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Evaluation of Some Plant Products of Tripura, North-East India for Piscicidal Property

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ABSTRACT

The study was carried out to examine the acute toxicity of leaves of *Moringa oleifera* (Drumstick), barks of *M. oleifera,* leaves of *Camellia sinensis* (Tea), barks of *Magnolia champaka* (Champak), fruits of *Melia azedarach* (Chinaberry), leaves of *Glycyrrhiza glabra* (Mulethi), leaves of *Millettia pachycarpa* (Bish lata), seeds of *Jatropha curcas* (Bherenda) and *Hevea brasiliensis* (Rubber), leaves of *Tephrosia purpurea* (Bannilgach), leaves of *Dioscorea alata* (Banalu) and fruits of *Trichosanthes tricuspidata* (Makal) with an aim of using them as low-cost piscicides for eradication of weed fishes during pond preparation for seed rearing. The products were locally collected, processed and their aqueous extracts were prepared (1:5) and then their potency was evaluated against *Puntius sophore* (Sophore barb), a weed fish. The test method followed was static bio-assay (24 hr and 48 hr). Test concentrations of the extracts were assessed through range finding study. Median lethal concentrations (LC_{50}) were estimated by probit analysis. All products were observed effective as piscicide. Tea leaves' extract was observed most effective followed by extract of *Tephrosia* leaves, *Jatropha* seed, *Trichosanthes* fruits, Rubber seed, *Moringa* barks, *Moringa* leaves, *Dioscorea* leaves, *Magnolia* barks, *Millettia* leaves, *Glycyrrhiza* leaves and fruits of *Melia*. The 24h LC_{50} varied from 11.0 to 247 ml/L and 48h LC_{50} from 9.2 to 202 ml/L. From, this study, it could be concluded that aqueous extracts of leaves of tea, *Tephrosia, Moringa, Dioscorea, Millettia* and *Glycyrrhiza*, seeds of *Jatropha* and Rubber, barks of *Moringa* and *Magnolia* and fruits of *Trichosanthes* and *Melia* possess piscicidal property; hence, they are useful as low-cost bio-piscicides.

Introduction

Aquaculture is one of the fastest growing food producing sectors in North-East India. As per the latest data published by the Handbook of Fishery Statistics, Govt. of India (2020), it is a major source of livelihood for more than 26.3 lakh people of the region. The contribution of aquaculture is more than 85% in the annual fish production of North-East India (0.5 MMT). Aquaculture is considered as a lucrative livelihood enterprise, however due to hilly terrains interspersed with valley and plains, inadequate infrastructures, poor supply in raw materials, weak supply chains, conventional approach and transboundary diseases etc, the growth rate in the sector is not uniform across different states and region. Where states like Tripura and Assam are highly progressive in technology assessment, refinement and adoption, rest are laggard.

Quality fish seeds are the 'heart of aquaculture'. To make aquaculture more outreach and progressive, hence, emphasis shall be laid on quality fish seed production and strengthening of their supply chains. The average survival of fish seeds, mainly from spawn to fry stage in the carps is 20-30% due to various reasons. There are scopes to increase the productivity of fish seeds through technological interventions. But most of the fish seed growers of NE India in general and Tripura in particular encounter huge loss due to unscientific pond preparation and management. Pond needs eradication of predatory and weeds fishes such as murrels (*Channa* spp.), barbs (*Puntius* spp.), gobies (*Glossogobius giuris*), minnows (*Danio*, *Aplocheilus*, etc), etc before seed stocking; if they are not eradicated, they immensely hamper the growth and production of target species (mainly Indian major carps in Tripura and NE India) by competing with food, living space and dissolved oxygen of latter. Fishes such as Murrels and Gobies are so dangerous that the presence of their few individuals turns the success of nursery fish rearing into compete failure because they are predaceous and ferocious. In commercial fish seed rearing loss due to presence of predatory and weed fishes is 30 to 40% (Pillay and Kutty, 2005), therefore, proper attention must be paid towards their removal well in advance of stocking seed of target species such as Indian major carps, Silver carp, Grass carp, common carp, etc. To eradicate the predatory and weed fishes, repeated netting of pond, use of hooks and lines or application of piscicides can be followed.

Repeated netting is not an effective method whereas application of piscicides is highly effective. The most effective piscicide is mohua oil cake (Rath, 2011). But problem is that it is not readily available in many places, particularly in the region of Northeast India. As an alternative farmers go for application of bleaching powder or combination of urea and bleaching powder for eradication of unwanted fishes from pond before seed stocking (Ayyappan *et al.*, 2019). But long-term use of bleaching powder makes aquatic ecosystems caustic and unsuitable for the production of fish food organisms like plankton, benthos, earthworms, beneficial soil microbes etc, hence, finding alternative materials is important.

Northeast India is very rich in medicinal plants and herbs (Mao and Hynniewta, 2000) and many of them posses fish stupefying property due to their bioactive compounds (Wise, 2006); for example, Tea seeds and leaves contain saponin (Minsalan and Chiu, 1986), *Jatropha* and *Tephrosia* contains flavonoid compounds, rubber seed contains cyanogenic glycoside (Lieberei, 1986), *Moringa* barks and leaves contain moringine and moringinine (Wise, 2006), *Dioscorea* contain dioscorine (Lu *et al.*, 2012), *Glycyrrhiza* contain glycyrrhizin and glycyrrhetinic acid etc.

These materials can be useful for the removal of unwanted fishes from pond before seed stocking, but, due to lack of systemic efforts and integrated approaches, their usefulness remained unexplored. In the present study, some plant products locally available in Tripura, Northeast India was evaluated for piscicidal property so that they can be popularized as alternative piscicides for NE farmers.

Materials and Methods

The experiment was conducted by ICAR Research Complex for NEH Region, Tripura Centre, Lembucherra. Twelve materials such as *Moringa* leaves, *Moringa* barks, tea leaves, Champak barks, *Melia* fruits, *Glycyrrhiza* leaves, *Millettia* leaves, *Jatropha* seed, Rubber seed, *Tephrosia* leaves, *Dioscorea* leaves and *Trichosanthes* fruits were tested. The materials were collected from different location of Tripura, Northeast India (Table 1) and then processed for testing. Processing steps involved were cleaning the materials, slicing into small pieces, removal of shell and collection of kernel in case of seeds, pounding, grinding etc.

After that, they were soaked in distilled water for overnight (one part material + five parts distilled water). Next day (approximately after 18-24 hrs.), the soaked materials were filtered through a fine muslin cloth and aqueous extracts were collected. The extracts were estimated for pH (using a digital pH meter), colour (by eye estimation), odour (by sensory) and acid-neutralizing capacity (by titrating with NaOH). The extracts were immediately evaluated after preparation to ensure its freshness and potency.

Puntius sophore (Sophore barb) was used as 'test fish' in this experiment. It is a weed fish and breed profusely in pond. It has immense negative effect on the growth and survival of target species. Further making pond getting rid of them is difficult because of its concealing habit and hardiness. This is the fish which do late response to the application of piscicides. It could be assumed that the pond became weed fish free once they died. Therefore, in the present, it was used as a model test fish.

Around 3000 nos. of *P. sophore* were collected from the farm ponds of ICAR NEH, Tripura, transported and acclimatized in FRP pools over a period of one week with regular feeding, aeration and water exchange before bioassay experiment. Only healthy fish were used for bioassay study (APHA, 1985). The experiment was performed in aquaria (each 20 litres capacity) containing ten litres water and ten fish (avg. weight 0.2±0.1g and avg. length 3.5±0.02 cm). Their supplementary feeding was terminated 24 hr before starting the assay. The test concentrations of the extracts were kept between their safe limit (the concentration at which there is no fish mortality) and lethal limit (the concentration at which all fish die) for fish (Table 2) which was determined by conducting range-finding study (Nanda *et al.,* 2009).

Fish behaviours (swimming, skin colour, air engulfing, reflex, mucus secretion etc) was noted at regular interval (1 hr, 6 hr, 12 hr, 24 hr and 48 hr.) after exposing them to extracts. Their inability to respond to external stimuli and mechanical prodding was considered 'death of the fish'. Dead fish were removed and counted and mortality (%) was calculated for each concentration of extracts.

Each test was performed three times before final conclusion. 24 and 48 hr median lethal concentration (LC_{50}), i.e., the concentration at which 50% of fish dies and 50% of fish survives was calculated by plotting fish mortality against the test concentrations in MS Excel and probit analysis (Finney, 1971).

Results and Discussion

In this experiment, twelve plant products i.e., *Moringa* leaves, *Moringa* barks, tea leaves, Champak barks, *Melia* fruits, *Glycyrrhiza* leaves, *Millettia* leaves, *Jatropha* seed, Rubber seed, *Tephrosia* leaves, *Dioscorea* leaves and *Trichosanthes* fruits were tested for piscicidal property using Sophore barb (*P. sophore*) as a test fish. All the products were found possessing piscicidal property against the tested fish. From this finding, it can be state that the tested materials can be applicable as piscicide for eradication of unwanted fishes from pond during pond preparation for seed rearing.

The responses the test fish showed after exposing to the extracts were found dependent on test concentrations of the extracts. Fish had minimal signs of discomfort and distress when concentrations of the extracts were low; however, when the concentrations were elevated, fish started developing greater signs of distress and discomforts in the form of discolouration of skin, excessive mucus secretion, ataxia, no response to mechanical prodding, clouding of eye, etc.

This indicated that with the increase of test concentrations in water, the levels of bio-active compounds available in the extracts got increased in water and that reflected in the biological/physiological activities of fish deviated from normal. Similar observations were reported by Ayotunde *et al.,* (2010).

The first sign of physiological distress fish displayed after exposing to the extracts was erratic swimming, later there was difficulty in their breathing performance (fishes were observed doing engulfing at the surface of aquarium water), development of dark patches on skin, excessive mucus secretion, and loss of reflex (Table 3).

The ulterior mechanisms remained behind such physiological distresses in fish could not study in this experiment due to resource constraints, however, it could be assumed that such physiological distresses happened due to damage of gills and disturb in respiratory functions, impairments in their metabolic and nervous activities, activation of melanocyte stimulating factors and dispersion of melanin pigments in skin (Akinwande *et al.,* 2007).

From the probit analysis, it was observed that tea leaves' extract was most effective against the fish followed by *Tephrosia* leaves, *Jatropha* seed, fruits of *Trichosanthes,* Rubber seed, *Moringa* barks, *Moringa* leaves, *Dioscorea* leaves, *Magnolia* barks, *Millettia* leaves, *Glycyrrhiza* leaves, and *Melia* fruits (Table 4). The overall 24h LC_{50} varied from 11.0 to 247 ml/L and 48h LC_{50} varied from 9.2 to 202 ml/L with their extracts. Similar observations were reported on other fishes with the plant products (Adesina and Omitoyin, 2011; Adelakun *et al.,* 2017).

In this study, the effectiveness of the materials could be attributed to the presence of bio-active compounds in them. Though we could not assess the bio-active compounds present in those materials but based on the secondary literatures it could be attributed to saponin in case of tea leaves (Minsalan and Chiu, 1986), prenylated flavonoid compounds in case of *Jatropha* seed and *Tephrosia* leaves (Subramanian *et al.,* 1971; Chen *et al.,* 1978), cyanogenic glycoside compound, linamarin in rubber seed (Lieberei, 1986), moringine, moringinine, tannins, saponins and glucosinolates in *Moringa* (Wise, 2006), dioscorine in *Dioscorea* (Lu *et al.,* 2012), tannins, saponins, glycosides, flavonoids compounds in *Trichosanthes* and *Michelia* (Geetha *et al.,* 2011; Kumar *et al.,* 2012), glycyrrhizin and glycyrrhetinic acid in *Glycyrrhiza* (Gulati *et al.,* 2016), and melatonin in *M. azedarach* (Oelrichs *et al.,* 1984). These bioactive compounds caused death in fish by altering their blood chemistry and impairing their nervous system (Gulati *et al.,* 2016).

Aquaculture as a livelihood enterprise will be more sustainable if local resources are integrated with it. Several studies were carried out to assess the toxicity of plant-origin products against livestock animals, mollusks, bacteria, fungi etc (Orwa *et al.,* 2009), but, studies are greatly limited when it comes for lower vertebrates like fish, plankton, etc (Kiat, 1969; Singh and Singh, 2002; Nasiruddin *et al.,* 2012; Chiotha *et al.,* 2012; Das *et al.,* 2013; Vanichkul *et al.,* 2014). It is the first attempt to explore the piscicidal potential of selected plant products widely naturalized in Tripura and many other NE States of India. Tea and rubber cultivation are growing fast in Tripura; during tea processing, 20-30% leaves get wasted and during rubber cultivation, 150-200 kg rubber seeds/ha are generated as residues; further plants like *Moringa, Dioscorea¸ Tephrosia* etc are widely naturalized in this region of India and used as vegetables, fodder, medicines, etc (Kamble *et al.,* 2014).

Table.1 Plant products evaluated for piscicidal property

Scientific name	Family	Habit	Common name	Parts useful	Collection site	GPS Coordinates
Moringa oleifera	*Moringaceae*	Tree	Drumstick/ Sajna	Leaf, bark, root	Lembucherra, West Tripura	23.9064^0N, 91.3132^0E
Camellia sinensis	*Theaceae*	Shrub	Tea	Seed, leaf	Durgabari Tea Estate, Mohanpur, West Tripura	23.8917°N, 91.2915°E
Magnolia champaka	*Magnoliaceae*	Tree	Champak	Seed, bark	Kamalghat, West Tripura	23.9285°N, 91.3385°E
Melia azedarach	*Meliaceae*	Tree	Chinaberry	Seed, leaf, bark	Lembucherra, West Tripura	23.4505°N, 91.2146°E
Glycyrrhiza glabra	*Fabaceae*	Herb	Mulethi	Leaf	Lembucherra, West Tripura	23.3023°N, 91.2165°E
Millettia pachycarpa	*Fabaceae*	Climber	Bish lata	Leaf	Melaghar, South Tripura; Rupashnagar, North Tripura	23.4973°N, 91.3313°E; 23.9248°N, 91.8465°E
Jatropha curcas	*Euphorbiaceae*	Shrub/ small tree	Bherenda	Seed, Leaf	Lembucherra, West Tripura	23.4454°N, 91.5634°E
Hevea brasiliensis	*Euphorbiaceae*	Tree	Rubber	Seed kernel	Santir Bazar, South Tripura	23.3121°N, 91.5620°E
Tephrosia purpurea	*Fabaceae*	Herb/ shrub	Bannilgach	Leaf	Lembucherra, West Tripura	23.8566°N, 91.3065°E
Dioscorea alata	*Dioscoreaceae*	Climber	Banalu	Leaf	Lembucherra, West Tripura	23.9082°N, 91.3195° E
Trichosanthes tricuspidata	*Cucurbitaceae*	Climber	Makal, Bitter Snake gourd	Fruit	Lembucherra, West Tripura	23.4436°N, 91.2132°E

Fig.1 Some plant products locally collected for evaluation of their piscicidal property

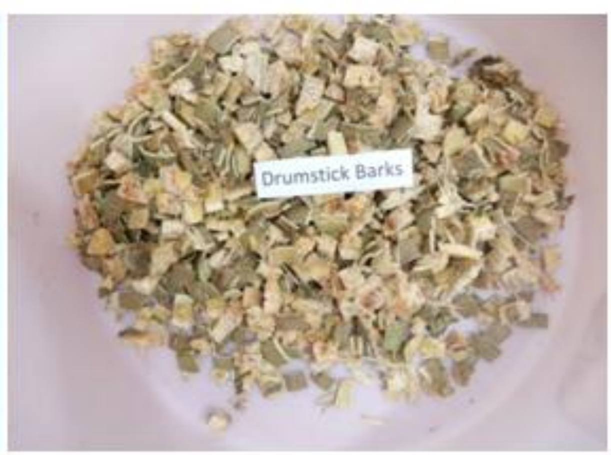

Table.2 The quality parameters of aqueous extracts and their test concentrations against Sophore barb (*Puntius sophore*).

Sl. No.	Extracts	pH	Colour	Odour	Alkali requires to neutralize it (ml)	Test concentrations	Safe limit (ml/L)	Lethal limit (ml/L)
1.	Leaves of *Moringa*	4.83	Greenish	Musky	12.5	0, 10, 20, 40, 80, 160, 320	10	320
2.	Barks of *Moringa*	3.93	Brownish	Pungent	14.4	0, 10, 20, 40, 80, 160, 320	10	320
3.	Residues of tea leaves	3.84	Tainted	Sweet musky	14.6	0, 5, 7, 9, 11, 13, 15	5	15
4.	Barks of *Magnolia*	3.47	Brownish	Pungent	15.5	0, 50, 100, 150, 200, 250, 300	50	300
5.	Fruits of *Melia*	4.00	Greenish-brown	Waxy	13.8	0, 50, 100, 150, 200, 250, 300, 350	50	350
6.	Leaves of *Glycyrrhiza*	4.38	Greenish	Musky	12.8	0, 50, 100, 150, 200, 250, 300, 350	50	350
7.	Leaves of *Millettia*	4.13	Greenish	Musky	13.3	0, 50, 100, 150, 200, 250, 300	50	300
8.	*Jatropha* seed kernel	3.85	White	Waxy	14.5	0, 5, 10, 15, 20, 25, 30	5	30
9.	Rubber seed kernel	4.03	Blackish	Waxy	13.6	0, 5, 10, 50, 75, 100	10	100
10.	*Tephrosia* leaves	3.94	Greenish	Musky	14.2	0, 5, 7.5, 10, 15, 20	5	20
11.	*Dioscorea* leaves	4.69	Greenish	Musky	12.2	0, 50, 100, 150, 200, 250	50	250
12.	Fruits of *Trichosanthes*	4.34	Blackish	Pungent	12.6	0, 10, 20, 30, 40, 50	10	50

Table.3 Behavioural response of Sophore barb (*Puntius sophore*) against aqueous extracts of different plants of Tripura.

Extracts	Responses of fish	Exposure time (hr)				
		1	6	12	24	48
***Moringa* leaves**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	-	+	+
	Air engulfing	-	-	+	+	+
	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	-	+	+	+
***Moringa* barks**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	-	+	+	+	+
	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	-	+	+	+
Tea leaves	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	-	+	+	+	+
	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	-	+	+	+
Champak barks	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	-	-	+	+	+
	Erratic swimming	-	+	+	+	+
	Excessive mucus	-	-	+	+	+
***Melia* fruits**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	-	+	+	+	+
	Erratic swimming	-	+	+	+	+
	Excessive mucus	-	-	+	+	+
***Glycyrrhiza* leaves**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	-	+	+	+	+
	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	-	+	+	+
***Millettia* leaves**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	+	+	+	+	+
	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	-	+	+	+
***Jatropha* seed**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	-	-	+	+	+

	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	-	+	+	+
Rubber seed	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	-	-	+	+	+
	Erratic swimming	-	+	+	+	+
	Excessive mucus	-	-	+	+	+
***Tephrosia* leaves**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	+	+	+	+	+
	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	+	+	+	+
***Dioscorea* leaves**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	-	+	+
	Air engulfing	-	-	+	+	+
	Erratic swimming	-	+	+	+	+
	Excessive mucus	-	-	+	+	+
***Trichosanthes* fruits**	Loss of reflex	-	-	-	-	+
	Skin discoloration	-	-	+	+	+
	Air engulfing	+	+	+	+	+
	Erratic swimming	+	+	+	+	+
	Excessive mucus	-	+	+	+	+

Fig.2 Preparation of extracts

Table.4 Median lethal concentration of different extracts against Sophore barb (*Puntius sophore*)

Sl. No.	Extracts	24 hr	48 hr	LC_{50} (ml/L)	
				24 hr	48 hr
1.	Leaves of *Moringa*	y = 0.2673x + 9.2732 R^2 = 0.8555	y = 0.3195x + 11.717 R^2 = 0.8701	152.4	120.0
2.	Barks of *Moringa*	y = 0.2924x + 21.303 R^2 = 0.7475	y = 0.2924x + 21.303 R^2 = 0.7475	145.6	98.2
3.	Residues of tea leaves	y = 7.5076x - 19.113 R^2 = 0.8918	y = 7.5076x - 19.113 R^2 = 0.8918	11.0	9.2
4.	Barks of *Magnolia*	y = 0.369x - 12.5 R^2 = 0.961	y = 0.369x - 12.5 R^2 = 0.961	187.0	170.0
5.	Fruits of *Melia*	y = 0.2579x - 13.889 R^2 = 0.9358	y = 0.3016x - 11.111 R^2 = 0.955	247.0	202.0
6.	Leaves of *Glycyrrhiza*	y = 0.2579x - 9.7222 R^2 = 0.9358	y = 0.3056x - 5.5556 R^2 = 0.9571	231.6	181.8
7.	Leaves of *Millettia*	y = 0.369x - 10.119 R^2 = 0.9688	y = 0.369x - 10.119 R^2 = 0.9688	198.0	163.0
8.	*Jatropha* seed kernel	y = 3.4524x - 8.9286 R^2 = 0.9471	y = 3.4524x - 8.9286 R^2 = 0.9471	19.8	17.0
9.	Rubber seed kernel	y = 0.7611x + 0.1124 R^2 = 0.9577	y = 0.9634x + 0.3532 R^2 = 0.9853	65.5	51.5
10.	*Tephrosia* leaves	y = 4.7619x - 9.5238 R^2 = 0.9124	y = 5.551x - 3.1973 R^2 = 0.8847	12.5	9.6
11.	*Dioscorea* leaves	y = 0.3333x - 11.111 R^2 = 0.9292	y = 0.419x - 13.492 R^2 = 0.9429	183.4	151.6
12.	Fruits of *Trichosanthes*	y = 1.6667x - 11.111 R^2 = 0.9292	y = 2.0952x - 13.492 R^2 = 0.9429	36.7	30.3

Fig.3 Extracts prepared

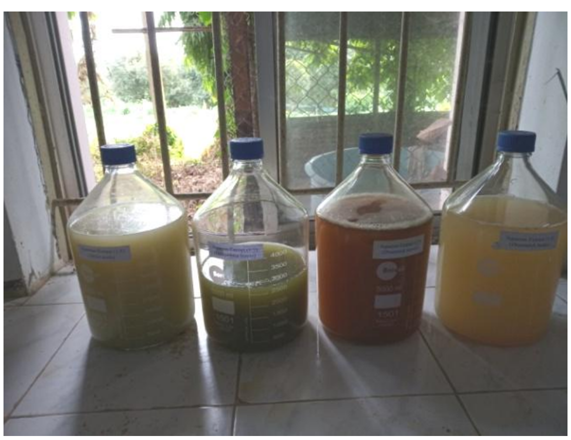

Fig.4 Test fish (*Puntius sophore*)

If these materials are used or promoted as piscicides in fish farming, on one hand it will reduce the cost of cultivation and on the other hand it will make aquaculture more eco-friendly and sustainable. In conclusion, it could be said that leaves and barks of drumstick, leaves of Tea, barks of Champak, fruits of Chinaberry, leaves of Mulethi, leaves of Bish lata, seed of Bherenda and Rubber, leaves of Bannilgach, leaves of Banalu and fruits of Makal possess piscicidal property; they are effective against the weed fish.

Being locally available, they impose low cost on aquaculture and being biodegradable, they leave no residual effect on the ecosystems. Thus their use is recommended as piscicides for eradication of unwanted fishes during pond preparation for seed rearing. Further location-specific studies are recommended to identify similar other plant products and to promote sustainable aquaculture.

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