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Research Article

PISCICIDAL EFFECTS OF THREE PLANT SEED EXTRACTS ON TWO PREDATORY FISH, *HETEROPNEUESTES FOSSILIS* (BLOCH) AND *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT

Piscicidal properties of distilled water, 50% ethyl alcohol and 100% ethyl alcohol extracts of dry seeds of *Albizzia procera* (Benth), *Annona reticulata* (Linn.) and dry seed kernels of *Achras zapota* (Linn.) were studied on two predatory fish, *Heteropneustes fossilis* (Bloch) and *Channa punctatus* (Bloch) under normal laboratory conditions. Percentage mortality varied depending upon the different types of plant extracts, dosages and fish. The order of activity of the extracts was: distilled water < 50% ethyl alcohol < 100% ethyl alcohol. On the basis of LC_{50} values the relative toxicities of the piscicides on the two fish were found to be in the order *A. procera* > *A. reticulata* > *A. zapota* excepting 100% ethyl alcohol extract of *A. reticulata*. In case of *A. procera* seed extract *H. fossilis* was more tolerant and less sensitive than *C. punctatus*. But in case of *A. reticulata* seed and *A. zapota* seed kernel extracts (excepting distilled water extract of both the plants) *C. punctatus* was more tolerant and less sensitive than *H. fossilis*. *A. procera* seed extracts were more toxic as to mortality in both the fishes excepting 100% ethyl alcohol extracts of *A. reticulata* seeds which was more toxic than *A. procera* in *H. fossilis.* This study reveals that the dry seed extracts of *A. procera*, *A. reticulata* and seed kernel extracts of *A. zapota* are potentially good piscicides.

KEY WORDS: Piscicidal effects, plant extracts, mortality, *Heteropneustes fossilis, Channa punctatus.*

INTRODUCTION

Predatory and undesirable fishes create a great problem in the aquaculture ponds by predating on commercial fish fries and competing for food and space. Eradication of these predatory fishes from aquaculture ponds is a good management practice to increase fish production. Use of pesticides to eradicate predatory and undesirable fishes is a common practice. Most of the pesticides used in the third world countries are imported from the developed countries. Moreover these pesticides are biohazardous. However, the most effective and safe method is the use of plant origin fish toxicants as these satisfy all the requirements of ideal fish poison (Chowdhury *et al*. 1981). Botanicals are natural biocides (Burkill 1985). Natural pesticides of indigenous plant origin can reduce the mass use of imported costly foreign pesticides. Herbal toxicants do not create hazards like those experienced in synthetic ones. Also plants are virtually an inexhaustible source of structurally diverse and biologically active substances (Batabyal *et al*. 2007).

Several literatures are available on the piscicidal effects of plant extracts on fishes in different countries of the world (Fafioye 2005, Obomanu *et al*. 2007, Jothivel and Paul 2008, Singh and Singh 2010 and Ayotunde *et al*. 2011). In Bangladesh, piscicidal studies on such aspect were carried out to some extent (Latifa *et al*. 1987, 1988, 1992, 1997, 2002), Latifa and Begum (1993) and Nasiruddin *et al*. 1997, 1998, 2006, 2009, 2012).

In view of these considerations, the present study was undertaken to study the piscicidal effects of dry seed and seed kernel extracts of three indigenous plants namely *Albizzia procera* (Benth), *Annona reticulata* (Linn) and *Achras zapota* (Linn) on two predatory fishes, *Heteropneustes fossilis* (Bloch) and *Channa punctatus* (Bloch).These two fishes are predators on cultured fish fries in the aquaculture ponds. Findings

of this study may be useful to develop some low cost plant origin pesticides which may help to protect the fish fries from predators and thus to increase the fish production in commercial fish farms.

MATERIALS AND METHODS

The seeds of *Albizzia procera* (Family-Leguminosae), *Annona reticulata* (Family-Annonaceae) and *Achras zapota* (Family-Sapotaceae) were collected from Chittagong University campus, BCSIR, Chittagong campus and different fruit markets of Chittagong city. Then the seeds were washed, cleaned and dried under diffused sunlight for stocking. Dry seeds of *A. procera* and *A. reticulata* and dry seed kernels of *A. zapota* were used for the experiments.

Healthy and live fishes, *H. fossilis* and *C. punctatus* were collected from various fish markets of Chittagong city and acclimatized for 4±1 hours in laboratory conditions in glass aquarium (60X 30X30 cm^3) containing underground water. Only the healthy fishes were used for the experiments.

Dry seeds and seed kernels were pulverized into fine powder with a power driven grinder and sieved through a 0.0025 cm^2 sieve. Seed and seed kernel powder were dissolved in distilled water, 50% or 100% ethyl alcohol solvents separately. For experimental purposes the required amount of seed and seed kernel powder was weighed and mixed with relevant amount of the solvent in a magnetic stirrer for 3-4 hours. After extraction, the resultant liquid was filtered and the different test doses were prepared from this stock solution. Desired concentrations of different test solutions were obtained by appropriate dilution of the stock solution (APHA 1976).

Bioassays

The bioassays were run in a series (12) of glass aquaria (30 x 23 x 23 cm^3) each containing five liters of underground water and toxicant. To determine the LC_{50} and LC_{90} values of the seeds and seed kernels of the three plants for *H. fossilis* and *C. punctatus*, the extracts in different doses were added to the aquaria. Five concentrations of each extract were used in the final experiments which resulted in 1– 99% mortality. In each test a set of five test fishes were released at random and each dose of the relevant toxicant was replicated twice (Sharma and Latifa 1999) and the test fishes were kept for relevant test exposure period, i.e. 24 hours. In each experiment a control was maintained in underground water and under similar condition. All the bioassays were conducted in the laboratory at room temperature (29 ± 2^0C) and in diffused light. During the experiments, the behavior and the mortality of the fishes within 24 hours were recorded. There was no mortality in control groups.

Statistical analysis

Mortality data were subjected to probit analysis following the methods of Finney (1971). Probit analysis was used to determine the LC_{50} and LC_{90} values of each extract. The regression equation was calculated from the empirical probit, working probit, weighting probit, the values of which were taken from the tables given by Finney (1971). Expected probit was calculated from respective empirical probit. LC_{50} and LC_{90} with 95% confidence limits were calculated using a computer based probit analysis programme. The values of chi-square (χ^2) was determined and compared with tables of the statistics for n-1 degrees of freedom at 0.05 levels. One-way ANOVA of percentage mortality of fishes was made to estimate the variation among treatments at 0.05 levels. Relative potency of toxicity values were calculated from the LC_{50} values on the basis of potency which is reciprocal of the equitoxic doses.

RESULTS

Effect of the extracts on behaviour:

The behavioral activities of the experimental fishes when exposed to the plant seed or seed kernel extracts in the five concentrations of each of the three solvents were different from the control. Behavioral activities increased with increasing concentrations. In *H. fossilils* the affected fishes showed irregular swimming with vigorous movements and were repeatedly rising towards the surface for taking air. After erratic and dashing movements the fishes began to loose stamina, became balance less, fins stiffened and barbels were straightened. Then they slowly dropped to the bottom and ultimately died at different intervals. Slime secretion was observed. In case of *C. punctatus*, immediately after exposure in the toxicants, the fishes jumped upwards. Then the fishes swam erratically, and opercular movement was very rapid. Subsequently, their movement slowed down and finally stopped. Gradually they relaxed to the bottom and ultimately died at different intervals. Scales were seen to be shed off.

Effects of extracts on mortality:

The dose ranges of the two plant seed and one seed kernel extracts, the mortality ranges of the two fish species, the chi-square and ANOVA values and their significance at 0.05 levels, the regression equations of the slope lines, the LC_{50} and LC_{90} values of the different extracts are given in Table 1. In case of *H. fossilis* (Table 1) doses with *A. procera* seed extracts ranged from 5-200 ppm, with *A. reticulata* seed extracts from 5-600 ppm and with *A. zapota* seed

kernel extracts from 25-800 ppm. Mortality of the experimental fishes ranged from 10-90%. Chi-square values were insignificant at 0.05 levels with all the extracts excepting distilled water extract of *A. procera* seeds. The ANOVA values were significant with all the extracts. The slope line equations indicated an increase of mortality with an increase in the experimental doses. The LC_{50} and LC_{90} values showed that amongst all the experimental extracts 100% ethyl alcohol extract of *A. procera* seeds was the most toxic extract and distilled water extract of *A. zapota* seeds was the least toxic extract. In case of *C. punctatus* (Table 1) the dose ranges of *A. procera* seeds were from 1-75 ppm, for *A. reticulata* seeds from 25-400 ppm and for *A. zapota* seed kernels from 50-600 ppm. Mortality of the test fish ranged from 10-90%. The chi-square values for all the extracts were insignificant at 0.05 levels. The ANOVA values were significant with most of the extracts excepting 50% ethyl alcohol extract of *A. procera* seeds and distilled water extract of *A. reticulata* seeds. The slope line values also indicated an increasing mortality with increasing experimental doses. The LC_{50} and LC_{90} values showed that of all the tested extracts100% ethyl alcohol extract of *A. procera* seeds was the most toxic extract and distilled water extract of *A. zapota* seed kernels was the least toxic extract.

The relative potency values are reciprocals of the equitoxic extracts. The relative potency values of the distilled water, 50% ethyl alcohol and 100% ethyl alcohol extracts of *A. procera*, *A. reticulata* and *A. zapota* seed or seed kernels are presented in Table 2.

From Table 2 it is seen that for *H. fossilis*, 100% ethyl alcohol extract of *A. reticulata* seeds was the most toxic extract having a low LC_{50} (15.435 ppm) and high relative potency (23.293). The least toxic extract was the distilled water extract of *A. zapota* seed kernel with high LC_{50} (359.534 ppm) and low relative potency (1.000). On the basis of LC_{50} and relative potency values the relative position of the extracts were in the order: 100% ethyl alcohol extract of *A. reticulata* seeds > 100% ethyl alcohol extract of *A. procera* seeds > 50% ethyl alcohol extract of *A. procera* seeds > 50% ethyl alcohol extract of *A. reticulata* seeds > distilled water extract of *A. procera* seeds > 100% ethyl alcohol extract of *A. zapota* seed kernels > 50% ethyl alcohol extract of *A. zapota* seed kernels > distilled water extract of *A. reticulata* seeds > distilled water extract of *A. zapota* seed kernels.

In case of *C. punctatus*, 100% ethyl alcohol extract of *A. procera* seeds was the most toxic with a LC_{50} of 4.115 ppm and relative potency of 79.990. Distilled water extract of *A. zapota* seed kernels extract was the least toxic extract having a LC_{50} of 329.160 ppm and relative potency value of 1.000. On the basis of LC_{50} and relative potency values, the relative positions of the extracts were ranked in the order: 100% ethyl alcohol extract of *A. procera* seeds > 50% ethyl alcohol of *A. procera* seeds > distilled water extract of *A. procera* seeds > 100% ethyl alcohol extract of *A. reticulata* seeds > 50% ethyl alcohol extract of *A. reticulata* seeds > distilled water extract of *A. reticulata* seeds > 100% ethyl alcohol extract of *A. zapota* seed kernels > 50% ethyl alcohol extract of *A. zapota* seed kernels > distilled water extract of *A. zapota* seed kernels.

On the basis of LC_{50} and relative potency values, in *H. fossilis* the relative toxicities of the piscicides were found to in the order *A. procera* seeds > *A. reticulata* seeds > *A. zapota* seed kernels excepting 100% ethyl alcohol extract *A. reticulata* seeds which had an reverse effect. In *C. punctatus* the relative toxicities of the three plant extracts were also found to be in the order *A. procera* seeds > *A. reticulata* seeds > *A. zapota* seed kernels.

On the basis of LC_{50} values, the degree of tolerance of the two fish species to all the extracts of *A. procera* seeds and distilled water extracts of both *A. reticulata* seeds and *A. zapota* seed kernels was observed in the order *H. fossilis* > *C. punctatus* but the reverse order was observed with 50% and 100% ethyl alcohol extracts of *A. reticulata* seeds and *A. zapota* seed kernels.

DISCUSSION

All the three plant seed extracts used in this study was proved to be more or less toxic against the two fish species. Toxicities of plant extracts varied with different doses as well as with different solvents. The toxicity of the extracts of seeds or seed kernels of the three plants on both the fishes observed in the present study was ranked in the order 100% ethyl alcohol extracts > 50% ethyl alcohol extracts > distilled water extracts. This result is in consonance with the findings of Latifa *et al*. (1987, 1988, 1997), Latifa and Begum (1993), Sharma and Latifa (1999), and Nasiruddin *et al*. (2006, 2009, 2012). The toxic ingredients are strongly soluble in 100% ethyl alcohol, slightly soluble in 50% ethyl alcohol and almost insoluble in distilled water (Ameen *et al*. 1983).

Due to the piscicidal action several abnormal behaviors such as restlessness, loss of equilibrium, surfacing, stiffness of barbels and fins and slime secretion showed by the fishes were also observed by Latifa *et al*. (1987, 1988, 1997), Latifa and Begum (1993), Nasiruddin *et al*. (1997, 1998, 2006, 2009, 2012) and Ashraf *et al*. (2010) in different fish

species. Hyperactivity could be due to stimulation of surface perceptive organs caused by the toxicants as also opined by Gill (1989). Slime secretion might be due to the effect of toxicants and Bennet and Dooley (1982) regarded the secretion as a defense and respiratory response. Death of the fishes may be associated with respiratory distress. In the present observation, of the three plant extracts, *A. procera* and *A. reticulata* seed extracts were of higher effectivity as the doses ranged from 5-200 ppm in case of *A. procera* and 5-600 ppm in case of *A. reticulata*. *A. zapota* seed kernel extracts showed medium effectivity and the doses ranged from 25 to 800 ppm.

In the present work, on the basis of LC_{50} and relative potency values the degree of tolerance of the test fishes to all the extracts of *A. procera* seeds and distilled water extracts of both *A. reticulata* seeds and *A. zapota* seed kernels was in the order: *H. fossilis* > *C. punctatus. H. fossilis* is more tolerant and less sensitive than *C. punctatus.* So, the susceptibility pattern is *C. punctatus* > *H. fossilis.* Same type of susceptibility was observed by Nasiruddin *et al*. (2012). On the other hand the reverse susceptibility was observed in case of 50% and 100% ethyl alcohol extracts of both *A. reticulata* and *A. zapota* seeds and seed kernels and same findings was noticed by Latifa *et al*. (1987,1988,), Latifa and Begum (1993) and Sharma and Latifa (2009).

In the present observation, all the chi-square values were insignificant at 0.05 levels excepting distilled water extract of *A. procera* seeds on *H. fossilis*, indicating that there was no significant difference between the observed and expected mortalities. As to ANOVA test, all the extracts showed significant result at 0.05 levels excepting 50% ethyl alcohol extract of *A. procer*a seeds and distilled water extract of *A. reticulata* seeds on *C. punctatus* indicating a good relationship between the doses of the extracts applied and mortalities of the fishes obtained.

CONCLUSION

The present study revealed the potentiality of dry seed extracts of *A. procera, A. reticulata* and seed kernel extracts of *A. zapota* as bright piscicide. Effectivity and availability of these plants offer a possibility of using these as piscicides of unwanted fishes in the nursery, rearing and stocking ponds of fish culture farms. It can be concluded that toxicity based studies in the laboratory conditions of crude plant products can give near optimal information regarding the spectrum of its toxic effects and offers the possibility of being used commercially.

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Table 1: Toxicity parameters of dry seed or seed kernel extracts of *A. procera, A. reticulata* and *A. zapota* on *H. fossilis* and *C. punctatus.*

Fish	Plant part	Solvent	Dose range (ppm)	Mortality range (%)	χ^2 value	P-value (χ^2)	ANOVAv alue	P-value (ANOVA)	Regression equation	LC_{50} (ppm)	LC_{90} (ppm)
H. fossilis	*A. procera* (seed)	Distilled Water	25-200	10-90	19.54	P<0.05	15.50	P<0.05	-0.808+3.063x	74.793	251.005
		50% ethyl alcohol	10-150	20-90	3.99	P>0.05	18.25	P<0.05	2.512+16.060x	35.225	218.599
		100% ethyl alcohol	5-100	20-90	0.46	P>0.05	41.00	P<0.05	3.066+1.610x	15.834	99.134
	A. reticulata (seed)	Distilled Water	200-600	30-90	3.57	P>0.05	9.50	P<0.05	-3.853+3.580x	297.027	649.066
		50% ethyl alcohol	10-150	10-90	3.62	P>0.05	10.00	P<0.05	1.674+1.994x	46.395	199.488
		100% ethyl alcohol	5-75	20-90	1.71	P>0.05	8.20	P<0.05	2.983+1.696x	15.435	87.124
	A.zapota (seed kernel)	Distilled Water	100-800	10-90	7.56	P>0.05	14.00	P<0.05	-1.923+2.712x	359.534	1043.773
		50% ethyl alcohol	50-600	10-90	3.16	P>0.05	10.00	P<0.05	0.083+2.154x	191.604	753.503
		100% ethyl alcohol	25-400	10-90	1.66	P>0.05	14.00	P<0.05	0.721+2.175x	92.774	362.685
C. punctatus	*A. procera* (seed)	Distilled Water	5-75	20-90	7.50	P>0.05	10.25	P<0.05	2.879+1.634x	19.908	120.448
		50% ethyl alcohol	2.5-50	30-90	3.82	P>0.05	4.07	P>0.05	3.920+1.272x	7.072	70.272
		100% ethyl alcohol	1-25	20-90	4.05	P>0.05	13.83	P<0.05	4.051+1.542x	4.115	27.615
	A. reticulata (seed)	Distilled Water	75-400	20-90	7.01	P>0.05	3.65	P>0.05	-0.175+2.380x	150.086	508.093
		50% ethyl alcohol	50-300	10-90	4.96	P>0.05	10.00	P<0.05	-1.007+2.910x	116.331	315.190
		100% ethyl alcohol	25-200	30-90	6.83	P>0.05	5.45	P<0.05	1.289+2.168x	51.498	202.674
	A.zapota (seed kernel)	Distilled Water	200-600	20-90	2.14	P>0.05	41.00	P<0.05	-6.23+4.460x	329.160	642.888
		50% ethyl alcohol	100-500	10-90	7.46	P>0.05	10.00	P<0.05	-3.340+3.436x	268.399	629.969
		100% ethyl alcohol	50-400	20-80	3.27	P>0.05	14.25	P<0.05	1.164+1.1752x	154.386	830.935

Table 2: Relative potencies of distilled water, 50% ethyl alcohol and 100% ethyl alcohol extracts of *A. procera, A. reticulata* dry seeds and *A. zapota* seed kernels on *H. fossilis* and *C. punctatus*.

Plants	*H. fossilis*			*C. punctatus*		
	Extracts	LC_{50} (ppm)	Relative potency	Extracts	LC_{50} (ppm)	Relative potency
A. procera (seed)	Distilled water	74.793	4.807	Distilled water	19.908	16.534
	50% ethyl alcohol	35.227	10.206	50% ethyl alcohol	7.072	46.544
	100% ethyl alcohol	15.834	22.706	100% ethyl alcohol	4.115	79.990
A. reticulata (seed)	Distilled water	297.027	1.210	Distilled water	150.086	2.193
	50% ethyl alcohol	46.395	7.749	50% ethyl alcohol	116.331	2.829
	100% ethyl alcohol	15.435	23.293	100% ethyl alcohol	51.498	6.392
A. zapota (seed kernel)	Distilled water	359.534	1.000	Distilled water	329.160	1.000
	50% ethyl alcohol	191.604	1.876	50% ethyl alcohol	268.399	1.226
	100% ethyl alcohol	92.774	3.875	100% ethyl alcohol	154.386	2.132