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PISCICIDAL EFFECTS OF *ALBIZZIA PROCERA* (BENTH) SEEDS, *SWIETENIA MAHAGONI* (JACQ) AND *AZADIRACHTA INDICA* (A. JUSS) SEED KERNELS AND *CAMELLIA SINENSIS* (WILD) SEED CAKE EXTRACTS ON *OREOCHROMIS MOSSAMBICUS* (PETERS) AND *CHANNA PUNCTATUS* (BLOCH).

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ABSTRACT

Piscicidal effects of distilled water and fifty percent ethyl alcohol extracts of *Albizzia procera* seeds, *Swietenia mahagoni and Azadirachta indica* seed kernels and *Camellia sinensis* seed cake were bioassayed upon *Oreochromis mossambicus* and *Channa punctatus* under normal laboratory conditions. Due to the toxic effects, the piscicide treated fishes showed irritability, excitement and gradual loss of balance. Mortality in both the species showed dose dependent increase with increasing concentrations. From the LC_{50} values, the relative toxicities of the plant extracts for both the fishes were found to be *A. procera* seeds > *C. sinensis* seed cake > *S. mahagoni* seed kernels> *A. indica* seed kernel extracts. The degree of tolerance of the test fishes to any of the two extracts was in the order *C. punctatus* > *O. mossambicus*.

Key words : Piscicide, Plant extract, Toxicity, Mortality, LC_{50}.

INTRODUCTION

Presence of predatory and weed fishes create a great problem in the aquaculture of nursery ponds by preying upon fish fry and competing with culture fishes for food, thus directly affecting the productivity of fish (Jhingran 1977). Eradication of these fishes from nursery and stocking fish ponds and farms prior to fry release of economically important fish species is an important operation in any fish culture practice. A great number of investigations have been done on the development and practical use of piscicides, as there is a great demand for selective toxicants to control undesirable fishes. As chemical pesticides, which are used as piscicides, are highly toxic and ecologically ruinous, so the use of biodegradable fish poison of plant origin will be environment friendly and an effective method for removing undesirable fish from fish ponds.

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Plant products, such as, rotenone, nicotine, saponin, sabadilla, quassia, ryania, pyrethrum have pest control properties and also have piscicidal effects. Marking and Bills (1976) reported that the piscicides of plant origin has been favoured for the removal of undesirable fishes from ponds. A considerable number of indigenous plants having piscicidal effects have been reported by Nayar (1955), Jhingran (1977) and Chopra *et al*. (1985). Research based on botanical piscicides has been carried out by some workers at home viz., Haque and Tilton (1970), Chowdhury *et al.* (1981), Ameen and Shahjahan (1987), Ameen *et al.* (1987), Latifa *et al*. (1987, 1988, 1992, 1997, 2002, 2004), Latifa and Begum (1993) and Nasiruddin *et al*. (1997, 1998, 2006).

There is need for further research to find out the action of herbal piscicides on different fishes. In view of this, the present paper deals with the piscicidal action of dry seeds of *Albizzia procera* (Benth), seed kernels of *Swietenia mahagoni* (Jacq) and *Azadirachta indica* (A. Juss) and seed cake of *Camellia sinensis* (Wild) on *Oreochromis mossambicus* (Peters) and *Channa punctatus* (Bloch).

MATERIALS AND METHODS

Dry seeds of *Albizzia procera*, seed kernels of *Swietenia mahagoni* and *Azadirachta indica* and seed cake of *Camellia sinensis* were stocked for the experiments. Healthy and live fishes of *O. mossambicus* were collected from a local pond and *C. punctatus* from fish markets of Chittagong city. Fishes were maintained in aquaria (60x30x30 cm^3) containing tap water and acclimatized for 4±1 hours in laboratory conditions. Necessary arrangements were taken for aeration of the experimental aquaria. The average total length, standard length and weight of *O. mossambicus* were 10.39±1.74 cm, 8.01±1.11 cm and 22.95±7.25 g and that of *C. punctatus* were 14.3±3.23 cm, 13.4±3.22 cm and 31.32±4.76 g respectively.

Toxicants were extracted from pulverised dry seeds, seed kernels or seed cake powder with two solvents, namely distilled water and 50% ethyl alcohol. The proportion of dry seed, seed kernel or seed cake powder and the relevant solvent was 10 g/100 ml. The extract was filtered and the filtrate obtained was kept as stock solution from which different test concentrations were prepared.

The required concentrations of the test solutions were obtained by appropriate dilution of the stock solution (APHA 1976). For each set of experiment a certain (calculated) volume of the stock solution extract was added

to certain volume of tap water so that the final volume in the experimental aquarium was always 5 litre in each of the replicates. The concentrations were prepared in terms of ppm. Several preliminary screening tests were done to ascertain the final concentration ranges used in the final experiments.

Bioassays were done in the laboratory at room temperature ($30\pm3^{o}C$) and were conducted in a series of glass aquaria (30x23x23 cm^3). Different concentrations of the extracts were added to the aquaria to determine the LC_{50} values for *O. mossambicus* and *C. punctatus*. Five concentrations of each extract were used in the final experiments. In each test five test fishes were released at random. Each concentration of the relevant toxicant was replicated twice. The test fishes were kept in the aquaria for an exposure period of 24 hours. Behaviour of the fishes in control and in the highest concentration of each of the extracts was observed. Mortality (%) was counted after 24 hours exposure. There was no control mortality.

Mortality data was subjected to probit analysis. LC_{50} with 95% confidence interval was analysed in a computer based probit analysis programme. Regression equation of each extract was calculated from empirical probit, working probit, weighting probit, the values of which were taken from the Tables of Finney (1971). Expected probit was calculated from empirical probit. Values of chi-square were determined following Fisher and Yates (1963) at 0.05 level of significance. Estimation of variation among treatments at 0.01 level of significance was made by F test. Relative potencies were calculated on the basis of a potency which was taken as a reciprocal of the equitoxic doses.

RESULT AND DISCUSSION

Behaviour of the control fishes

Both the fish species in the control sets were well balanced, showing normal behaviour and morphology throughout the experiments. They remained healthy, active and alive with vigorous movement. *O. mossambicus* moved normally by regular opening of their operculum, keeping the dorsal fins straight and they did not jump upwards. *C. punctatus* swam moving their pectoral fins regularly and gently. No slime secretion was observed.

Effect of the extracts on the behaviour of O. mossambicus

Movement started immediately after the exposure to both the distilled water and 50% ethyl alcohol extracts of *A. procera* seeds at the highest concentrations 35 and 30 ppm respectively. Vigorous movement started within 5

minutes, dashing up and down rapidly. The fishes swam erratically in the aquaria for a while. After 30 minutes their movement slowed down, gradually relaxed and sank to the bottom. The almost motionless fishes ultimately died at different time intervals. Dead fishes were seen floating parallel or made an angle to the water surface. Colour of the fishes changed, some fins were broken and heavy slime secretion occurred. Scales of these fishes were seen drooping.

The first visible reaction started within half an hour of exposure to the distilled water extract of *S. mahagoni* seed kernels at the highest concentration 750 ppm and within 15 minutes after exposure to the 50% ethyl alcohol extract at 500 ppm. The fishes showed vigorous movement after 15 minutes, moving up and down and were repeatedly coming towards the surface for taking air. Later on irregular movement was seen and the fishes failed to keep their balance. Gradually their movement became slow. At last their movement ceased, fins became straight, and slowly settled to the bottom of the aquaria and ultimately died at different time intervals. Dead fishes remained at the bottom of the aquaria. Slime secretion after death was seen to occur.

The first visible reaction started within 45 minutes after exposure to 1200 ppm of distilled water and 1000 ppm of 50% ethyl alcohol extract of *A. indica* seed kernels. At times they rapidly moved up and down. Later on their movement slowed down, became motionless and died at different intervals. Less slime secretion was observed. Dead fishes laid flat in the bottom of the aquaria.

The fishes exhibited their agitated movement within 10 minutes of exposure to the highest toxicant (100 ppm for distilled water and 75 ppm for 50% ethyl alcohol) of *C. sinensis* seed cake. They showed vigorous movements after 10 minutes as they swam restlessly. Such movement continued for a while. After 45 minutes their movement became slow, gradually relaxed to the bottom and died at different intervals. Slime secretion was in lesser quantity than those exposed to *A. procera* seed extract. Most of the dead fishes were seen in floating condition.

Effect of extracts on behaviour of C. punctatus

The fishes started their irregular movement immediately within 2 minutes of exposure to the distilled water and 50% ethyl alcohol extracts of *A. procera* seeds at the highest concentrations 50 and 35 ppm respectively. After exposure to the toxicants the fishes jumped upwards and tried to escape from the aquaria. Later on the fishes moved rapidly up and down with jerky movement. After 45 minutes they became lethargic and their movement slowed down. Finally, the

fishes died at different intervals. Some of the dead fishes were seen floating near the water surface, with ventral side was turned upward. Slime secretion in this fish was less than in *O. mossambicus*.

Vigorous erratic movement started within 10 minutes of exposure to 1000 ppm of distilled water and 500 ppm of 50% ethyl alcohol extracts of *S. mahagoni* seed kernels. The fishes moved rapidly up and down for a while. Subsequently their movement became slow, gradually relaxed to the bottom and ultimately died at intervals. Some of the dead fishes were seen floating at an angle. Slime secretion was also observed to some extent.

The fishes exhibited their restless movement within 30 minutes after exposure to the seed kernel extract of *A. indica* at the highest concentrations of 1500 ppm of distilled water and 1200 ppm of 50% ethyl alcohol. The fishes tried to jump out of the aquaria. They swam irregularly and such movement continued for 2-3 hours, then they gradually lost their balance and died. Dead fishes remained at the bottom of the aquaria.

Vigorous movement started within 5 minutes of exposure to the highest concentrations 150 and 100 ppm of distilled water and 50% ethyl alcohol extracts of *C. sinensis* seed cake respectively. At times the fishes tried to jump out of the aquaria and at times they moved restlessly. After sometimes they became motionless, lost their balance and finally died. Dead fishes were seen floating near the surface.

The degree of abnormal behaviour of the species was regarded as an important parameter in assessing the extent of the toxic effects of the plant extracts. The behaviour of the fishes changed due to the toxic effect of the extracts. Upon exposure to the toxicants the fishes exhibited reactions with regards to their movement. The treated fishes showed irritability, excitement and died with gradual loss of balance. Slime secretion followed by death was seen to occur. These behavioural findings are almost similar to the findings of Latifa *et al*. (1987, 1988, 1992), Latifa and Begum (1993) and Nasiruddin *et al.* (1997, 1998, 2006). Agitated swimming behaviour in *Salmo gairdnerii* was observed by Wedemeyer (1970) and in *Pseudapocryptes dentatus*, *Gambusia affinis* and *Aphinis mento* by Sharma *et al*. (1978). The result of the present study also showed that an increase in toxicity level caused a decrease in the time of total loss of equilibrium in the two species.

Toxicological effects of the extracts

A particular concentration of an extract caused varying percentage of mortality in *O. mossambicus* and *C. punctatus*, the mortality of the fishes increased with the gradual increase of the concentrations of the different extracts (Table 1). The statistical analysis of the concentration and mortality parameters of both the species are given in Table 2. The LC_{50} values and relative potencies of distilled water and 50% ethyl alcohol extracts of *A. procera* seeds, *S. mahagoni* and *A. indica* seed kernels and *C. sinensis* seed cake in *O. mossambicus* and *C. punctatus* are shown in Table 2. Fifty percent ethyl alcohol extract of *A. procera* seeds showed the highest toxicity, $LC_{50,}$ the values for *O. mossambicus* and *C. punctatus* were 13.40 and 15.65 ppm respectively. Whereas, the least toxic were distilled water extract of *A. indica* seed kernels (LC_{50} = 301.74 ppm) for *O. mossambicus*, and distilled water extract of *S. mahagoni* seed kernel (LC_{50} value of 596.55 ppm) for *C. punctatus*. The relative toxicities for both the fishes were found as *A. procera* seeds > *C. sinensis* seed cake > *S. mahagoni* seed kernels > *A. indica* seed kernels (Table 3).

For *O. mossambicus* the relative position of the extracts on the basis of their relative potency values were: 50% ethyl alcohol extract of *A. procera* seeds > Distilled water extract of *A. procera* seeds> 50% ethyl alcohol extract of *C. sinensis* seed cake > Distilled water extract of *C. sinensis* seed cake > 50% ethyl alcohol extract of *S. mahagoni* seed kernels > Distilled water extract of *S. mahagoni* seed kernels > 50% ethyl alcohol extract of *A. indica* seed kernels > Distilled water extract of *A. indica* seed kernels.

In case of *C. punctatus* the relative position of the extracts on the basis of their relative potency values were: 50% ethyl alcohol extract of *A. procera* seeds > Distilled water extract of *A. procera* seeds > 50% ethyl alcohol extract of *C. sinensis* seed cake > Distilled water extract of *C. sinensis* seed cake > 50% ethyl alcohol extract of *S. mahagoni* seed kernels > 50% ethyl alcohol extract of *A. indica* seed kernels > Distilled water extract of *A. indica* seed kernels > distilled water extract of *S. mahagoni* seed kernels.

TABLE 1: DATA SHOWING THE PERCENTAGE MORTALITY, LC_{50} AND RELATIVE POTENCY VALUES OF *O. MOSSAMBICUS* AND *C. PUNCTATUS* AT DIFFERENT CONCENTRATIONS OF DISTILLED WATER AND 50% ETHYL ALCOHOL EXTRACTS OF *ALBIZZIA PROCERA* SEEDS, *SWIETENIA MAHAGONI* AND *AZADIRACHTA INDICA* SEED KERNELS AND *CAMELLIA SINENSIS* SEED CAKE AFTER 24 HOUR EXPOSURE.

Toxicant	Solvent	Log Conc.	Conc. (ppm)	Mortality (%)	Log Conc.	Conc. (ppm)	Mortality (%)
		Oreochromis mossambicus			*Channa punctatus*		
A. procera (seed)	Distilled water	0.69	5	10	0.69	5	10
		1.00	10	30	1.00	10	30
		1.17	15	50	1.30	20	50
		1.39	25	70	1.60	40	70
		1.54	35	90	1.69	50	90
	50% ethyl alcohol	0.69	5	20	0.39	2.5	10
		1.00	10	40	0.60	5	20
		1.17	15	50	1.17	15	30
		1.39	25	60	1.39	25	60
		1.47	30	90	1.54	35	90
S. mahagoni (seed kernel)	Distilled water	1.69	50	10	2.00	100	10
		2.00	100	20	2.39	250	20
		2.39	250	30	2.69	500	30
		2.69	500	60	2.87	750	50
		2.87	750	90	3.00	1000	90
	50% ethyl alcohol	1.00	10	20	1.69	50	10
		1.69	50	40	2.00	100	20
		2.00	100	50	2.30	200	60
		2.39	250	60	2.60	400	80
		2.69	500	90	2.69	500	90
A. indica (seed kernel)	Distilled water	2.30	200	10	2.00	100	10
		2.60	400	30	2.39	250	20
		2.90	800	60	2.69	500	40
		3.00	1000	70	3.00	1000	60
		3.70	1200	90	3.17	1500	90
	50% ethyl alcohol	2.00	100	10	2.00	100	20
		2.30	200	20	2.39	250	30
		2.60	400	50	2.69	500	50
		2.90	800	70	3.00	1000	80
		3.00	1000	90	3.07	1200	90
C. sinensis (seed cake)	Distilled water	1.00	10	10	1.39	25	10
		1.39	25	20	1.69	50	30
		1.69	50	40	1.87	75	60
		1.87	75	60	2.00	100	70
		2.00	100	90	2.17	150	90
	50% ethyl alcohol	0.69	5	10	1.00	10	20
		1.00	10	20	1.39	25	30
		1.39	25	40	1.69	50	60
		1.69	50	80	1.87	75	80
		1.87	75	90	2.00	100	90

TABLE 2: SHOWING THE STATISTICAL ANALYSIS OF THE TOXICITY PARAMETERS OF DISTILLED WATER AND 50% ETHYL ALCOHOL EXTRACTS OF *ALBIZZIA PROCERA* SEEDS, *SWIETENIA MAHAGONI* AND *AZADIRACHTA INDICA* SEED KERNELS AND *CAMELLIA SINENSIS* SEED CAKE ON *O. MOSSAMBICUS* AND *C. PUNCTATUS* AFTER 24 HOUR EXPOSURE.

Toxicant	Solvent	Conc. range (ppm)	Regression equation	Chi-square (P-value)	F-test (p-value)	LC_{50} (ppm)	Confidence limit (ppm)		Relative potency
							Lower	Upper	
Oreochromis mossambicus									
A. procera (seed)	Distilled water	5-35	1.71+2.84x	3.72 (P>0.05)	5.00 (P>0.01)	14.82	10.17	21.13	39.89
	50% ethyl alcohol	2.5-35	2.78+1.86x	31.90 (P<0.05)	17.83 (P<0.01)	15.65	9.29	29.32	38.12
S. mahagoni (seed kernel)	Distilled water	50-750	0.44+1.83x	20.05 (P<0.05)	14.88 (P<0.01)	301.74	181.62	555.90	1.96
	50% ethyl alcohol	10-500	2.99+1.06x	13.99 (P<0.05)	3.35 (P>0.01)	81.64	25.73	211.14	7.24
A. indica (seed kernel)	Distilled water	200-1200	-0.76+2.10x	20.17 (P<0.05)	12.75 (P<0.01)	591.17	396.11	821.55	1.00
	50% ethyl alcohol	100-1000	-0.9+2.27x	7.12 (P>0.05)	15.50 (P<0.01)	398.91	256.66	616.28	1.48
C. sinensis (seed cake)	Distilled water	10-100	1.10+2.32x	24.59 (P<0.05)	8.58 (P>0.01)	49.61	31.84	80.30	11.92
	50% ethyl alcohol	5-75	1.96+2.22x	9.41 (P>0.05)	31.75 (P<0.01)	23.75	14.64	38.11	24.89
Channa punctatus									
A. procera (seed)	Distilled water	5-50	2.23+2.20x	6.80 (P>0.05)	10.00 (P>0.01)	18.61	11.48	29.40	32.06
	50% ethyl alcohol	2.5-35	2.78+1.86x	31.90 (P<0.05)	17.83 (P<0.01)	15.65	9.29	29.32	38.12
S. mahagoni (seed kernel)	Distilled water	100-1000	-3.12+2.98x	32.24 (P<0.05)	12.50 (P<0.01)	596.55	365.59	1051.63	1.00
	50% ethyl alcohol	50-500	-1.14+2.87x	29.72 (P<0.05)	10.58 (P>0.01)	175.78	114.39	258.82	3.39
A. indica (seed kernel)	Distilled water	100-1500	-0.54+2.01x	14.39 (P<0.05)	5.15 (P>0.01)	581.56	353.04	1018.55	1.03
	50% ethyl alcohol	100-1200	0.18+1.88x	11.97 (P<0.05)	6.64 (P>0.01)	376.96	200.35	621.70	1.58
C.sinensis (seed cake)	Distilled water	25-150	-0.65+3.12x	2.58 (P>0.05)	12.75 (P<0.01)	65.97	46.53	89.07	9.04
	50% ethyl alcohol	10-100	1.80+2.12x	9.27 (P>0.05)	7.75 (P>0.01)	32.75	17.89	50.03	18.22

TABLE 3: SHOWING THE COMPARISONS AMONGST THE DIFFERENT EXTRACTS WITH THEIR RELATIVE POTENCY VALUES IN *O. MOSSAMBICUS* AND *C. PUNCTATUS*. (DWE= DISTILLED WATER EXTRACT AND 50% EAE =50% ETHYL ALCOHOL EXTRACT).

Comparison	Relative potency	
	O. mossambicus	C. punctatus
DWE of *A. procera* seed relative to 50%EAE of *A. procera* seed	1.106	1.189
DWE of *A. procera* seed relative to DWE of *S. mahagoni* seed kernel	0.049	0.033
DWE of *A. procera* seed relative to 50%EAE of *S. mahagoni* seed kernel	0.182	0.106
DWE of *A. procera* seed relative to DWE of *A. indica* seed kernel	0.025	0.032
DWE of A. procera *seed relative to 50%EAE of* A. indica *seed kernel*	0.037	0.049
DWE of *A. procera* seed relative to DWE of *C. sinensis* seed cake	0.299	0.282
DWE of *A. procera* seed relative to 50%EAE of *C. sinensis* seed cake	0.624	0.568
50%EAE of *A. procera* seed relative to DWE of *S. mahagoni* seed kernel	0.044	0.027
50%EAE of *A. procera* seed relative to 50%EAE of *S. mahagoni* seed kernel	0.164	0.089
50%EAE of *A. procera* seed relative to DWE of *A. indica* seed kernel	0.023	0.027
50%EAE of *A. procera* seed relative to 50%EAE of *A. indica* seed kernel	0.034	0.042
50%EAE of *A. procera* seed relative to DWE of *C. sinensis* seed cake	0.270	0.237
50%EAE of *A. procera* seed relative to 50%EAE of *C. sinensis* seed cake	0.564	0.478
DWE of *S. mahagoni* seed kernel relative to 50%EAE of *S. mahagoni* seed kernel	3.696	3.240
DWE of S. mahagoni *seed kernel relative to DWE of* A. indica *seed kernel*	0.510	0.979
DWE of *S. mahagoni* seed kernel relative to 50%EAE of *A. indica* seed kernel	0.756	1.512
DWE of *S. mahagoni* seed kernel relative to DWE of *C. sinensis* seed cake	6.082	8.634
DWE of *S. mahagoni* seed kernel relative to 50%EAE of *C. sinensis* seed cake	12.705	17.390
50%EAE of *S. mahagoni seed kernel relative to DWE of A. indica seed kernel*	0.138	0.302
50%EAE of *S. mahagoni* seed kernel relative to 50%EAE of *A. indica* seed kernel	0.205	0.467
50%EAE of *S. mahagoni* seed kernel relative to DWE of *C. sinensis* seed cake	1.646	2.665
50%EAE of *S. mahagoni* seed kernel relative to 50%EAE of *C. sinensis* seed cake	3.437	5.367
DWE of *A. indica* seed kernel relative to 50%EAE of *A. indica* seed kernel	1.482	1.544
DWE of *A. indica* seed kernel relative to DWE of *C. sinensis* seed cake	11.916	8.816
DWE of *A. indica* seed kernel relative to 50%EAE of *C. sinensis* seed cake	24.891	17.757
50%EAE of *A. indica* seed kernel relative to DWE of *C. sinensis* seed cake	8.041	5.710
50%EAE of *A. indica* seed kernel relative to 50%EAE of *C. sinensis* seed cake	16.796	11.501
DWE of *C. sinensis* seed cake relative to 50%EAE of *C. sinensis* seed cake	2.089	2.014

On the basis of relative potency values the relative toxicities of the plant extracts on both the fishes were found to be – extract of *A. procera* seeds > *C. sinensis* seed cake > *S. mahagoni* seed kernels > *A. indica* seed kernels. The comparative analysis values amongst the different extracts of *A. procera* seeds, *S. mahagoni* and *A. indica* seed kernels and *C. sinensis* seed cake in terms of LC_{50} values of each toxicant for both the fishes are given in Table 3.

The toxic effects of different concentrations of the extracts varied in the two fishes which showed a dose dependent increase with increasing concentration. The possible mechanism of the toxicants which cause mortality might be (i) the toxicants may alter the rate of heart beat and ultimately cause

muscle paralysis (Patton 1963), (ii) the compounds may affect the conduction of nervous system (Fukami 1962).

The degree of tolerance of the test fishes to any of the two extracts of *A. procera* seeds, *S. mahagoni* seed kernels and *C. sinensis* seed cake as observed from the LC_{50} values was in the order *C. punctatus* > *O. mossambicus* but was the opposite for *A. indica* seed kernel extract. So, *O. mossambicus* was more susceptible to the toxic effect of the different extracts used compared to *C. punctatus*. Moreover, both *O. mosambicus* and *C. punctatus* were more susceptible to the toxic effect of *A. procera* seeds and *A. indica* seed kernel extracts than *H. fossilis* but the order was reversed for *A. testudineus*, and on the other hand both the fishes were more tolerant to the toxic effect of *S. mahagoni* seed kernel extracts than *H. fossilis* and *A. testudineus* (Nasiruddin *et al.* 1997, 1998). The differential toxicities of the plant extracts indicated that probably the physiology of the test fishes played an important role in their response to toxicants (Macek and Mc Allister 1970).

Laboratory based toxicity study of crude plant products can give near optimal information regarding the spectrum of the plant's toxic effect. From the present study it has appeared that the potentiality of the two plant extracts might be helpful to control the predatory or undesirable fish species in aquaculture ponds of commercially valuable fish species.

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