Asian Fisheries Science **19**(2006):75-89
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2006.19.1.009

Asian Fisheries Society, Manila, Philippines

Use of *Derris trifoliata* (Leguminosae) Root Extracts for Fishpond Management

FLORENTINO C. SUMERA* **and MARLON T. CONATO**

Institute of Chemistry, College of Science,
University of the Philippines,
Diliman, Quezon City, Philippines

Abstract

This study aims to determine whether *Derris trifoliata* (Leguminosae) locally known as "sila-sila", can be used in a formulation for fishpond management, compared with the usual commercial source "tubli" (*Derris elliptica*, Leguminosae). Thus, prior to the formulation of the root extracts, the rotenone content of the roots of *Derris trifoliata* was determined and compared to two other *Derris* species, one of which is *Derris elliptica*. Of the three derris species, *D. trifoliata* was found to have the lowest rotenone content of 0.019% compared to that of *Derris elliptica*'s 5.09%. Although *D. trifoliata* has very low rotenone content, the extract of its root bark and formulation with acetone resulted in 1 L formulations with comparable toxicity as that of *D. elliptica* or that of a commercial insecticide (illegally used as piscicide). For example, formulations from about 15 kg of root or root bark and 20 L of acetone as extractant produced formulations of about 10 x 1 L for *D. trifoliata* (rotenone conc. = 0.180 mg•ml^{-1}) and 12 x 1 L for *D. elliptica* (rotenone conc. = 4.90 mg•ml^{-1}). Both formulations can kill fish such as *Oreochromis niloticus* (Cichlidae) fingerlings within 30 minutes comparable to a commercial insecticide Telothion 40. The median lethal concentration of the formulation for a 96-hour bioassay against *O. niloticus* for *D. trifoliata* was LC_{50} = 0.03 ppt while for *D. elliptica*, LC_{50} = 0.005 ppt. Sensitivity of nine different unwanted fishes near the location of the test fishponds was determined using a *D. trifoliata* reconstituted formulation. The unwanted fishes appeared more sensitive during summer months when the salinity of water was higher than during the rainy months when the salinity was much lower. Different species of unwanted fishes at different life stages appeared to have different tolerance to the toxicity of the extract. Application of the different formulations previously bioassayed was successful in cleaning several fishponds from unwanted fishes. Cost analysis showed that fish farmers (whose fishponds are located near colonies of *D. trifoliata* plants) could economize by using extracts of this *Derris* plant instead of insecticides harmful to the environment or dangerous poisons like sodium cyanide.

Introduction

Every time a fishpond is prepared for fish culture, unwanted fishes that may compete or prey on the fish to be cultured, have to be eliminated. This important step may be accomplished with the use of selective fish poisons ordinarily absent in the market but substituted with commercial insecticide or sodium cyanide by fishpond owners. These substitute chemicals are usually persistent in the environment or too dangerous for humans to handle and when released from the fishpond can destroy other beneficial fish species present in the aquatic ecosystem. There are, however, safer alternatives such as the use of plant derivatives

* Corresponding author. Tel.: +632 981 8500 local 3653, Fax: +632 920 5427
E-mail address: florentino.sumera@up.edu.ph

like teaseed cake, which is relatively non-toxic to humans, biodegradable and selective to fish although its supply locally is not enough because it has still to be imported.

A natural product extracted from plants and well known for its selective fish toxicity is rotenone, which is approved for fishpond management in developed countries such as the US (APHA 1998). Rotenone is considered to be environmentally safe, biodegradable and easily neutralized. Rotenone can be extracted from the *Derris* plant, a wild vine that may be cultivated and is endemic in the Philippines. The use of rotenone as piscicide was in fact inspired by aboriginal fishermen in Asia, Africa, Australia and South America who used the pounded bark and leaves of certain mangrove plants to stupefy fish. In the Philippines, some fishermen use the pounded extracts of *Derris* roots (tubli) for fishing during spring tides near the seashore. Some rural families even cultivate this plant in their backyard for this purpose. There are several species of the *Derris* plant in the Philippines and most prominent members of this genus are known locally as tubli (*D. elliptica* [Roxb.]Benth.), upi (*D. philippinensis* Merr.), tuglon (*D. polyantha* Perk.). malasaga (*D. scandens* [Roxb.] Benth.), and tubling tatlong dahon or sila-sila (*D. trifoliata* Luor.) (Quisumbing 1947). Local fishermen prefer to use tubli, recognized from the rest by the shape and color of its leaves, and the characteristic milky sap that exudes from its roots when twisted and pressed. In fact, its rotenone and rotenoid content are really much higher than those of other *Derris* species (Tee 1976; Dubouzet 1988). Reports from Cebu (Central Philippines) where the plant is cultivated, disclosed that *Derris* root powder (10 ppm) is effective in controlling 14 species of fish in brackish water without harming prawns and shrimps (*Peneus monodon* and *Peneus indicus*, Penaedae) at the concentration tested (Tumanda 1980). Guerrero et al. (1990) observed that application of fine *Derris* (collected from the Bicol region) root powders (10 and 20 ppm) proved effective in killing five freshwater fish including *Oreochromis niloticus* within an hour. In the US and Europe, commercial formulations of the plant (used in eradicating wild and stunted fish in ponds) can be purchased from most farm supply or feed and seed stores by anyone with a pesticide applicator permit. In all likelihood the above authors used similar *Derris* species, *Derris elliptica* or tubli commonly known among native fisherfolk and ordinarily obtained from secondary forests at the foot of hills and mountains and is used here and abroad. Another *Derris* plant that was investigated and whose fish toxicity was compared with other mangrove plants was sila-sila (*D. trifoliata*). Its toxicity to fish (*O. niloticus*) exceeded those from other mangrove plants such as *Excoecaria agallocha* (Euphorbiaceae), *Aegiceras corniculatum* (Myrsinaceae) and *Heritiera littoralis* (Sterculiaceae) known to be used also as piscicides. *D. trifoliata* is said to retain its toxicity even after air-, oven-, and solar drying and its root bark extract was found to be most active (Dela Cruz et al. 1984; Gomez et al. 1986).

The objective in this present study is thus to determine whether another species such as *D. trifoliata* which is abundant in mangrove swamps close to local fishponds in coastal Central Luzon, and which is not commonly utilized by fisherfolk as fish poison, could be used for fishpond management. The other objective is to use it as an organic solvent extract similar to how common insecticides have been used with the possibility of improving the rotenone concentration of the extract. The toxicity to fish as well as commercial viability of the extract of *D. trifoliata* will also be compared with the two other *Derris* plants, *D. elliptica* (tubli) and *Derris* “uwak” with respect to their effectivity for fishpond management.

Materials and Methods

Sample collection and identification

The plant, *Derris trifoliata*, was obtained from the mangrove swamps of San Roque, Paombong, Bulacan. The roots of the mature plant (0.9–1.1 cm diameter) were cut into about 15-cm pieces prior to their transport to the UP Natural Sciences Research Institute (NSRI) laboratory (University of the Philippines, Diliman, Quezon City) where the bark was stripped for further extraction. Specimens of the plant were identified by a plant taxonomist Mr. Leonardo Co of the Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines at Diliman.

The plants, *Derris elliptica* and *Derris* "uwak", were collected at the foot of the hills of Bato, Catanduanes with the help of villagers who know and use the plant for clandestine fishing during spring tides on nearby shores. The diameter of the roots collected was 2–3.5 cm for *D. elliptica* and 3–4 cm for *Derris* "uwak". The roots were cut into about 15-cm pieces prior to transport to the UP NSRI laboratory where they were further chopped to smaller pieces just before extraction. Plant specimens were identified by a plant taxonomist Dr. Edwino Fernando of the Makiling Center for Mountain Ecosystem, University of the Philippines, Los Baños, Laguna.

Fingerlings (3-4 cm, 0.4-1.0 g) of the Nile tilapia (*O. niloticus*) were obtained from a nursery of a commercial tilapia fingerling distributor, Mr. Arnold Billanes of Bgy. Pulo, Malolos City, Bulacan. The fish were acclimated in laboratory holding tanks before the bioassay experiments.

The fish species collected during the application of the formulation in the fishponds were identified by a fish taxonomist Dr. Robert Pagulayan of the Institute of Biology, University of the Philippines at Diliman.

Rotenone content

Extraction of rotenone

Fresh or air-dried plant parts (200 g each) of *Derris* plant were ground and extracted three times overnight, each time with 800 ml of ethanol. The ethanol extract was concentrated, and after all the ethanol solvent had been removed the resulting aqueous concentrate was extracted with $CHCl_3$. The chloroform layer was further concentrated and analyzed for rotenone by high pressure liquid chromatography (HPLC).

Quick bioassay

About 0.5 g of the plant $CHCl_3$-extract dissolved in 2 ml ethanol was poured and immediately stirred into an aquarium containing 10 L of conditioned tap water and five *O. niloticus* fingerlings. The temperature of the water was measured, and the time of death of the fingerlings as well as their weight was noted.

HPLC analysis of rotenone

About 50-200 mg of plant extract from the extraction process above were dissolved in 25 ml acetonitrile. The rotenone concentration in the extract was determined by comparison with calibrated concentration of rotenone standard using HPLC. The standard procedure used was from the Manual of Analytical Methods of the National Institute for Occupational Safety and Health (NIOSH 1994).

Standard rotenone (Sigma) was calibrated into the following set of solutions in 10-ml acetonitrile: 0.01, 0.03, 0.05, 0.07 and 0.095 mg•ml^{-1}. The HPLC column used was μ-Bondapak C18, with UV at 290 nm detector and mobile phase 60% methanol: 40% water with a flow rate of 1.0 ml•min^{-1}. The rotenone peak of the *Derris* extracts was identified by the standard addition technique and mass spectrometrical method.

Mass Spectrometrical Identification of Rotenone from Plant Extract

HPLC fractions from the root or root bark extract corresponding to the retention time of rotenone were collected and subjected to mass spectroscopy (Finnegan LC-MS). The MS spectrum of this fraction was then compared to the MS spectra of standard rotenone.

Formulation studies

Formulation

Several kilograms of *Derris* root or root bark were extracted with CP grade acetone (~20 L) or ethanol (~20 L) by soaking about two kilograms of new plant part (root or root bark) every after twenty four hours. The extraction was stopped only when the bioactivity of the extract reached its maximum or when the activity was comparable to a reference fish toxin. The extracts' piscicidal activities were monitored by comparing activity with a known insecticide (Telothion-40 EC, Shell Chemicals) used illegally also as fish poison by fishpond owners. The volume of extract needed for fishpond application was determined from the comparison with Telothion.

For the bioassay, a given volume (mL) of the extract was poured and immediately stirred into a glass tank containing 10 L of tap water and five tilapia (*O. niloticus*) fingerlings. The time when all the five fingerlings died was noted. The insecticide (1 mL) was used as the reference fish poison.

Table 1. Formulated extracts from derris plants

Extract Label	Extraction Solvent	Source	Plant part used
DT-1	ethanol	*D. trifoliata*	root
DT-2	ethanol	*D. trifoliata*	root bark
DT-3	acetone	*D. trifoliata*	fresh root bark
DT-4	acetone	*D. trifoliata*	dried root bark
DT-5	acetone	*D. trifoliata*	root
DE	acetone	*D. elliptica*	root
DU	acetone	*Derris* "uwak"	root
DT-6	acetone	*D. trifoliata*	root bark

Shown on Table 1 is a list of the different extracts formulated for use in fishpond management.

Reconstituted extract DT-6 (Acetone Extract)

In one carbouy (20 L) of acetone, approximately 2 kg of *D. trifoliata* root bark were soaked for at least 24 hr, removed and the fresh sample added. This process was repeated until about 6.7 kg of root bark had been extracted and the volume of acetone reduced to 13.48 L from the process. At this point, the extract was bioassayed for its toxicity to *O. niloticus* fingerlings.

The acetone-extract was evaporated and the aqueous residue extracted with $CHCl_3$. The total acetone recovered from evaporation was around 10.80 L. The remaining volume of the extract was mainly aqueous solution which was then extracted with $CHCl_3$. The total $CHCl_3$ extract recovered was 48.49 g. This extract and the water insoluble solid from the emulsion layer were combined and redissolved in 1 L of acetone labeled as DT-6A which was bioassayed.

The 1 L-acetone extract was further diluted to a 2 L-acetone extract, and labeled DT-6B. Five hundred ml of DT-6B were further diluted to 1 L corresponding to a 4 L-dilution (of the original DT-6A) and labeled as DT-6C.

Determination of LC_{50} (96 h) of the stabilized formulations

Formulations DT-4 and DT-6B of *D. trifoliata*, and formulations DE of *D. elliptica* and DU of *Derris* "uwak" were bioassayed and their median dose concentration (LC_{50} 96 h) against *O. niloticus* were determined. Fingerlings (3–4 cm, 0.4–1.0 g) of *O. niloticus* used as test animals and obtained from a fishpond in Malolos City, Bulacan were acclimated in laboratory holding tanks before the experiment.

The standard semi-static bioassay (APHA, 1975) was used to determine the relative toxicity of the formulation to the fish. The LC_{50} was estimated using the formula of Ward and Parrish (1982),

$$LC_{50} = (AB)^{1/2}$$

where A is the highest toxin concentration in which none of the organisms died and B is the lowest toxin concentration in which all the organisms died. The confidence level (C.L.) is calculated using the formula:

$$C.L. = 100(11 - 2(1/2)^{N})$$

where N is the number of the test fish exposed i.e. ten fish were used as was done in all the experiments and C.L. is 99.8%.

Application of formulation

Fish sensitivity study

Fish samples were taken from the fishpond or nearby river and placed in a 20 L-tank filled with 10 L of fishpond water with no aeration. One ml of DT-6B extracted from *D. trifoliata* was added to the tank containing the fish and the time when the fish died was noted. Several fish species considered as unwanted fish were used to determine their sensitivity to the formulation. The most sensitive fish provided the shortest time of death.

Application of the DE formulation in a pond

A formulation (DE) of comparable toxicity with Telothion (as obtained from the bioassay in laboratory water tank with *O. niloticus*) was applied to a pond with 14 m length and 7.1 m width and with 0.33 m average depth of water. The same concentration of the formulation as was used in the bioassay was applied. The required volume of the formulation needed by the pond was first mixed with water (4 x the volume of the formulation) before spreading the mixture over the pond surface. A total of 1.64 L of formulation was required for treatment of the pond (14 m x 7.1 m x 0.33 m x 1 ml per 10L ÷ 2). (The activity of the formulation was such that 1 ml of formulation killed all five fish in ten minutes compared to 1 ml of Telothion that killed all the fish in about 20 min in a 10 L water aquarium. For this reason the formulation was divided by half to approximate the toxicity of Telothion.) The time in minutes that distressed fish made their appearance after application of the mixture was recorded. The fish were collected, counted and weighed. The temperature of the pond was also measured.

General application for larger ponds

The water level of the fishpond treated was first reduced to its lowest level (5 – 10 cm). The extract was then applied directly and slowly over the area where water remained

stagnant. Fishpond conditions in several applications of formulations are shown in Table 2. In more general way, the extract was mixed first with water in a container in the proportion of 1 L extract to 4 L water, and placed in plastic bottles with caps provided with a small hole. The emulsion was then spread (sprinkled or squirted) over the pond of water. Reapplication was made only when no visible effect was observed on the pond such as the appearance of distressed fish within 10 minutes. The time of application was done just before noon when the sun started to warm up the pond (except in fishpond 3 where more time was needed in pumping out water). The distressed and dead fish were collected, counted and weighed within an hour after application. The salinity, temperature and depth of the pond were noted as well as the time of application and collection. The weather in all these experiments was sunny.

Table 2. Fishpond conditions during application of formulations

Fishpond	Place*	Area (ha.)	Time	Temp. (^{o}C)	Salinity (ppt Cl^{-})	Formulation (Volume Used)
I	Bungalon	3.5	9:30 AM	35	1.258	DT-1 (7.2 L)
II	Bungalon	2	9:06 AM	33-34	3.415	DT-6 (1L)
III	Manilad	3.5	4:00 PM	32	0.163	DT-3 (3 L)
IV	Bungalon	0.35	10:48 AM	28	10.2	DE (0.7 L)
V	Bungalon	0.40	12:02 PM	33	11.5	DU (0.5 L)

* The site is in San Roque, Paombong, Bulacan

Methods for cost/return analysis

The cost of plant collection from the site, the cost of debarking the *D. trifoliata* roots, the cost of cutting *D. elliptica* and *Derris* "uwak" to pieces, the cost of extraction and the cost of packaging the formulation were noted. The total cost of production of the extract and the market price of the insecticide used illegally were then compared.

Results and Discussion

Rotenone content of the Derris roots

Out of three *Derris* plants collected, the percentage rotenone found in the root was least in *Derris trifoliata*. The highest rotenone content was found in *D. elliptica* collected from Bato, Catanduanes where it is used traditionally in catching fish. *Derris* "uwak" contains also a significant amount of rotenone although it is not preferred by the natives compared to *D. elliptica* (Table 3). The natives have a way of differentiating these two plants by twisting their roots and examining their root sap. The one that exudes a milky sap is the preferred *D. elliptica*, while *Derris* "uwak" (and the *D. trifoliata* plant) exudes clear and non-milky saps.

Table 3. Rotenone content of some fresh *Derris* roots

Derris plant (source)	% Rotenone in roots
D. trifoliata (sila-sila) (Paombong, Bulacan)	0.0196 ± 0.0001
Derris "uwak" (Bato,Catanduanes)	1.580 ± 0.002
D. elliptica (tubli) (Bato, Catanduanes)	5.09 ± 0.04

Significant increase in rotenone content was, however, observed in the root bark when *D. trifoliata* was air dried for a week. This increase in rotenone in the root bark compared to the extract from other plant parts was also observed by Gomez et al. (1986). The leaves extract showed the least ichthyotoxicity, although the leaves showed slightly higher rotenone content than the stem. This discrepancy is due to the higher amount of resin extracted from the leaves compared to the other plant parts while an equal amount of resin extract from each were taken for bioassay (Table 4). It can be seen, however, that as the rotenone content in the extract increases, the time of death of tilapia fingerlings (*O. niloticus*)

diminishes. The root bark extract showed even greater activity than 1 ml of the insecticide Malathion (57%).

Table 4. Rotenone content and bioassay of air-dried *D. trifoliata*

Plant Parts	% Rotenone in plant part	% Rotenone in extract	Toxicity*	Time of death (min)
Leaves	0.00383	0.0578	1086	1086
Stems	0.00218	0.115	52	47
Root	0.00454	0.343	29	32
Root bark	0.0852	4.68	15	16
Reference**	-	-	31	45

* 0.5 g of extract was used and dissolved in a 10 L of water containing five *O. niloticus* fingerlings
** One ml of Malathion (57 %) was used.

The rotenone in each of the *Derris* species and *D. trifoliata* plant parts were identified by their characteristic intense base peak at m/e 393 ($M^{\circ +}$– 1) in the MS of the rotenone peaks of the extracts similar to the MS of standard rotenone. The rotenone peaks of the extracts were also identified by HPLC using the standard addition technique.

Extraction and formulation

Comparison between ethanol and acetone formulations

Several batches of extraction of the plant part of *D. trifoliata* which contain the greatest concentration in rotenone (the root bark) were made either in acetone or in ethanol.

Table 5. Summary of Data on Formulations Using *Derris* Root or Root Bark in Ethanol and Acetone

Formulation (in solvent)	Wt. of root/root bark extracted (kg)	Init. Vol. of Solvent (L)	Final Vol. of Extract(L)	Bioactivity: mL (min)	Date of pond application (volume applied)
Ethanol Extracts					
DT-1:*D. trifoliata* (root)	10.70	20.0	9.2	5(21,27) Telothion (40,34)	10/27/03 (7.2L)
DT-2:*D. trifoliata* (root bark)	13.05	20.5	13.0	5(32,24) Telothion (30,34)	
Acetone Extracts					
DT-3*: D.trifoliata* (fresh root bark)	15.55	20.5	10.0	1(26,31) Telothion (30,34)	11/30/03 (3L)
DT-4:*D.trifoliata* (dried rootbark)	14.70	20.0	7.0	1(29), 2(14) Telothion (35)	
DT-5:*D.trifoliata* (whole root)	14.90	20.0	11.0	2(19,23) Telothion (37,25)	
DE:*D. elliptica* (root)	17.15	20.0	12.4	1(17,14,14) Telothion (27,25)	2/07/04 (700mL)
DU:D."uwak" (root)	19.20	20.0	11.6	1(19,13,15) Telothion (27,25)	3/06/04 (500mL)

The *Derris* parts extracted with ethanol such as formulation DT-2 (ethanol) and formulation DT-1 (ethanol) shown in Table 5 provided final extracts with an activity similar to 1 ml Telothion at extract volumes of about 5 ml. The activity seemed to find its maximum at these volumes inspite of the addition of more plant parts for extraction. The volume of extract also tended to decrease as more root bark was extracted without improvement on toxicity. At this point the effectiveness of formulation DT-2 (ethanol) may be considered to be 13.0L/5 L multiplied with that of 1 L of Telothion. This means that there are only 2.6 x 5 L extract with equivalent toxicity to 1 L Telothion 40. That of formulation DT-1 (ethanol) would be 9.2L/5 L multiplied with that of 1 L of Telothion. This means that there are only 1.84 x 5 L extract with equivalent toxicity as 1 L Telothion 40. The 13.0 L and 9.2 L are the final volumes of their extracts. The insecticide Telothion although banned is used here as reference fish poison.

Fishpond owners use one liter of this insecticide to clear an approximately dried up 3-hectare fishpond.

The root bark extracted with acetone such as in formulation DT-3 (acetone) and formulation DT-4 shown in Table 5 exhibited bioactivities of their final extract at a volume of 1 ml, even greater than that of Telothion. Note that this was not achieved when the extractant used was ethanol. Thus, the effectiveness of the acetone formulation would be 10.0L/1 L multiplied with that of 1 L Telothion for formulation DT-3 and 7.0L/1 L multiplied that of 1 L Telothion for formulation DT-4. This implied that in using acetone as extractant, 1-L volumes of the acetone extract can be produced that has the same or even greater activity than 1 L Telothion. This conclusion as to which is better extractant, ethanol or acetone is supported by the fact that the solubility of rotenone in acetone is much greater (1/12) than in ethanol (1/300).

Whole root or root bark (fresh or dried) formulation

Using acetone as the better extractant or solvent, the result in the preparation of the extract of fresh and dried root bark can be compared with that of the whole root. If we look at the summary of data on formulations on Table 5, the weight of *D. trifoliata* root and root bark extracted were almost the same, 15 kg, and the initial volumes of solvent acetone were also similar (~ 20 L). However, the final volume of the extracts and their toxicities were not the same. The whole root extract cannot reach the equivalent toxicity of 1 ml Telothion and instead reached its maximum toxicity only at 2 ml. This was not the case with the root bark formulations which reached the 1 ml equivalent toxicity of Telothion. Between the fresh and the dried root bark extract, however, the final volumes of their extract were different. More 1L extracts (10L/1L) with equivalent toxicity as telothion can be produced from the fresh root bark.

D. trifoliata (sila-sila) vs. D. elliptica (tubli) and Derris "Uwak" *formulations*

While the fresh and dried root bark acetone extracts of *D. trifoliata* reached the equivalent toxicity of 1 L Telothion, it was expected that the *D. elliptica* and *Derris* "uwak" root extracts would surpass those of *D. trifoliata* because of their much higher rotenone content. In fact, the volume of their extracts with equivalent activity as Telothion was greater, around 12 L compared to *D. trifoliata*'s 10 L. It was also observed that the weight of root extracted was greater. The effective volume for these tubli extracts is therefore 12L/1 L (times that of Telothion). This means that 12 L of extract of comparable activity with Telothion can be produced compared to *D. trifoliata*'s 10 L.

A comparison of the rotenone concentrations among the formulations in acetone is shown in Table 6.

Table 6. Rotenone concentration of the formulations

Formulation	Rotenone Conc. (mg/mL)
DT-4 (*D. trifoliata*)	0.180 ± 0.001
DU (*Derris* "uwak")	2.48 ± 0.03
DE (*D. elliptica*)	4.90 ± 0.03

Reconstituted formulation (DT-6)

A separate formulation was made on *D. trifoliata* whereby the final extract was evaporated until all the solvent acetone was removed. The remaining aqueous extract was then extracted with chloroform which in turn was evaporated leaving a black resinous extract. This extract was taken up with acetone to make the formulations as shown on Table 7. Only about one half of the total weight of root bark from the previous formulation was extracted and the final volume in acetone was 13.48 L.

Table 7. Reconstituted formulation of chloroform extract from *D. trifoliata*

Formulation (in acetone)	Wt. of root bark extracted	Init. vol. of solvent (L)	Final vol. of extract (L)	Bioactivity: mL (min)	Date of pond application.
DT-6	6.7	20	13.48	1(>720), 5(17) 10(15), Telothion (41,51)	
DT-6A	-	-	1	1(19,22) Telothion (37,33)	
DT-6B	-	-	2	1(22,27) Telothion (37,33)	
DT-6C	-	-	4	1(47,50) Telothion (37,33)	10/14/03

About 54 % of the acetone was recovered and the 1 L reused in the reconstitution of the formulation. A two and a four-liter dilutions were also made to determine the effective volume that approximates the toxicity of Telothion. Table 7 shows that the two-liter dilution of the extract DT-6B approximates the toxicity of 1 ml Telothion. This two-liter dilution will be the subject of subsequent tests.

Fish toxicity of the formulations

Two formulations from *D. trifoliata* and one each from *D. elliptica* (tubli) and *Derris* "uwak" were tested on *O. niloticus* with as many as ten concentrations but only the mortalities in the levels bracketed by the highest concentration which did not cause any mortality and the lowest concentrations in which all the fish died are shown in Table 8. The determination was made possible by getting first the lethal concentrations of the formulations prior to the determination of the median lethal concentrations (LC_{50}). The median lethal concentrations or LC_{50} values for 96-hour exposure are also summarized in Table 8. On the basis of biological activity on tilapia fingerlings, the formulation DE (*D. elliptica*) with LC_{50} of 0.005 ppt was more toxic than the formulation DU (*Derris* "uwak") with LC_{50} of 0.009 ppt. Both formulations from Catanduanes plants were, however, much more toxic than the formulations from *D. trifoliata* with LC_{50} of 0.02 and 0.03 ppt for formulations DT-6B and DT-4, respectively. The toxicities were seen to parallel the rotenone concentrations of the formulations as can be seen in Table 6.

Table 8. Median lethal concentrations (LC_{50}) of the formulations on *O. niloticus* fingerlings calculated by the Binomial Test ($LC = (AB)^{1/2}$) of Ward and Parish (1982).

Derris Formulation	A* (ppt)	B** (ppt)	LC_{50}*** $(AB)^{1/2}$
D. trifoliata			
DT-4	0.01	0.09	0.03
DT-6B	0.009	0.03	0.02
D. elliptica			
DE	0.001	0.03	0.005
Derris "uwak"			
DU	0.003	0.03	0.009

*A is the highest toxin concentration in which none died (0% mortality)
**B is the lowest toxin concentration in which all died (100% mortality)
***Confidence limit is at 99.8%.

On exposure to the formulations, the fish behaved abnormally gasping for air and exhibiting frenzied and spasmodic movements at the water surface. Their opercula flared out and their gills and the base of their pectoral fins became abnormally reddish. These reactions were reported to be normal observations on fish affected by rotenone (Dela Cruz et al. 1984).

Field applications of the formulations

Order of tolerance of fish to the formulations

Different fish species had different responses to the *Derris* extracts as shown in Table 9. An order of fish tolerance cannot, however, be presented due to the different life stages of the different fishes as caught on site. However, a simple summary according to the various times of total mortality for the different fishes (of different life stages collected on site) bioassayed can be deduced from the table as fish tolerance was arranged from lowest (top) to highest tolerance (bottom) for fish found in brackish water.

Table 9. On site fish sensitivity to *D. trifoliata* extract (formulation DT-6B)

Fish	Date of experiment and (time of death)			
	April 2004 (min)	May 2004 (min)	June 2004 (min)	Aug. 2004 (min)
Elops hawaiiensis (f) (Elopidae)		13	11	
Mugil cephalus (f) (Mugilidae)			(16,14)	
Leiopotherapon plumbeus (a) (Terapontidae)	16	(17,17)		36
Zenarchopterus dispar (a) (Hemiramphidae)	12		(30,35)	
Xiphophorus maculates (j) (Poeciliidae)	17	(22,20)	(36,41)	
Xiphophorus maculates (a)			(47,52)	
Ophicephalus striatus (j) (Channidae)				(64,57,200)
Oreochromis mossambicus (j) (Cichlidae)	19	(35,36)	(188,180)	503
Glossogobius.giurus (j) (Gobiidae)	35			
Creisson validus (j) (Gobiidae)	77	240		
Chronophorus sp.(a) (Gobiidae)	97		405	
Salinity (ppt Cl-)	12	8	6	5

(f) = fingerling (j) = juvenile (a) = adult

As seen from Table 9, fish sensitivity to the extract of *D. trifoliata* generally decreases from April to August (see *O. mossambicus*, *L. plumbeus*, *X. maculatus*), where killing the same species of fish takes longer time. This could be due to changes in salinity of the pond waters from summer (April) to rainy season (August) where fresh water fishes are more resistant. It should be noted that the rotenone concentration and toxicity of the formulation (laboratory bioassay) were relatively constant within these periods.

Application of formulation DE in a pond

In the pond test, distressed fish were observed and collected every fifteen minutes after application of the formulation DE. The same concentration in ppm of the formulation was shown to work in the field test as in the laboratory test. The effect of the *Derris* extract on brackishwater fish in an earthen pond is shown in Table10. The trend as to the tolerance of the fish to the formulation agrees well with the order found in Table 9. In this case, the maximum number and weight for a specific species appeared in the first fifteen minutes of exposure for *X. maculatus* (kataba) and *M. cephalus* (aligasin), the next fifteen minutes to *O. mossambicus* (Mozambique tilapia) and *G. giurus* (biyang puti) and then next to *C. validus* (biyang lungga) in the last fifteen minutes.

Formulation application in larger ponds

It can be difficult to achieve an even distribution of rotenone for an effective fish kill in large ponds and it is also expensive to treat large volumes of water. For these reasons, the pond water surface area and volume had to be reduced as much as possible before application of the formulation. In large earthen ponds when water was reduced almost completely, the remaining water stays in puddles, canals or pools where measurement of area and volume of water was not feasible and practical. A simpler way without measuring the volumes of water

is to treat the small bodies of water by sprinkling them with the emulsion or mixture of formulation, and stirring the surface of the water for the toxin to spread. This was the case done in the following applications of the formulations to large fishponds using the different formulations prepared. Also, from the experience shared by fishpond owners, approximately 1 L of the insecticide Telothion 40 can be used to clean a 3- ha fishpond, it was assumed that 1 L of *Derris* extract had the equivalent toxicity as Telothion.

Table 11 shows the data gathered when each particular formulation was applied taking note of the formulation's toxicity compared to that of Telothion and applying the information for use by fishpond owners. Each of the fishponds had been previously harvested and thus required cleaning prior to restocking. This meant that the fishponds had been emptied before hand from the primary species such as bangos (*Chanos chanos,* Chanidae) and sugpo (*P. monodon,* Penaedae) and most of the secondary species such as the Nile tilapia (*O. niloticus*). The fish were collected within an hour. Collection of fish was done (if possible every 15 min) and was completed after about an hour. Additional dead fish were collected the day after. Small and distressed fish that cannot be caught by net were left to the predator birds like seagulls and herons that preyed on them during the night. All the applications were successful in that the fishpond owners who used them reported that their ponds were completely eradicated of unwanted fish. This was checked and verified about a week after the application.

Cost and return analysis

Cost of collection

The cost of collection of *D. trifoliata* roots was estimated to be P 6.25 kg^{-1}. This was deduced from a collection of four sacks of roots (13 kg•$sack^{-1}$) per day by a person hired at P300 day^{--1} using a motorized banca to reach and come back from the source with P 25 of gasoline as fuel. The cost of collection of *D. elliptica* and *Derris* "uwak" was estimated to be P 72 kg^{-1} root. This consisted of collecting the plant from the mountain site and bringing it down to the nearest accessible town. The main cost was transporting the plant from the mountain through rough roads by a motorized tricycle.

Cost of debarking

The cost of debarking *D. trifoliata* was around P200 kg^{-1} of bark. This was the average cost when three different sets of people (a family of five, fishpond laborers, and out of school youths) were hired. *Derris elliptica* and *Derris* "uwak" were not debarked but simply cut into small pieces as they already contain a high concentration of rotenone. The work cost only around P300 kg^{-1} for the whole 17 – 19 kg root used in this formulation.

Cost of extraction

The cost of extraction depended largely on the cost of one carbouy (~20 L) of acetone (P 1,800 $carbouy^{-1}$) and soaking the root or root bark for five to six days at P 500 for the whole operation.

Cost of packaging

The brown bottle container (1 L) was purchased at P 13 $bottle^{-1}$ and the label at about P2.00 $piece^{-1}$ or a total of P 15 L^{-1}•$bottle^{-1}$. A 10 L formulation was then divided into 10 x 1 L bottle for a total cost of P 150.

The total cost of production of the formulation for each *Derris* plant root extract is shown on Table 12. The cost analysis of the process shown in the table is based on the data on formulation given in Table 5 for DT-3 (*D. trifoliata*), DE (*D. elliptica*) and DU (*Derris*

"uwak"). For example, since 15.55 kg of root bark of *D. trifoliata* was extracted and the root bark is 45% of the whole root, then 15.55 kg ÷ 0.45 = 34.56 kg of whole root was needed. This means that 34.56 kg x P 6.25 kg^{-1} = P 216 is the cost of collection. Since the cost of debarking is around P 200 kg^{-1}, the total cost of debarking 15.55 kg would be P3,110.00. The cost of extraction will depend largely on the cost of one carbouy of acetone and to a lesser extent to labor cost that totaled P 2,300. Since packaging cost was only P 150 for the 10 L extract, the whole production cost was P 5,776.00 for the *D. trifoliata* extract.

Table 5 shows that 1 ml of acetone extract from the formulation has the same or even greater toxicity than 1 ml of insecticidal Telothion. Based on this, it is safe to assume that 1 L of acetone extract would have comparable toxicity with 1 L of insecticidal Telothion. This meant that to become more profitable, formulations DE, and DU could still be diluted to have the same activity as the insecticide. This dilution should be at around 0.2 $mg \cdot ml^{-1}$ rotenone concentration, the same rotenone concentration of formulation DT-3 when it had comparable activity with 1ml Telothion. Considering the cost of the banned insecticide Telothion which is P 1,500 ($ 26.79) per liter, fishpond owners can economize if they use natural piscicide because one liter of *D. trifoliata* extract costs only P 577.60 or $ 10.31, one liter of *D. elliptica* costs only P 334.56 or $ 5.97, and one liter of *Derris* "uwak" costs only P 346.87 or $ 6.19.

Table 10. Application of formulation DE (*D. elliptica*) in a fishpond* in Paombong

Fish Collected	Number (Weight)			
	15 min	30 min	45 min	60 min
O. mossambicus				
Juvenile	9 (100g)	8 (170g)	5 (90g)	3 (60g)
L. plumbeus				
Fingerling	7 (<10g)	1 (<10g)	-	1 (<10g)
G. giurus				
Fingerling	2 (<10g)	-	-	-
Juvenile	1 (50g)	-	-	7 (50g)
Adult	-	8 (140g)	4 (150g)	-
X. maculatus				
Fingerling	195 (120g)	-	-	-
Juvenile	-	90 (50g)	22 (10g)	4 (<10g)
Adult		8 (30g)	-	-
M. cephalus				
Fingerling	10 (10g)	10 (30g)	-	2 (<10g)
C. validus				
Juvenile	-	14 (130g)	18 (180g)	15 (120g)
Mixed small fish	-	35(30g)	16(10g)	25(20g)

* Area: 14.0m x 7.10m; Average depth: 0.33m; Place: Bungalon, San Roque; Date: May 31, 2004; Time of application: 12:57 PM; Temp. of pond: 34^{o}C; Salinity: 7.540 ppt chloride; Volume of extract used: 1.640 L)

Table 11. Application of formulations in different fishponds

Fish species	Number of Affected Fish				
	Fishpond I*	Fishpond II*	Fishpond III*	Fishpond IV*	Fishpond V*
C. carpio					
Juveniles	1	1	-	-	-
Adults	-	-	15	-	-
E. hawaiensis					
Juveniles	-	-	-	-	66
G. giurus		Too many to			
Fingerlings	-	count(0.4kg)	-	-	-
Juveniles	-	-	-	33	5
L. plumbeus					
Fingerlings	12	-	-	-	-
Juveniles	-	-	-	4	-
Adults	-	-	-	25	-
M. cephalus					
Juveniles	1	-	45	-	-
M. cyprinoides					
Adults	-	-	4	-	-
O. mossambicus					
Fingerlings	-	35	-	-	36
Juveniles	-	42	-	25	-
O. niloticus					
Fingerlings	539	47	-	24	-
Juveniles	456	140	811	253	81
Adults	61	6	518	-	-
O. striatus					
Juveniles	2	3	504	-	-
Adults	6	-	3	-	-
X. maculatus					
Juveniles	-	-	-	-	310
Adults	237	-	-	-	-
Mixed small fish	Too many to count(5.3kg)	Too many to count(1.4kg)	Too many to count(4.8kg)	-	-

*Formulations Used: I = DT-1(EtOH), II = DT-6B, III = DT-3, IV = DE, V = DU

Table 12. Cost analysis of formulations from *D. trifoliata*, *D. elliptica*, and *Derris* "uwak"

Process	DT-3(*D. trifoliata*) Formulation	DE(*D. elliptica*) Formulation	DU(*Derris* "uwak") Formulation
Collection	P 216.00	P 1,234.80	P 1,382.40
Debarking	P 3,110.00	P 300.00	P 300.00
Extraction	P 2,300.00	P 2,300.00	P 2,300.00
Packaging	P 150.00	P 180.00	P 180.00
Total Cost	P5,776.00 per 10 L	P4,014.80 per 12 L	P 4,162.40 per 12L

Summary and Conclusion

Although the rotenone content of *D. trifoliata* roots is much too low compared with the other *Derris* species, root bark of *D. trifoliata* where rotenone was found to be concentrated can be used in the formulation. Acetone instead of ethanol should be used to maximize the rotenone extraction in preparing the formulation as rotenone is more soluble in such solvent. Relatively fresh root bark should be used to maximize the extraction and prevent loss from absorption of solvent by the dried root bark. If the *D. elliptica* and *Derris* "uwak" are present in the locality then they should be preferred over *D. trifoliata*. The rotenone concentration of the formulations from *D. elliptica* and *Derris* "uwak" are relatively high and their

formulations can still be diluted to meet just the correct toxicity to improve on cost. The formulation using *D. trifoliata* had a median lethal concentration (96 h) of about LC_{50} = 0.03 ppt while those *using D. elliptica* and "uwak" had LC_{50} = 0.005 ppt and LC_{50} = 0.009 ppt respectively, showing that the *D. trifoliata* formulation is about 3 to 6 times less toxic than the *D. elliptica* and *Derris* "uwak" formulations. The processed formulation, however, for *D. trifoliata* is enough to kill fish within 30 minutes comparable to the commercial insecticide used by fishpond farmers.

The tolerance of the different species of unwanted fish from the lowest to highest in brackish water as shown in Table 9 where fish sensitivity to the DE extract generally decrease from periods of high salinity in summer to periods of low salinity during rainy season.

When the formulation with bioactivity equivalent to Telothion in the laboratory experiment was used in a fishpond with measured volume of water (measured concentration of formulation), the trend as to the tolerance of fish (present) to the formulation agreed well with the order found in Table 9. All types of formulations (acetone or alcohol) of *D. trifoliata*, *D. elliptica*, and *Derris* "uwak" applied were successful in clearing the fishpond from unwanted fish. The formulation's toxicity compared to that of Telothion and its capacity to clear (1L/ 3ha) were used as basis in clearing larger ponds. The same method can be used with these formulations as when applying the synthetic insecticide. Considering the high cost of the banned insecticide Telothion, fishpond owners can economize if they use natural piscicide from *Derris* plants, more so if they are available in their vicinity (like *D. trifoliata*) or if they are cultivated near their fishponds.

Acknowledgment

We would like to acknowledge the generous funding from the Department of Agriculture – Bureau of Research and the Natural Sciences Research Institute of the University of the Philippines. This funding would have not been realized without the help of Dr. IreneVillasenor, research representative of the Institute of Chemistry, University of the Philippines at Diliman.

References

APHA, 1975. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Waterworks Association, Water Pollution Control Federation. 1975. 14th Edition, APHA Press, Washington, D.C. 1193 pp.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Waterworks Association, Water Environment Federation publication Office. 20th Edition, (ed. L. Clesceri, A. Greenberg, A. Eaton), APHA Press, Washington, D.C., 2005 –2605.

Dela Cruz, A., G. Gomez, H. Miles, G. Cajipe, and V. Chavez, 1984. Toxicants from Mangrove Plants. 1. Bioassay of Crude Extracts. International Journal of Ecology and Environmental Sciences 110:1–9.

Dubouzet, J. 1988. Characterization of Vegetative and Rotenoid Yield of Various Philippine Derris. M.S. Thesis, University of the Philippines at Los Banos, Los Banos, laguna, Philippines. 85 pp.

Gomez, E., A. de la Cruz, V. Chavez, H. Miles, D. Cajipe, 1986. Toxicants from Mangrove Plants: II. Toxicity of Aqueous Extracts to Fish. The Philippine Journal of Science 115:81.

Guerrero, R. III, L. A. Guerrero and L. O. Basmayor, 1990. Use of Derris Root Powder for the Control of Fish Competitors and Predators in Ponds. In: 21st Annual Meeting of the Pest Control Council of the Philippines. Bacolod City: May 7 – 10, 1990.

NIOSH. 1994. Manual of Analytical Methods. 4th edition (ed. P.C. Schlecht and P.F. O'Connor), DHHS National Institute for Occupational Safety and Health (NIOSH) Publication (August, 1994).

Quisumbing, E. 1947. Vegetable Poisons of the Philippines. Philippine Journal of Forestry. 5:145–172.

Tee, B. 1976. Analysis of Rotenone Content and Toxicity of the Powdered Roots of the Common Species of Derris in the Province of Cebu. M.S. Thesis, University of San Carlos, Cebu City, Philippines. 51 pp.

Tumanda, M. 1980. Effects of Rotenone Containing Derris Root Extract on the Mortality of Some Predator Fishes of Pond Culture Prawns under Different Water temperature – Salinity Combination. M.S. Thesis. University of San Carlos, Cebu City, Philippines. 89 pp.

Ward, G.S. and P.R. Parrish. 1982. Manual of methods in Aquatic Environment Research. Part 6. Toxicity Tests. FAO, Fish Tech. Pap. 185, 23 pp.