

The Toxicity of Five Native Thai Plants to Aquatic Organisms

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Abstract

Five species of native Thai plants were bioassayed as toxicants against the cladoceran *Moina* sp., the fishes *Oreochromis niloticus* and *Anabas testudineus*, the shrimp *Penaeus merguiensis*, and the snail *Cerithidea cingulata*. The 24-h LC₅₀ of each plant to each test species was determined. *Diospyros diepenhorstii* is the least effective plant extract against all five test organisms, while *Maesa ramentacea* and *Sapindus emarginatus* are the most effective plant extracts against *Moina* sp., *O. niloticus* and *A. testudineus*. The lethal concentrations of all plant extracts to test fish are greater than that to shrimp. Moreover, all plant extracts are more effective against *O. niloticus* than *A. testudineus*. *Pittosporum ferrugineum* and *S. emarginatus* have potential against snail pests. Among the five plants, *M. ramentacea* showed the highest toxicity to *O. niloticus*. Toxicity to *O. niloticus* of *M. ramentacea* (15-30 mg·l⁻¹) was about half that of tea seed cake (6-15 mg·l⁻¹). However, improvement of *M. ramentacea* toxicity is feasible. These results are useful for developing botanical pesticides for selective killing of undesired aquatic organisms.

Introduction

One factor that influences aquaculture production is the presence of pests, predators and competitors of cultivated organisms. Most of the enemies are carnivores. Those commonly found in freshwater fish farms include the catfish (*Clarias* spp.), snakehead (*Ophicephalus striatus*) and climbing perch (*Anabas testudineus*). In marine shrimp farms, the major predators are *Lates calcarifer*, *Eleutheronema tetradactylum*, *Oreochromis mossambicus*, *Therapon theraps*, *Scatophagus argus*, *Glossogobius giurus* and *Mystus* spp. (Thambuppa 1981).

Application of piscicides is one method used to increase aquaculture production. There are many kinds of piscicides of differing effectiveness. They can be classified into two groups - chemical reagents such as PCP-Na and malachite green (Terazaki et al. 1980), sodium cyanide (Gribgratok 1981) and

antimycin (Marking 1992), and natural products such as tea seed cake (Tang 1961), derris root, tobacco waste (Chakroff 1976) oil cake from the seed of the plant mahua (*Bassia latifolia*) (Bhatia 1970), ripe fruit of *Catunaregam spinosa* and leaves of *Polygonum hydropiper* (Kulakkattolickal 1989a). Piscicides derived from plants are popular since they are available locally, the toxicity degrades very rapidly, and thus they are safe to use. The tolerance limits of different aquatic organisms to any piscicide are different (Rajitparinya et al. 1975; Tharnbuppa 1981; Tharnbuppa 1982; Kulakkattolickal 1989b). One of the most important desired characteristics of a piscicide is its ability to kill only the unwanted species.

Five species of native plants were selected for further testing after a study on toxicity to tilapia fry (Chiayareesajja et al. 1987), after considering the toxic levels and the availability of the plants. This article compares the effectiveness of these five plants in killing various species of aquatic organisms with that of commercial tea seed cake, a popular piscicide used in marine shrimp farms (Terazaki et al. 1980).

Materials and Methods

The five plants tested were *Diospyros diepenhorstii*, *Maesa ramentacea*, *Pittosporum ferrugineum*, *Sapindus emarginatus* and *Schima wallichii* (Fig. 1). The fruits of *S. emarginatus* and the leaves of the other four plants were air-dried for one week then mixed with aerated tapwater and minced with an electric blender for one minute. The extract was filtered through muslin cloth and Whatman filter paper No. 40 with suction. Dilutions of plant extract were prepared for testing with water flea (*Moina* sp.), Nile tilapia (*Oreochromis*

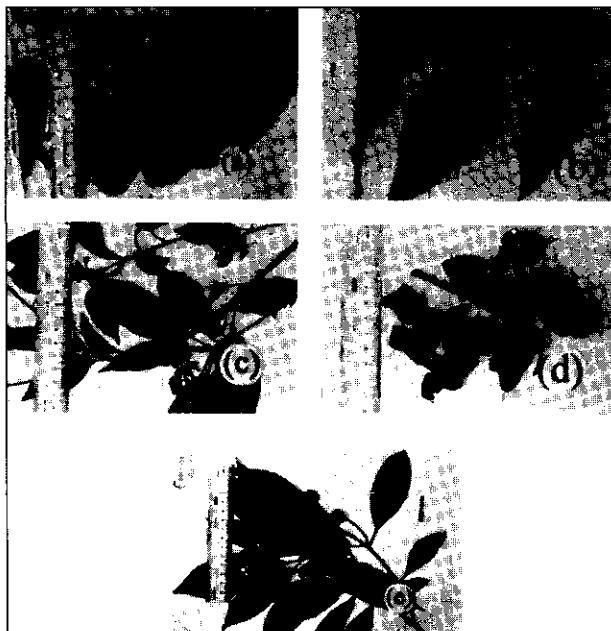


Fig. 1. Five native Thai plants tested as piscicides (a) Nian *Diospyros diepenhorstii*, (b) Kradookkai *Maesa ramentacea*, (c) Kela *Pittosporum ferrugineum*, (d) Prakumdekwai *Sapindus emarginatus*, and (e) Kunchoke *Schima wallichii*.

niloticus), climbing perch (*Anabas testudineus*), banana shrimp (*Penaeus merguiensis*) and brackishwater snail (*Cerithidea cingulata*).

A static bioassay technique, according to APHA et al. (1981) was used to determine median lethal concentrations (LC_{50}). Six concentrations (10,000, 1,000, 100, 10, 1 and 0 $\text{mg}\cdot\text{l}^{-1}$ dry leaves or fruits) of each plant extract were used for range-finding or exploratory tests. In the full-scale or definitive tests, six concentrations of each plant extract were selected from an arithmetically spaced series of concentrations between the highest concentration that killed no test animals and the lowest concentration that killed all test animals.

Toxicity of Five Plants to *Moina* sp.

Six concentrations of each plant extract, including a control (freshwater only), were prepared in 145 ml freshwater and 5 ml of concentrated *Moina* sp. culture added to each. Three replicates were used for each concentration. After 24 h, the proportion of dead *Moina* was recorded.

Toxicity of Five Plants to *O. niloticus* and *A. testudineus*

The same methodology was used for *O. niloticus* and *A. testudineus*. Aged tap water that had been aerated for 3-4 d was used in preparing test solutions. Six concentrations of each plant extract, including a control (tap water only), were prepared. Ten fish (*O. niloticus* or *A. testudineus*, total length 5-7.5 cm) were immersed in glass jars with 10 l of the treatment solutions, three replicates for each concentration. The numbers of dead fish were recorded, and removed from the jars at 1, 3, 6, 12 and 24 h after immersion.

Toxicity of Five Plants to *P. merguiensis*

Six concentrations of each plant extract, including a control, were prepared in 18 glass jars with 10 l of 20 ppt seawater. Each glass jar was divided into 10 compartments with an aluminum frame with blue netting and each compartment had one shrimp, 5 cm long (isolated to prevent cannibalism). Three replicate jars (30 shrimp) were used for each concentration. The numbers of dead shrimp were recorded at 1, 3, 6, 12 and 24 h after immersion.

Toxicity of Five Plants to *C. cingulata*

Six concentrations of each plant extract, including a control, were prepared in 30 ppt seawater in 1-l beakers. Ten snails, 2.5 cm long, were immersed in each beaker, three replicate beakers per concentration. Numbers of dead snails were recorded at 1, 3, 6, 12 and 24 h after immersion. The snails were collected either from shrimp farms or from Songkhla beach.

Relative Toxicity of *Maesa ramentacea* and Tea Seed Cake to *O. niloticus*

Six concentrations of *M. ramentacea* and tea seed cake including controls were prepared in jars with 10 l of 20 ppt seawater. Ten tilapia (5-7.5 cm long)

were then released into each jar. Fish mortality in each solution was plotted against concentration at various time intervals on a semi-log paper to evaluate the lethal concentrations (LC) at chosen times: 3-h LC₁₀₀, 6-h LC₁₀₀ and 24-h LC₅₀ as outlined by APHA et al. (1981).

Data were subjected to t-test or analysis of variance (ANOVA) and multiple comparison using the New Duncan's Multiple Range Test at 5% significance level (Steele and Torrie 1960).

Results

Each plant was effective in killing the test organisms at different concentrations. *D. diepenhorstii* is the least effective plant extract against all five test organisms. The 24-h LC₅₀ of *D. diepenhorstii* to *Moina* sp., *O. niloticus* and *A. testudineus* are significantly higher than the other four plants (Table 1). In addition, *M. ramentacea* and *S. emarginatus* are the most effective plant extracts against *Moina* sp., *O. niloticus* and *A. testudineus*. The plants that killed *Moina* sp., *O. niloticus*, *A. testudineus*, *P. merguiensis* and *C. cingulata* at the lowest concentration were either *M. ramentacea* or *S. emarginatus* (Table 1). *Moina* sp. and *O. niloticus* had lower tolerances to all test solutions than did *A. testudineus*, *P. merguiensis* and *C. cingulata*. The plant extracts are more effective against *O. niloticus* than air-breathing *A. testudineus*. Among the five species of selected plants, the greatest toxicity to *O. niloticus* was shown by dry leaves of *M. ramentacea*. Furthermore, *P. ferrugineum* and *S. emarginatus* have potential against snail pests.

Tea seed cake showed a potency about twice that of *M. ramentacea* extract against *O. niloticus*. The lethal concentrations of *M. ramentacea* to *O. niloticus* are significantly higher than tea seed cake (Table 2). Increased piscicide concentrations kill fish faster.

Discussion

The results of this study are similar to those of Rajitparinya et al. (1975), Terazaki et al. (1980), Tharnbuppa (1981, 1982), Minsalan and Chiu (1986), and Kulakkattolickal (1989b) who reported different tolerance limits of various aquatic organisms to various piscicides. The tolerance limit of crustaceans to saponin extracted from tea seed cake was higher than that of fish (Rajitparinya et al. 1975; Terazaki et al. 1980; Tharnbuppa 1981). These differences in tolerance limits could be applied to the selective eradication of aquatic organisms by using each plant at different concentrations against different pests.

Saponins were identified as the active constituent in *M. ramentacea*, *P. ferrugineum*, *S. wallichii* (Wiriyachitra and Towers 1988) and *S. emarginatus* (Mahabusarakam et al. 1990); whereas the active constituent in *D. diepenhorstii* is a proanthocyanidin polymer (Balza et al. 1989). Saponin has a strong hemolytic action on red blood cells (Budavari 1989). Saponin thus affected oxygen uptake, and exposed animals desperately tried to surface and gulp air.

Table 1. Toxicity (24-h LC₅₀) of five native plants to five aquatic organisms.

Plant extract	Lethal concentration of plant extract (mg·l ⁻¹) to test organisms				
	<i>Moina</i> sp.	<i>Oreochromis</i> <i>niloticus</i>	<i>Anabas</i> <i>testudineus</i>	<i>Penaeus</i> <i>merguiensis</i>	<i>Cerithidea</i> <i>cinctulata</i>
<i>Diospyros diepenhorstii</i>	365±144 ^a	260±17 ^a	2,433±17 ^a	> 3,000	> 10,000
<i>Maesa ramentacea</i>	67±5 ^b	17±2 ^e	315±20 ^c	> 3,000	> 10,000
<i>Pittosporum ferrugineum</i>	106±22 ^b	120±6 ^b	176±8 ^{cd}	> 3,000	683±143 ^a
<i>Sapindus emarginatus</i>	69±10 ^b	41±5 ^d	43±6 ^d	1,210±144	420±12 ^a
<i>Schima wallichii</i>	80±1 ^b	66±6 ^c	1,035±153 ^b	> 3,000	> 10,000

The 24-h LC₅₀ of *D. diepenhorstii*, *M. ramentacea* and *S. wallichii* to *P. merguiensis* and *C. cinctulata* and *P. ferrugineum* to *P. merguiensis* were not determined because the concentrations required were too high. Mean±SE (n=3) with different superscripts in the same column are significantly different (P<0.05).

Table 2. Comparison of the toxicity of *Maesa ramentacea* extract and tea seed cake to *Oreochromis niloticus*.

Piscicide	Lethal concentration (mg·l ⁻¹)		
	3-h LC ₁₀₀	6-h LC ₁₀₀	24-h LC ₅₀
<i>Maesa ramentacea</i> extract	30±3 ^a	24±2 ^a	15±1 ^a
Tea seed cake	15±2 ^b	9±1 ^b	6±2 ^b

Mean±SE (n=3) with different superscripts in the same column are significantly different (P<0.05).

The potency of *M. ramentacea* against *O. niloticus* was only around half that of tea seed cake (Table 2), whose active principle is also saponin. The saponin content in air dried *M. ramentacea* powder was measured on a water high pressure liquid chromatography with a C₁₈ reverse phase column using methanol-water as eluant and found to be 5.96% (Wiriyachitra and Towers 1988), not much different from tea seed cake with 5.2-7.2% (Terazaki et al. 1980). Minsalan and Chiu (1986) reported that 15 mg·l⁻¹ tea seed cake was adequate to kill fish within 6 h of application in ponds.

M. ramentacea and *S. emarginatus* are the most effective plant extracts against *O. niloticus* and *A. testudineus*. The lethal concentrations of both plant extracts to test fish are much less than that to shrimp. Both plant extracts have potential to be used as piscicides for shrimp farming. However, *S. emarginatus* is too expensive for use as piscicide because it is already commercialized as an herb. Furthermore, the fruit of *S. emarginatus* is used. It is seasonal and not a productive source of piscicide compared to *M. ramentacea* whose leaf is used. Therefore, further study on the Thai piscicidal plant, *M. ramentacea* has been emphasized. *M. ramentacea* is a widespread weedy tree in Thailand and should present no difficulty in cultivation in Indochina.

Our recent study has revealed that 6 mg·l⁻¹ of *M. ramentacea* elite clone obtained from genetic selection and tissue culture techniques caused 100% mortality of *O. niloticus* within 12 h. Hence, substitution of *M. ramentacea* for

tea seed cake is feasible. The innovative use of *M. ramentacea* as a piscicide would benefit fish or shrimp farmers in tropical Asian countries where *M. ramentacea* is native in that the importation of tea seed cake may be reduced.

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References

APHA (American Public Health Association). 1981. Standard methods for the examination of water and wastewater. 15th Edition. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington, D.C. 1,134 pp.

Balza, F., Z. Abramowski, G.H.N. Towers and P. Wiriachitra. 1989. Identification of proanthocyanidin polymers as the piscicidal constituents of *Mammea siamensis*, *Polygonum stagninum* and *Diospyros diepenhorstii*. *Phytochemistry* 28: 1827-1830.

Bhatia, H.L. 1970. Use of mahua oil cake in fishery management. *Indian Farming* 20: 39-40.

Budavari, S., Editor. 1989. The Merck Index. 11th Edition. Merck and Co., Inc., Rahway. 1,606 pp.

Chakroff, M. 1976. Freshwater fish pond culture and management. A joint Peace Corps and Volunteers in Technical Assistance Publication. Volunteers in Technical Assistance, Mt. Rainier. 196 pp.

Chiayvareesajja, S., W. Mahabusarakam, J.F. Maxwell, P. Wiriachitra and G.H.N. Towers. 1987. Thai piscicidal plants, I. *Journal of the Science Society of Thailand* 13: 29-45.

Gribgratok, S. 1981. The role of cyanide on the fisheries. *Thai Fisheries Gazette* 34: 499-506. (In Thai.)

Kulakkattolickal, A.T. 1989a. Piscicidal plants of Nepal: ripe fruit of *Catunaregam spinosa* (Thunb.) (Rubiaceae) and leaves of *Polygonum hydropiper* L. (Polygonaceae) as fish poisons. *Aquaculture* 78: 293-301.

Kulakkattolickal, A.T. 1989b. Piscicidal plants of Nepal: toxicity to air-breathing predatory fish (*Ophiocephalus punctatus*, *Clarias batrachus*, and *Heteropneustes fossilis*) and the duration of risk to cultivated fish. *Aquaculture* 78: 285-292.

Mahabusarakam, W., G.H.N. Towers, P. Tuntiwachwuttikul and P. Wiriachitra. 1990. Piscicidal triterpenoid saponins of the pericarps of *Sapindus emarginatus*. *Journal of the Science Society of Thailand* 16: 187-189.

Marking, L.L. 1992. Evaluation of toxicants for the control of carp and other nuisance fishes. *Fisheries* 17: 6-12.

Minsalan, C.L.O. and Y.N. Chiu. 1986. Effects of tea seed cake on selective elimination of finfish in shrimp ponds. *The First Asian Fisheries Forum*: 79-82.

Rajitparinya, C., Y. Jiamcharoensin and W. Imjaisook. 1975. Toxicity of tea seed cake to some species of brackishwater fish, shrimps and crab. *Thai Fisheries Gazette* 28: 397-399. (In Thai.)

Steele, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York. 481 pp.

Tang, Y.A. 1961. The use of saponin to control predacious fishes in shrimp ponds. *Progressive Fish-Culturist* 23: 43-45.

Terazaki, M., P. Tharnbuppa and Y. Nakayama. 1980. Eradication of predatory fishes in shrimp farms by utilization of Thai tea seed. *Aquaculture* 19: 235-242.

Tharnbuppa, P. 1981. An experimental study on application of tea seed for eradication of enemies of shrimp. Section of Experiment and Research for Aquaculture, Division of Brackishwater Fisheries, Department of Fisheries, Bangkok. Technical Report No. 6/1981. 16 pp. (In Thai.)

Tharnbuppa, P. 1982. An experimental study on application of derris root for eradication of fish in shrimp farms. Section of Experiment and Research for Aquaculture, Division of Brackishwater Fisheries, Department of Fisheries, Bangkok. Technical Report No. 7/1982. 23 pp. (In Thai.)

Wiriyachitra, P. and G.H.N. Towers. 1988. Fish poisons (UBC/Thailand). Final report for period June 1, 1985 to December 1, 1987. Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand. 99 pp.