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Full Length Article

Replacement of Rotenone by Locally Grown Herbal Extracts

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ABSTRACT

The studies were conducted to assess the piscicidal activity of 10 locally available plants on freshwater trash fishes. The studies were focused on the laboratory determination of lethal concentrations (LC_{50}) through a "static bioassay test". Leaf extracts of *Nerium oleander* (Family Apocynaceae), *Cannabium sativum* (Bhang, Family Cannabaceae), *Datura alba* (Family Solanaceae), *Adenophyllum* spp. (Berri patta, Family Asteraceae), *Nicotiana tabacum* (Tobacco, Family Solanaceae) and *Ricinus communis* (*Arind*, Family Euphobiaceae), while root extracts of *Parthenium* spp. (Family Compositeae), *Calotropis procera* (Family Asclepiadaceae), *Achyranthes aspera* (puth kanda, Family Amaranthaceae) and seed extracts of *Ricinus communis* and *Strychnos* were utilized for these studies. Based on the 96 h lethal concentration, *Ricinus communis*, *Datura alba* and *Strychnos nux vomica* (Kutchla, Family Loganiaceae) showed the strongest piscicidal activity to fish. During exposure, fish exhibited discoloration, gulping for air, erratic swimming, loss of reflexes, slow opercular movement and ultimately settling at the bottom motionless. The trial toxicity tests showed that locally available plants have the potential to be used as piscicides, which can be an alternate to an expensive and scarcely available imported rotenone for eradication of undesirable fish species present in fish ponds. © 2010 Friends Science Publishers

Key Words: Grown herbal extracts; Fish toxicants

INTRODUCTION

Unwanted fish may enter fish culture ponds through water supplies, birds or along with fish seed brought into fish farm and can account for up to 40% losses in the commercial fish and shrimp harvest (Pillay & Kutty, 2001; Personal observations). Some producers battle this problem by using cyanide (a poison) or any other poison of similar nature that can have serious impact on other organisms in food chain, including humans. Some use tea seed cake to control predators and trash fishes. Some drain ponds, which is usually not feasible and also ineffective in controlling and eradicating unwanted fishes at commercial scale. Sometimes inlets are screened to stop the entry of eggs or larvae of wild fish fauna if source is canal water. This too is not as effective as it is normally thought (Bardach *et al*., 1972). Ideally it is advisable that ponds should be sun dried and pond bottom cracked to get rid of unwanted fish fauna. However this practice is not always possible particularly during the wet season. The best way of ensuring total obliteration of unwanted fishes is through the use of fish toxicants in pond water (Guerrero & Guerrero, 1986).

Fish toxicants (piscicides) can be herbal or synthetic. Synthetic piscicides are not degradable, pose the problems of environmental resistance, pest resurgence and have detrimental effects on non-target organisms (Fafioye *et al*., 2005). Herbal toxicants do not create hazards like those experienced in synthetic ones. Secondly plants are virtually an inexhaustible source of structurally diverse and biologically active substances (Batabyal *et al*., 2007). Plant poisons called botanicals are extracted from flowers, bark, pulp, seeds, roots, leaves and even the entire plant (Sirivam *et al*., 2004). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties (Wang & Huffman, 1991).

Contradictory to synthetic fish toxicants, herbal toxicants are believed to be more environment friendly, because they are easily biodegradable and leave no residues in the environment. During the last several years, rotenone (herbal piscicide) has been used as fisheries management tool to rehabilitate lakes, ponds, streams and other waters (Ling, 2003). Rotenone is an active component in several terrestrial insecticides and for use in the aquatic environment as a piscicide in static and flowing waters to kill fish. It is a natural alkaloid extracted from the roots of tropical plants of pea family. It exhibits its pesticidal action by uncoupling oxidative phosphorylation in the cell mitochondria through blocking electron transport at complex I (Medda *et al*., 1995; Turner *et al*., 2007). Rotenone can be applied by a variety of aerial or ground application methods. It is relatively insoluble in water and has low volatility. It rapidly degrades in the environment primarily through hydrolysis (Turner *et al*., 2007). However the cost and availability limit its further use. The study therefore was planned to assess the piscicidal activity of extracts from selected locally available plants on trash fishes

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and finally replace rotenone by most effective herbal extract to eradicate undesirable fishes. During the course of study, a few trials were extended to selective Indian and Chinese major carps, for comparison purposes.

MATERIALS AND METHODS

Collection of plants: The survey of sub-urban area of Lahore city spanned through March 2000 to January 2001. Samples of 10 poisonous plants (orally known through artisanal fishermen & other relevant organizations) found at different locations, were collected and taken to Fisheries Research and Training Institute Lahore. Available literature and data from various sources were consulted for their proper identification.

Plant extracts: About 1000 g of leaves of each plant *Nerium oleander*, *Cannabium sativum* (Bhang), *Datura alba*, *Adenophyllum* (Berri patta), *Nicotiana* tabacum (Tobacco) and *Ricinus communis* (Arind), were pounded using mortal and pestle, soaked in 450 mL of ethyl alcohol for 15 days and then kept in oven at 35-40^{0}C for 15 days. Whole Plants of *Parthenium hysterophorus, Calotropis procera*, *Achyranthes aspera* (puth kanda) were removed from ground. Required quantity of soft roots were trimmed off with sharp knife and treated according to the methodology exercised in leaf extraction. Similarly, seeds of *Ricinus communis* and *Strychnos nux vomica*, were soaked in ethyl alcohol for same duration and kept in oven for 20 days to ensure sufficient penetration of alcohol through tough outer coating for reasonable extraction. The mixture was sieved through 200 µm sieve to remove broken pieces of plants and other extraneous material, if present. The extract was kept in refrigerator till use to avoid loss/evaporation of useful products.

Experimental design and toxicity tests: Toxicity trials were managed in 40 L glass aquaria at room temperature. Fresh water was added for each trial. There were 10 fish in each aquaria. All the treatments had three replicates, while control had one. Performance tests lasted for 96 h. Duration however was reduced, where total bereavement was observed. Fish was checked for mortality at different time intervals. The dead fish if found were removed continuously to alleviate pollution related effect and were counted for determination of LC_{50}.

Application rates differed from plant to plant and fish species exposed. The detail of herbs, fish species used and respective dosages have been presented in Table I. Temperature, pH and dissolved oxygen were monitored throughout the exposure period to determine any tangible water quality deterioration effect on fish, which might confound treatment effects (Table II).

Experimental animals: Four species of trash fish viz. *Tilapia* (1.0 to 5.2 gm), *Colisa lalia* (0.95-1.4 gm), *Channa punctatus* (0.7-13 gm) and *Ambassis ranga* (0.7-1.2 gm) collected from wild and two species of Indian carps, *Labeo rohita* (1.2-8.0 gm) and *Catla catla* (4.0-9.5 gm) and Chinese major carp, *Ctenopharyngodon idella* (12.0-14.3 gm) acquired from Central fish Hatchery, Lahore, Pakistan, were exposed to various herbal extracts for 96 h. Mortality was counted at 24 h intervals for determination of LC_{50} and LC_{100} values (Table III).

Statistical analysis: Data collected were subjected to Probit/Logit statistical test (Steel & Torrie, 1996) for determination of significance among treatments. Differences were considered significant at $p<0.05$.

RESULTS

Behavioural changes: Indian and Chinese major carps displayed abnormal behaviuor at 3, 5 and 2 ppm concentrations of *Nerium oleander*, *Cannabium sativum* and *Parthenium hysterophorus* leaf extracts respectively. Disturbed swimming, rapid opercular movements, loss of balance, incessant gulping of air, darkening of the whole body and fish settling at the bottom motionless, were very common.

Toxicity tests: Fingerlings of *Labeo rohita*, when exposed to *Nerium oleander* leaf extract @ 3 ppm, lost their balance after 6 min and died within an hour of exposure. Same was true for *Catla catla* fingerlings (Table I) when exposed to *Cannabium sativum* leaf extract @ 5 ppm concentration. *Tilapia* on the other hand tolerated up to 15 ppm and 12 ppm concentration when exposed to *Cannabium* or *Datura* leaf extract, respectively (Table III). *Colisa lalia* and *Ambasis ranga* tolerated upto 3 and 5 ppm when exposed to *Datura alba* leaf extract. Mortality then started and both the fishes were dead at 16 and 14 ppm, respectively (Table I). *Channa punctatus* performed much better than all the other fish species (Table III). Whenever *Datura alba* extract was added to fish holding container, fish started surfacing and jumping outside of water. *Adenophyllum, Nicotiana tabacum* (Tobacco), *Parhenium hysterophorus*, *Calotropis procera* (Auk) and *Achyranthes aspera* (Puth kanda) leaf extract did not affect fish behavior even at very high concentrations (up to 100 ppm; hence data not presented). *Ricinus communis* seed extract was comparatively more effective in killing fishes though mortality started relatively at higher concentrations (22 ppm; Table I). No mortality was observed in *Adenophyllum, parthenia, Nicotiana tabacum* and *Calotropis procera*. Highest LC_{50} values for *Datura alba* leaf extract were observed in trials on *Channa Punctatus*, while the lowest in Indian and Chinese carps (Table III). *Strychnos nux vomica (*Kutchla*)* was found most effective in current studies as it had the lowest LC_{100} value (2.5 ppm) for *Tilapia* (Table III). There were minute differences in water quality parameters, which probably do not have any bearing on toxicity trials (Table II).

DISCUSSION

Our studies revealed that fish exposed to toxicants exhibit marked behavioural changes like swift opercular movement, sudden jerky swimming body movements, which demonstrated a sensitive indicator of physiological

Table I: Type of plants and detail of herbal extract dosages applied to various fish species in toxicity trials

Name of Plant	Part used/extract	Fish species used	Dosage applied (ppm)
Nerium oleander extract)	Leaf extract	*Labeo rohita*	3
Canabium sativum	-do-	*Catla catla*	5
-Do-	-do-	*Tilapia* sp.	2,5,8,10,12,15,188,20,22,24,26
Datura alba	-do-	-do-	2,3,4,5,6,7,8,9,10,12,13,14,15,16,18,20,22,25,27,28,30,32,34,36,38,40,42,44,46,48,50
-do-	-do-	Grass carp	2,8,10,12,14,16,20,25,30,35,40,45,48,50,60,62,65,70,75
-do-	-do-	*Colisa lalia*	2,4,6,8,10,12,14,16
-do-	-do-	*Channa punctatus*	2,4,6,8,10,12,15,18,20,23,25,28,30,32
-do-	-do-	*Ambasis ranga*	2,4,6,8,10,12,14
Strychnos nux vomica (Kutchla)	Seed extract	*Tilapia* sp.	2,2.5,3
Adenophyllum	Leaf extract	*Tilapia* sp.	2,3,4,5,6,7,8,10,12,15,18,20,22,24,26
Nicotiana tabacum	-do-	-do-	2,5,8,10,12,15,18,20,22,24,26
Parthenium hysterophorus Parthenia	-do-	Grass carp	2,5,10,15,20,25,30,35,40,45,50,55,60,65,70
-do-	Root extract	-do-	2,4,6,8,10,12,14,16,18,20,25,30,35,40,45,50,55,60,65,70,75,80,85,90,95,100
Calotropis procera	Flower extract	*Tilapia* sp.	2,5,8,10,12
-do-	Fruit extract	*Labeo rohita*	2,5,8,10,13,15,20,25,30,34,36,38,40,42,45,,50,52,54,58,60,65,70,72,75,78,80
Riccinus communis	Leaf extract	*Tilapia* sp.	2,4,6,8,10,12,14,16,18,20,22,24,26,28,30
-do-	Seed extract	O.aureus	2,4,6,8,10,11,16,18,20,22,24,28,30
Achyranthus aspera	-do-	*Labeo rohita*	2,5,8,10,12,15,18,20,22,25,28,30,32,34,36,38,40,42,44,46,48,50

Table II: Ranges of water quality parameters during toxicity trials

Toxicant	Fish species	Size of fish (g)	Temperature (^{o}C)	pH	DO(ppm)
Nerium oleander (leaf extract)	*Labeo rohita*	1.2-8.0	25-29	7.5-8.0	3.0-3.8
Canabium sativum (leaf extract)	*Catla catla*	4.0-9.5	26-28	7.6-8.0	3.0-3.8
-do-	*Tilapia* sp.	1.0-5.2	25-27	7.5-7.8	2.5-3.0
Datura alba	-do-	5.2	26-30	7.5-7.8	2.5-3.0
-do-	*Colisa lalia*	0.95-1.4	25-27	7.6-8.1	2.99-3.0
-do-	*Channa punctatus*	0.7-13	26-30	7.6-8.1	2.99-3.0
-do-	*Ambassis ranga*	0.7-1.2	25-27	7.5-7.8	2.5-3.0
Strychnos nuxvomica (Kuchla)	*Tilapia*	1.0-5.2	25-27	7.6-8.1	2.99-3.0
Adenophyllum	*Tilapia*	1.0-5.2	25-27	7.5-8.0	2.5-3.0
Nicotiana tabacum (Linn)	-do-	1.0-5.2	26-30	7.6-8.1	2.99-3.0
Parthenium hysterophorus	Grass carp	12.0-14.3	25-27	7.5-8.0	2.5-3.0
Calotropis procera	*Tilapia* sp.	1.0-5.2	26-30	7.6-8.1	2.99-3.0
Ricinus communis	-do-	1	25-27	7.6-8.1	2.99-3.0
-do-	-do-	2.1	26-30	7.6-8.1	2.99-3.0
Achyranthus aspera	*Labeo rohita*	1.2-8.0	25-29	7.5-7.9	3.1-3.2

Table III: LC_{50} and LC_{100} of various plant extracts applied on various trash as well as commercial fish species

S.No.	Plant	Extracted from	Fish species	LC_{50}	LC_{100}
1	*Nerium oleander* (leaf extract)	Leaf	*Labeo rohita*	2 ppm	3 ppm
2	*Canabium sativum* (leaf extract)	Leaf	*Catla catla*	3 ppm	5 ppm
	-do-	Leaf	*Tilapia*	15 ppm	26 ppm
3	*Datura alba*	leaf	-do-	12 ppm	20 ppm
	-do-	-do-	*Colisa lalia*	6 ppm	16 ppm
	-do-	-do-	*Channa punctatus*	18 ppm	32 ppm
	-do-	-do-	*Ambassis ranga*	8 ppm	14 ppm
4	*Strychros nux vomica* (Kuchla)	seeds	*Tilapia*	1.5 ppm	2.5 ppm
5	*Adenophyllum*	Leaf	*Tilapia*	No mortality	
6	*Nicotiana tabacum* (Linn)	Leaf	-do-	-do-	
7	*Parthenium hysterophorus*	Leaf + root	Grass carp	No mortality up to 70 ppm	
8	*Calotropis procera*	Flowers	*Tilapia* sp.	No mortality up to 12 ppm	
9	*Ricinus communis*	Leaf extract	-do-	26 ppm	30 ppm
10	*Achyranthus aspera*	Seed extract	*Labeo rohita*	12 ppm	28 ppm

stress in fish. Davis (1973) has observed similar behavior when fish were subjected to sub-lethal concentrations of pollutants. The behavioural responses observed in current studies can be favourably compared with those observed by Pascual *et al.* (1994) in formalin test on seabass (*Lates calcarifer*) fry. Similarly, Lin and Liu (1990) reported abnormal movement and high respiration rate in hybrid Tilapia (*Oreochromis mossambicus*) induced by ammonia indicating neurological dysfunction and gill damage. The erratic behaviour prior to death in the present and past studies can be conveniently associated with the impact of toxicants on fish.

Guerrero and Guerrero (1986) reported 96 h LC_{50} of 10-20 ppm for *Oreochromis niloticus* fingerlings exposed to *Derris* root powder though much lower values (5-8 ppm) were observed in our field trials (Personal observations). Regular studies were not conducted, because it was out of scope of our work. Chiayvareesajja *et al.* (1997) evaluated the toxicity of five native Thai plants to aquatic organisms. They bio-assayed toxicants against *Oreochromis niloticus*

and *Anabas testudineus* and found *Diospyros diepenhorstii* least effective, while *Moesa ramentacea* and *Sapindus emarginatus* were the most effective plant extracts against test fish. Onusiriuka and Ufodike (1998) reported that *Blighia sapida* bark extract is more toxic to *C. gariepinus* than K*igelia Africana* bark extract. Tiwari and Singh (2003) when exposed *Channa punctatus* to diethyl ether extract of *Nerium indicum* found LC_{50} in the range of 13.58 mg L^{-1} to 17.34 mg L^{-1}. The extract significantly affected both aerobic and anaerobic pathway of fish respiration, which further confirmed its potent piscicidal activity against fish. The LC_{50} values worked out in current studies were comparatively higher than those of reported in literature, which may be due to different plants used and variation in environmental conditions. Studies of Tiwari and Singh (2004) further confirms this notion that method of extraction and solvent used matters a lot, because when he tried the alcoholic extract of same plant on same fish observed LC_{50} values much higher (66.32 mg L^{-1}) than observed in diethyl ether extract. Batabyal *et al*. (2007) however, has reported little lower LC_{50} values when they exposed *Anopheles stephensi* larvae to methanolic extract of *Azadirachta indica* and *Ricinus communis*, which is closer to our observations on the same plant (Table III). Recently Cheng *et al*. (2008) made similar observations when they exposed mosquito species to methanolic extract of red heartwood (*Cryptomeria japonica*) containing tectoquinone as a major ingredient (although there is lot of difference due to its invertebrate nature but can be compared from plant toxicity point of view). The variations observed in these studies can be attributed to fish species used, size differences environmental factors, food or water parameters, type of solvent used and selective action of toxicants. It reveals that leaf extract of each plant or even extract from parts of same plant varies in toxicity. Studies of Fafioye and Adebisi (2001) further verify this phenomenon who proved *Raphia vinifera* pods to be more toxic than *Parkia biglobosa* bark on Nile Tilapia (*Oreochromis niloticus*).

Current studies have revealed similar findings and proved the selective toxicity of the chosen plants or their parts as in *Parthenia* (Table III), which may be inferred to the type of active ingredient it contains, which will be objective of next studies. Wang and Huffman (1991) and Alade and Irobi (1993) in their studies has proved that varied toxicities were due to the presence of different active ingredients, which strongly support our view point.

In crux, active ingredients in plants, in this study, caused physiological impairment in the fish. Therefore, for any final decision regarding replacement of rotenone, further comprehensive studies are needed on the estimation of biochemical constituents of these plants or their parts and residual effects of these herbal extracts in water, soil and experimental animals. More efforts are required for chemical analysis and their toxicity on different fish species at biochemical and tissue level. Moreover, control of predators or trash fishes is not selective.

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