EVALUATION OF BOTANICAL PISCICIDES ON NILE TILAPIA *Oreochromis niloticus* L. AND MOSQUITO FISH *Gambusia affinis* BAIRD AND GIRARD

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Abstract

The study assessed the piscicidal activity of ten locally available plants to two freshwater fishes; Nile tilapia (*O. niloticus* L.) and mosquito fish (*G. affinis* Baird and Girard*)*. It focused on the laboratory determination of lethal concentrations (LC_{50} and LC_{100}) through a static bioassay test. The ten plants tested were ampalaya *Momordica charantia*, adelfa *Nerium indicum*, agave *Agave cantala*, kalamansi *Citrus mitis*, lagundi *Vitex negundo,* madre de cacao *Gliricidia sepium,* makabuhai *Tinosphora rhumpii,* neem *Azadirachta indica*, physic nut *Jatropa curcas* and sambong *Blumea balsamifera*.

Based on the 24-hour lethal concentration (LC_{100}**)**, the plants with the strongest piscicidal activity to Nile tilapia and mosquito fish were makabuhai and kalamansi, respectively. The toxicity to Nile tilapia of the ten plants arranged in the order of decreasing toxicity is as follows: makabuhai (0.82 ml l^{-1}) > adelfa (1.06 ml l^{-1}) > ampalaya (2.59 ml l^{-1}) > kalamansi (5 ml l^{-1}) > neem (12.4 ml l^{-1}) > physic nut (26.67 ml l^{-1}) > lagundi (31.5 ml l^{-1}) > agave (74.29 ml l^{-1}) > madre de cacao (90 ml l^{-1}) > sambong (125.71 ml l^{-1}). For mosquito fish, the toxicity of the ten plants arranged in the order of decreasing toxicity is as follows: kalamansi (3 ml l^{-1}) > makabuhai (6 ml l^{-1}) > adelfa (7.87 ml l^{-1}) > neem (8.31 ml l^{-1}) > ampalaya (13.5 ml l^{-1}) > lagundi (50 ml l^{-1}) > sambong (80 ml l^{-1}) > physic nut (81.67 ml l^{-1}) > agave (102.08 ml l^{-1}) >madre de cacao (117.5 ml l^{-1}).

The results of the study showed that locally available plants in the Philippines have the potential to be used as piscicide which can be an alternative to harmful chemical piscicides to eradicate unwanted fishes in the ponds.

Introduction

Unwanted fishes may enter aquaculture farms through water supplies or along with seed brought into the fish farm. Occasional draining of the pond and fishing are usually inadequate to control and eradicate unwanted fishes. Screening is the standard method, but it does not stop the entry of predatory fishes in larval form. In ponds where water enters through pipes, screens may significantly restrict the flow of water (Bardach *et al.,* 1972). The best way of ensuring total eradication of unwanted fishes is through the use of fish toxicants (piscicide) in the pond water (Chakroff, 1976). The use of piscicides as a tool in pond management during pond preparation to get rid of predators before fish stocking is an important tool. Ideally, ponds should be sundried and the pond bottom cracked dried to help get rid of fish predators. However, this practice is not always possible particularly during the wet season. Moreover, farmers who are always in a hurry to prepare their ponds always resort to the use of inorganic fish toxicants.

In the country today, there is no legally registered fish toxicant except for some organics such as tea seed cake and tobacco dust. In view of this, farmers resort to non-conventional and unregistered fish toxicants such as agro-pesticides and sodium cyanide because they are fast acting and readily available in the market. However, these chemicals may have negative effects on the environment and farmers' health. Hence, there is a need to explore other environment and health-friendly fish toxicants such as botanical plants with molluscicidal activity.

Plants are virtually inexhaustible source of structurally diverse biologically active substances (Istvan, 2000). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties. Unlike synthetic chemical pesticides which leave harmful residues in the aquatic environment (Koesomadinata, 1980; Cagauan, 1990; Cagauan and Arce, 1992), botanical insecticides are believed to be more environment friendly because they are easily biodegraded and leave no residues in the environment. Since some of these pesticidal compounds present in plants were also toxic to fishes, botanical pesticides can be used as piscicide to eradicate unwanted fishes in the pond.

Many plants from different families have been applied for catching fish all over the world. Examples of these plants are of the genera *Derris, Tephrosia* and *Lonchocarpus* of the family L*eguminosae*. The toxic parts of plants employed as fish poisons include roots, seeds, fruits, bark, latex or leaves. Some plants have been reported to have molluscicidal action (Rejesus and Punzalan, 1997) hence, they may have high piscicidal action. The study assessed the piscicidal activity of extracts from selected botanicals on Nile tilapia *O. niloticus* and mosquito fish *G. affinis*, two fishes commonly eradicated during pond preparation.

Methodology

Experimental set up

Rectangular plastic containers, each measuring 16 cm x 11 cm x 5 cm and provided with net covers, were used. Each container was filled with 500 ml non-chlorinated tap water. The water was pre-aerated for 20 minutes to full oxygen saturation before the different volumes of the plant extract were added.

Test plants

Ten locally available plant species used in the study are found in Table 1. The plants *T. rumphii, J. curcas*, and *A. cantala* were obtained from the University of the Philippines at Los Baños, Laguna and grown at FAC, CLSU, whereas the other plants were obtained within the vicinity of Nueva Ecija.

Collection of the plant materials was done mostly in the morning. The leaves were the main plant part used except for *J. curcas* which had only few leaves but having a very fleshy stem, therefore the stem was used.

Table 1. Plants used as treatments.

English/Local Name	Scientific Name	Family Name	Parts Used
Adelfa	*Nerium indicum* Mill.	Apocynaceae	leaves
Neem	*Azadirachta indica* A. Juss	Meliaceae	leaves
Makabuhai	*Tinospora rumphii* Boerl.	Menispermaceae	leaves
Physic Nut/tuba	*Jatropha curcas* L.	Euphorbiaceae	stem
Sambong	*Blumea balsamifera* L.	Asteraceae	leaves
Kalamansi	*Citrus mitis* Blanco	Rutaceae	leaves
Agave	*Agave cantala* Roxb.	Agaveceae	leaves
Lagundi	*Vitex negundo* L.	Verbaceae	leaves
Ampalaya	*Momordica charantia* L.	Cucurbitaceae	leaves
Madre-de-cacao	*Gliricidia sepium* (Jacq.) Steudel	Leguminosae	leaves

Plant materials for assay were prepared in water-extracted form. Fresh plant material was weighed using the Mettler balance and then processed in a food blender. Distilled water was added to the chopped plant material before grinding. The ratio of plant material to the volume of the distilled water added was 1:2 or 100 g of plant material was added with 200 ml of distilled water. The extract and solid plant materials were separated by hand squeezing using cheesecloth. The plant extract was used immediately after extraction to ensure its freshness.

Test fish species

Fish species *O. niloticus* and *G. affinis* were obtained from the ponds of the Freshwater Aquaculture Center. Care was observed to minimize stress incurred by the test fish. The test fishes were acclimatized as prescribed in APHA *et al*. (1971; 1980). Nile tilapia (average weight: 0.17 g) and mosquito fish (average weight: 0.19 g) were stocked at ten fish per container after the addition of the plant extract. According to Murty (1986), fish weighing 0.5 to 5 grams or less than 7.62 cm in length are used in toxicity tests; and all fish must be of the same size. The recommended total fish biomass in the test container was followed based on Murty (1986) i.e. 0.8 mg l^{-1} at a temperature of 17^{o}C and 0.5 mg l^{-1} at higher temperatures.

For each plant, six different concentrations, each replicated three times, were used. The different test concentrations used are found in Table 2.

Table 2. Test concentrations used for the different plants tested on Nile tilapia and mosquito fish.

Plant	Test concentration (ml l^{-1})	
	Nile tilapia	Mosquito fish
Agave	0, 10, 30, 50, 70, 100	0, 20, 50, 100, 150, 200
Neem	0, 2, 4, 6, 8, 10	0, 2, 4, 6, 8 , 10
Madre-de-cacao	0, 8, 40, 60, 90, 125	0, 50, 80, 100, 140, 200
Ampalaya	0, 1, 2, 3, 4, 5	0, 2, 6 10, 12, 15
Adelfa	0, 0.1 , 0.5, 1.0, 1.5, 2.0	0, 2, 4, 6, 8, 10
Makabuhai	0, 0.1, 0.2, 0.4, 0.6, 0.8	0, 2, 4, 6, 8, 10
Sambong	0, 40, 60, 80, 120, 150	0, 10, 40, 80, 100, 120
Lagundi	0, 10, 15, 20, 30 40	0, 5, 10, 20, 36, 50
Kalamansi	0, 0.5, 1, 2, 5 10	0, 0.5, 1, 2, 3, 5
Physic Nut/tuba	0, 5, 10, 14, 20, 30	0, 20, 50, 60, 80 , 90

Standard static bioassay procedures were employed based on APHA, AWWA and WPCF (1971; 1980). Fish mortalities were observed and recorded at 24, 48, 72 and 96 hours from stocking. Dead fish were removed immediately. A fish is considered affected by the plant toxicant when it manifests erratic swimming behavior, hyperactivity, hyperventilation and pronounced ataxia coinciding with decreased capacity to respond to visual stimuli. A fish is considered dead when it does not respond to mechanical prodding.

Lethal concentrations

The lethal concentrations (LC_{50} and LC_{100}) of each of the test plant were determined by plotting concentrations of the plant against fish mortality within 24 hours, 48 hours, and 96 hours after exposure to the treatment. Interpolation between two concentrations where the mortality occurred at less than and greater than 50 % was done. LC_{50} or median lethal concentration is the concentration at which 50 % of the test fish survived and 50 % died. It is the basis of most toxicity and tolerance tests. LC_{100} is the lowest concentration at which 100 % of the fish died. It is the basis of the piscicidal activity of test plants because the purpose of using a piscicide is to ensure total eradication of unwanted fishes. Trendline analysis using linear regression in Microsoft Excel was used to estimate LC_{50} and LC_{100}.

Water quality

Temperature, pH and dissolved oxygen (DO) were monitored initially after the addition of toxicant, before fish stocking and 24 hours thereafter. Temperature and DO concentrations were analyzed using YSI model 50 while pH with pen-type digital pH meter.

Results and discussion

Generally, the plants with fleshy leaves had more extracts. The plants with the highest volume of extract obtained were sambong *B. balsamifera,* agave *A. cantala* and madre de cacao *G. sepium.* Although these plants gave more extracts, they had the least piscicidal activity. The leaves of the plants adelfa (*N. indicum*), neem (*A. indica*), and lagundi (*V. negundo*) had less water content, thus less extract was obtained.

Toxicity expressed as LC_{50}

The 96-hr toxicity test, also called short-term or acute toxicity test is one of the most commonly used tests in the evaluation of toxicity (Murty, 1986).

Based on the 96-hour median lethal concentration (LC_{50}), the most toxic plant to Nile tilapia and mosquito fish was adelfa (0.083 ml l^{-1}) and kalamansi (2.38 ml l^{-1}), respectively. The least toxic plant extract to Nile tilapia and mosquito fish was madre de cacao, with LC_{50} values of 52 ml l^{-1} and 92 ml l^{-1}, respectively.

The toxicity to Nile tilapia of the ten plants arranged in the order of decreasing toxicity is as follows: adelfa (0.083 ml l^{-1}) > makabuhai (0.44 ml l^{-1}) > ampalaya (0.45 ml l^{-1}) > neem (2.57 ml l^{-1}) > lagundi (2.93 ml l^{-1}) > kalamansi (3.12 ml l^{-1}) > sambong (5.11 ml l^{-1}) > physic nut (12.8 ml l^{-1}) > agave (30 ml l^{-1}) > madre de cacao (52 ml l^{-1}). The results were comparable to the results of the study conducted by Leaño and Cagauan (1994) which stated that *T. rumphii* was more toxic to Nile tilapia than *A. indica*, *V. negundo*, *B. balsamifera* with 96-hour LC_{50} values of 0.77, 1.59, 4.95, and 1.54 g/l, respectively.

The toxicity to mosquito fish of the ten plants used arranged in the order of decreasing toxicity is as follows: kalamansi (2.38 ml l^{-1}) > adelfa (2.85 ml l^{-1}) > neem (3 ml l^{-1}) > agave (3.12 ml l^{-1}) > makabuhai (4.7 ml l^{-1}) > ampalaya (9.16 ml l^{-1}) > lagundi (14 ml l^{-1}) > sambong (20.43 ml l^{-1}) > physic nut (28.57 ml l^{-1}) > madre de cacao (92 ml l^{-1}).

Piscicidal effect expressed as LC_{100}

Based on the 24-hour lethal concentration (LC_{100}), the plant with the greatest piscicidal effect to Nile tilapia and mosquito fish was makabuhai (0.82 ml l^{-1}) and kalamansi (3 ml l^{-1}), respectively (Tables 3 and 4).

The piscicidal effect of the ten plants to Nile tilapia arranged in the order of decreasing activity is as follows: makabuhai (0.82 ml l^{-1}) > adelfa (1.06 ml l^{-1}) > ampalaya (2.59 ml l^{-1}) > kalamansi (5 ml l^{-1}) > neem (12.4 ml l^{-1}) > physic nut (26.67 ml l^{-1}) > lagundi (31.5 ml l^{-1}) > agave (74.29 ml l^{-1}) > madre de cacao (90 ml l^{-1}) > sambong (125.71 ml l^{-1}).

The piscicidal effect of the ten plants to mosquito fish arranged in the order of decreasing toxicity is as follows: kalamansi (3 ml l^{-1}) > makabuhai (6 ml l^{-1}) > adelfa (7.87 ml l^{-1}) > neem (8.31 ml l^{-1}) > ampalaya (13.5 ml l^{-1}) > lagundi (50 ml l^{-1}) >

sambong (80 ml l^{-1}) > physic nut (81.67 ml l^{-1}) > agave (102.08 ml l^{-1}) > madre de cacao (117.5 ml l^{-1}).

Table 3. LC_{50} and LC_{100} values of the different test plants to Nile tilapia at different durations.

Plant	LC_{50}			LC_{100}		
	24-hrs	48-hrs	96-hrs	24-hrs	48-hrs	96-hrs
Agave	52.86	40.00	30.00	74.29	52.50	130.00
Neem	6.40	3.22	2.57	12.40	4.89	4.00
Madre de Cacao	75.00	73.93	52.00	90.00	90.00	72.00
Ampalaya	1.71	1.50	0.45	2.59	2.44	1.82
Adelfa	0.62	0.07	0.08	1.06	0.18	0.50
Makabuhai	0.69	0.53	0.44	0.82	0.68	0.64
Sambong	97.14	88.42	5.11	125.71	120	84.44
Lagundi	23.99	15.67	2.93	31.50	20.67	20.77
Kalamansi	3.50	3.33	3.12	5.00	5.00	5.00
Tuba	18.67	17.27	12.80	26.67	25.45	21.80

Table 4. LC_{50} and LC_{100} values of the different test plants to mosquito fish at different durations.

Plant	LC_{50}			LC_{100}		
	24-hrs	48-hrs	96-hrs	24-hrs	48-hrs	96-hrs
Agave	70.83	62.50	3.12	102.08	100.00	50.00
Neem	6.00	3.43	3.00	8.31	4.86	4.07
Madre de Cacao	98.75	120.00	92.00	117.50	140.00	112.00
Ampalaya	9.75	9.16	9.16	13.50	12.32	12.32
Adelfa	5.87	2.95	2.85	7.87	4.00	4.00
Makabuhai	5.00	4.93	4.70	6.00	6.00	6.00
Sambong	55.00	20.00	20.43	80.00	41.43	40.00
Lagundi	37.65	21.14	14.00	50.00	38.28	20.00
Kalamansi	1.71	2.44	2.38	3.00	3.00	3.00
Tuba	56.67	44.00	28.57	81.67	74.00	50.00

Test fish stocked in the higher concentrations of the test plant extracts exhibited erratic swimming behavior and rapid opercular movement. Later, the test fishes lost their balance, after which death occurred. This behavior was not evident in fishes stocked in the control and in the lower concentrations of the test plant extracts.

Direct comparison of the lethal concentration values suggests that mosquito fish more frequently had higher LC_{50} and LC_{100} than Nile tilapia. This indicates that mosquito fish is more tolerant to the test plants compared to Nile tilapia. The tolerance of mosquito fish is attributed to its age. The two test fishes were of nearly equal size but the mosquito fish is older than the tilapia since it can only attain a maximum length of 4 cm for males and 7 cm for females at maturity. Tilapia is a lot larger than mosquito fish when it reaches maturity. If both the fishes used are of the same age, the result may be otherwise, because larger fish tend to be more tolerant than smaller fish.

Water quality

The temperature values between replicates in tilapia and mosquito fish did not vary much throughout the duration of the test in all test plants. The highest and lowest temperature readings for Nile tilapia were 27.6^{o}C and 22.4^{o}C, respectively, while for mosquito fish, it was 30.0^{o}C and 22.4^{o}C. Murty (1986) reported that the toxicity of a certain toxicant is greater in higher temperatures. This may be attributed to increase in enzyme activity and metabolic rate of the fish with an increase in temperature.

The dissolved oxygen level in the tests was highest in the initial reading after the water was aerated for 20 minutes. The highest initial reading of DO for Nile tilapia was 8.19 mg/l while the lowest was 0.37 mg/l. For mosquito fish, highest and lowest initial DO concentrations were 8.19 mg/l and 0.82 mg/l, respectively. Drastic decline in dissolved oxygen concentration was observed within 24 hours after stocking. Treatments with higher concentrations of the plant extract had lower DO concentrations which can be attributed to the chemical oxygen demand of the extract incorporated. Higher extract doses may mean higher chemical oxygen demand. Further drop in DO concentrations was attributed to the oxygen consumption of the test fishes. According to Teichert-Coddington and Green (1993) cited in Egna and Boyd (1997), Nile tilapia can survive at DO concentrations of below 0.5 mg l^{-1} and can live for about 6 hours.

Summary, conclusions and recommendations

The control and eradication of unwanted fishes in the pond requires the use of effective piscicides. Most of the fish farmers resort to the use of chemical piscicides that prove to be very effective although these chemicals are rather dangerous to the environment and can do more harm than good. Alternative piscicides that are not hazardous to the environment and have shorter residual effects must be used. The results of the study showed that locally available plants in the Philippines have the potential to be used as piscicide which can be an alternative to harmful chemical piscicides that are widely used today to eradicate unwanted fishes in the ponds.

Future studies must be conducted to further explore the potential of other plants to be used as piscicide. The use of other extraction methods and other predatory fishes must be considered in future investigations.

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