the test fish was died and remaining 50% was survived. The lethal concentration (LC_{50}) was determined in MS Excel by plotting the test concentrations against the fish mortality within 24 hr. and 48 hr. after the experiment (Finney, 1971).

3. Results

Effect of leaf extract on fish mortality

The effect of leaf extract of drumstick (@ 0-640 ml/L) on the mortality of *P. sophore* is presented in Table 1. There was no fish mortality at 0 ml/L (control) and 10 ml/L throughout the experimental period of 48 hr. The mortality was initiated at 20 ml/L and it was increased with increased concentration of the extract. Concentration at which 100% fish died (LC100) was 320 ml/L. The relation between the concentrations of the leaf extract and fish mortality at 24 hr. was, $y = 25.762\ln(x) - 60.506$, $R^2 = 0.9783$, whereas, at 48 hr., it was $y = 24.047\ln(x) - 43.473$ $R^2 = 0.9515$ (Fig.1). The 24 hr. LC_{50} was calculated as 73.6 ml/L and 48 hr. LC_{50} as 50 ml/L.

Table 1. Effect of aqueous extract of Moringa leaf on the mortality of *P. sophore*

Conc. (ml/L)	No. of fish dead		Mortality (%)	
	24h	48h	24h	48h
0	0	0	0.0	0.0
10	0	0	0.0	0.0
20	1	2	16.7	33.3
40	2	3	33.3	50.0
80	3	4	50.0	66.7
160	4	5	66.7	83.3
320	6	6	100.0	100.0
640	6	6	100.0	100.0

Table 2. Effect of aqueous extract of Moringa barks on the mortality of *P. sophore*

Conc. (ml/L)	No. of fish dead		Mortality (%)	
	24h	48h	24h	48h
0	0	0	0.0	0.0
10	1	2	16.7	33.3
20	1	3	16.7	50.0
40	2	3	33.3	50.0
80	3	4	50.0	66.7
160	4	5	66.7	83.3
320	6	6	100.0	100.0
640	6	6	100.0	100.0

Effect of bark extract on fish mortality

The effect of bark extract of drumstick on the mortality of *P. sophore* is presented in Table 2. There was mortality at the dose of 0 ml/L (control) throughout the period of experiment of 48hr. Mortality was initiated at 10 ml/L and it was increased with increased dose of the extract. The concentration at which 100% mortality occurred (LC_{100}) was 320 ml/L. The relation between concentration of bark extract and fish mortality at 24 hr. was, $y = 23.181\ln(x) - 46.808$, $R^2 = 0.9492$, whereas, at 48 hr., it was $y = 17.178\ln(x) - 6.2332$, $R^2 = 0.9616$ (Fig. 2). From these equations, 24 hr. LC_{50} was calculated as 66.7 ml/L and 48 hr. LC_{50} was 27.2 ml/L.

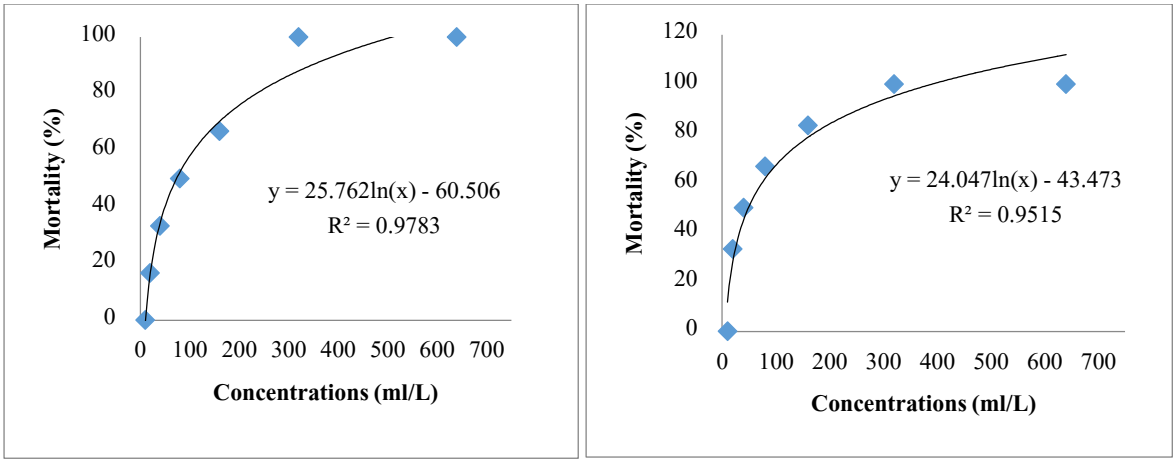

Figure 1. Relationship between the concentrations of leaf extract and fish mortality in 24 hr. (left) and 48 hr. (right)

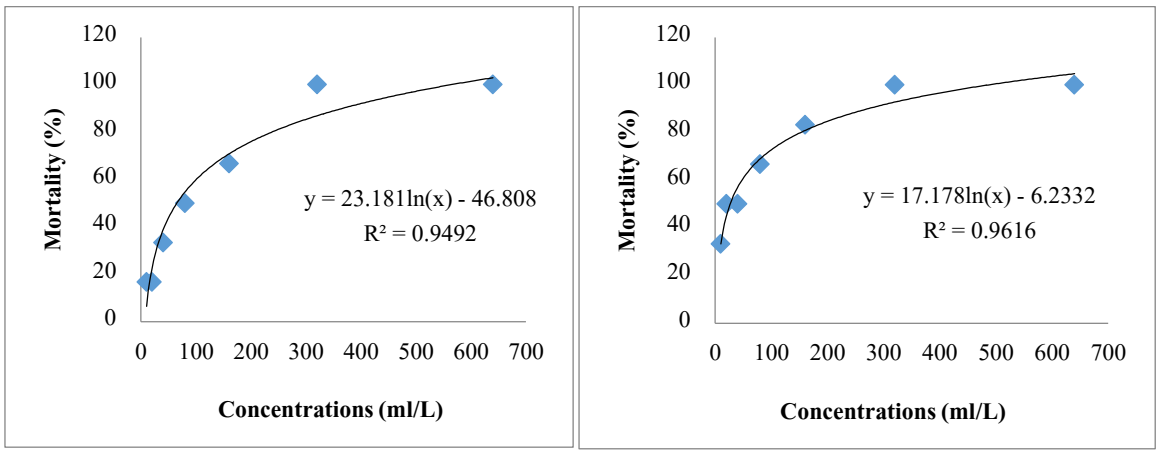

Figure 2. Relationship between the concentrations of bark extract and fish mortality in 24 hr. (left) and 48 hr. (right)

Discussion

The study demonstrated the piscicidal potential of aqueous extract of drumstick leaf and barks on *P. sophore* which is considered as a nuisance during nursery fish rearing. The water quality parameters of the aquaria namely, water temperature, pH, dissolved oxygen and total alkalinity were showed trivial varaiation and it remained within the ranges requiring for fish (Debnath *et al.,* 2015), thus fish mortality occurred in tanks during the trial, was the effect from the Moringa extracts rather variations in the water quality parameters. There was 100% fish mortality with leaf as well as bark extract at 320 ml/L during the initial 3 hr. of the experiment. This is almost similar to the finding of Adelakun *et al* (2017) where they recorded 90% mortality in the juveniles of African catfish (*C. gariepinus*). At other concentrations, during the initial 3 hr. of experiment, fish showed the signs of distress and respiratory impairment due to toxic secretions from the *Moringa*, however there was no fish mortality (Fafioye *et al.,* 2002). Fish were relatively unaffected at lower concentrations of the extracts.

But, as the concentration was increased, the signs of discomfort in the fish were increased. This indicated that the responses are dose-dependent (Ayotunde *et al.,* 2010). The fish mortality was increased with time with the increased concentration of the extracts. The LC_{50} for bark extract was calculated out to be 66.7 ml/L at 24 hr. and 27.2 ml/L at 48 h, whereas, the LC_{50} for leaf extract was calculated out to be 73.6 ml/L at 24 hr. and 50 ml/L at 48 hr. This indicated that the efficacy of bark extract is more than that of leaf extract in killing the barbs. Adelakun *et al* (2017) also reported higher potency in bark extract of *Moringa* in killing the juveniles of African catfish. They found relatively higher values *i.e.* 24 hr LC_{50} = 121.89 ml/L and 48 h LC_{50} = 110.92 ml/L when compared with our results which could be attributed to the inherent ability and genetic makeup of the species of the fish used for the trial. In our study, we used barbs which belong to the carp family and they used African catfish which belong to the catfish family and generally catfish are hardier than carps, thus it required higher doses of the Moringa extract to eradicate them.

From this, it could be concluded that the extracts of *M. oleifera* kills the weed fish like barbs through inciting a series of stressful physiological disorders. Bark extract of the plant contains more bioactive ingredients, thus it is more potent as

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piscicide than the leaf extract. *M. oleifera* could be a potential herbal piscicide for sustainable aquaculture. Thus it is recommended in the areas of Tripura and other places where the plant is widely naturalized. Further studies are recommended on the assessment of the plant parts against the predatory fishes of nursery fish rearing concern.

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