Piscicidal effects of *Pachyrhizus erosus* (yam bean) seed extracts

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Abstract

Pachyrhizus erosus (yam bean) seeds have been known to contain toxic compounds despite its high nutritional content. Along with the growth in the trend of plant-based pesticides and piscicides, this study was conducted to determine the effects of *Pachyrhizus erosus* seed extracts on *Chanos chanos* (milkfish) fingerlings to determine the probability of using it as an effective piscicide. The *Chanos chanos* fingerlings were subjected to the concentrations of 0.25 milligrams per liter (mg/L), 2.5 mg/L, 25 mg/L and were observed for 96 hours. The 1, 3, and 6-hour median Lethal Concentration (LC_{50}) values for the *Chanos chanos* fingerlings were calculated to be 2.03 mg/L, 0.25 mg/L, and 0.06 mg/L respectively. There were no calculated LC_{50} values in the 12 to 96-hour time range due to 100% mortality of the samples. Results showed a significant difference in the mortality rate of the fish subjected under said conditions at 95% confidence interval.

Keywords: *piscicidal effects, Chanos chanos fingerlings, Pachyrhizus erosus extracts, piscicide, LC_{50}*

Introduction. Plant-based insecticides, piscicides, and pesticides are gaining popularity in recent years as they are addressed to be better alternatives than chemical based due to their eco-friendliness, ease of availability, high efficiency, reduced toxicity to non-targeted animals, and rapid biodegradability [1]. The active substances of the majority of plant poisons are resin, tannin, rotenone and saponin [2].

The *Pachyrhizus erosus* (yam bean) plant is a leguminous plant cultivated in Southeast Asia [3] and has gained interest because of its nutritional content, agronomic yields selectivity, and low environmental hazard. The abundance of nutrients is found especially in its protein content as Abbas' [4] research quantified. Along with its high nutrient content comes a high toxicity content as reported by both Catteau [3] and Lautie [5]. This renders its high nutrient content invaluable. Rotenone, one of the active substances found in the seeds, along with resin, tannin, and saponin, is highly toxic to insects and fish but relatively non-toxic to plants and mammals [6, 7, 8, 9]. There are no reported effects of human consumers ingesting fish applied with rotenone [7]. Although it is less toxic to humans, ingesting a large amount of *Pachyrhizus erosus* seeds can lead to death. A Chinese-Taiwanese man died within two hours with respiratory failure after ingesting the seeds. The death was reported to be due to the seeds or rotenone toxicity, which is the most important toxic substance found in the seeds [10].

Many researches have tackled *Pachyrhizus erosus* seeds focusing more on the aspect of its rotenone content, with it being one of the most abundant compounds in the seeds [2]. There have already been many studies in rotenone's use as a piscicide and its effect on fish. Studies conducted by Cruz-Lacierda [11], Tobler [6], Rach [12] all have objectives to test this. They may be synthetic rotenone, similar to Cruz-Lacierda's [11] and Rach's [12], or be similar to Tobler's [6], which is rotenone derived from organic substances. Although there is still no study on *Pachyrhizus erosus* seeds as a piscicide, it has been tested as an insecticide. *Pachyrhizus erosus* seeds have also been quantified for their chemical contents, toxicity yields of extracts on different solvents, as well as the physicochemical characteristics [6] and properties degradation under different circumstances [3].

Using the data of its toxicity, as it has been used as an insecticide effectively with *Pachyrhizus erosus* seeds being most potent [13], it is a suitable piscicide candidate.

The use of piscicides has long since been practiced in the field of aquaculture. Rotenone, one of the main chemicals in *Pachyrhizus erosus* seeds has been known to be a selective piscicide. Such chemical has been used in eradicating unwanted fish, controlling fish, and mostly in pond preparation for the prevention of parasites and competition. This is why piscicides play a vital role in dealing with aquaculture [1].

About 98% of *Chanos chanos* (milkfish) production in the Philippines comes from aquaculture with only a very small amount from capture fisheries. Production from milkfish culture continued to increase contributing about 15% to the total aquaculture fish production of the country. From 2002 to 2011, production of milkfish in aquaculture grew at an average rate of about 3% [14], where production from brackish water fishponds was known to be the highest among the various production systems. Invasive species such as tilapia thrive in similar environments and water quality conditions with that of milkfish [15]. Therefore, this study deemed *Chanos chanos* fingerlings as a suitable subject, having easier to replicate commercialized conditions and considering the mortality of rotenone being used in pond preparations.

Studies were found bearing the toxicity amount on *Pachyrhizus erosus* seeds, but none showed data about it as a piscicide, including tests such as median Lethal Concentration (LC_{50}), and its effect on fish is still not known. Consequently, the toxicity could make *Pachyrhizus erosus* seeds a viable candidate for being a plant-based piscicide which could provide another use for the seeds aside from its general use which is for reproduction.

In the trend of the growing effectiveness of plant-based piscicides over synthetic ones, *Pachyrhizus erosus* seeds are an overlooked part of the plant because of its toxicity. This study aims to examine the seeds by comparing the effects of different concentrations of *Pachyrhizus erosus* seed extracts on *Chanos chanos* fingerlings.

The objective of this study was to determine the effects of the toxicity of *Pachyrhizus erosus* (yam bean) seed extracts on *Chanos chanos* (milkfish) fingerlings. It specifically aims

(i) To determine the mortality rate of *Chanos chanos* (milkfish) fingerlings from different concentrations (0.25 mg/L, 2.50 mg/L, 25 mg/L) of *Pachyrhizus erosus* (yam bean) seed extracts

(ii) To compare the mortality rate at 1, 3, 6, 12, 24, 48, and 96 hours exposure of *Chanos chanos* (milkfish) fingerlings from different concentrations of *Pachyrhizus erosus* (yam bean) seed extracts using Kruskal-Wallis test (H-test)

(iii) To calculate the median Lethal Concentration (LC_{50}) of *Pachyrhizus erosus* (yam bean) seed extracts on *Chanos chanos* (milkfish) fingerlings

This study was conducted primarily to observe the effects of the *Pachyrhizus erosus* seed extracts on the mortality of *Chanos chanos* fingerlings found in the Philippines and determine the median Lethal Concentration (LC_{50}) values. Extracts were only applied on already cultured *Chanos chanos* fingerlings. This study does not include the development of a piscicide but rather the possibility of using *Pachyrhizus erosus* seeds as a source of piscicide. Tests regarding the physicochemical properties of *Pachyrhizus erosus* seeds or the status of the *Chanos chanos* fingerlings aside from the mortality were not included in the objectives of this study.

Methods. This study determined the effects of *Pachyrhizus erosus* (yam bean) seed extracts as a piscicide on mortality of *Chanos chanos* (milkfish) fingerlings and observed the reactions of the fingerlings treated with extracts. Water quality parameters (salinity, pH, temperature, and dissolved oxygen) were timely monitored. The LC_{50} values of the seed extracts on the fingerlings were calculated.

Collection and Preparation of Fish Specimen. Acquisition of *Chanos chanos* fingerlings was done with Southeast Asian Fisheries and Development Center (SEAFDEC) Region VI's affiliated institution, Dumangas Brackishwater Station (DBS). The fingerlings were placed in a 20-liter tank with dimensions of 43.18 × 22.86 × 27.94 (l×w×h) centimeters. They were then set to acclimatize for 48 hours [8] under laboratory conditions. Water quality was maintained at a pH between 6 to 9, change in dissolved oxygen at 1 ppt to 3 ppt, and salinity at 0 ppt to 35 ppt [16]. The *Chanos chanos* fingerlings were fed with commercial fish pellets daily and starved for 24 hours prior to and during the experiment [11].

The water quality parameters (dissolved oxygen, pH, and temperature) were assessed using a PASCO PS-2230 water quality sensor, with an accuracy of ±0.6 mg/L right out of the box, provided by Philippine Science High School-Western Visayas Campus. Salinity was measured using an Original Equipment Manufacturer (OEM) brackish water salinity refractometer. The tests were done before and after the addition of extracts. Water quality was checked at 0, 1, 3, 6, 12, 24, 48, and 96 hours after application of extracts.

Preparation of Chemicals. Supply of materials, glasswares, equipment and chemicals were borrowed from PSHS-WVC laboratory and bought at Patagonian Enterprises.

Collection of Plant specimens. Acquisition of pre-identified *Pachyrhizus erosus* (yam bean) seeds were done with Tigbauan Local Government Unit (LGU) as referred by the Department of Agriculture (DA) - Region VI. The seeds were sun dried prior to acquisition.

Pulverization of Seeds. The sun-dried *Pachyrhizus erosus* seeds were cleaned by hand to remove extraneous matter. The *Pachyrhizus erosus* seeds were then washed with distilled water and air-dried [13, 9]. After air drying, the seeds were pulverized with the use of herb grinder. The powdered seeds were then stored in a sealed container at room temperature until extraction.

Extraction. The powdered seeds, weighing 50 grams in total, were loaded into the thimble, with 500 mL of 95% acetone. A solvent-solid ratio of 10 mL/g was used [9]. They were then heated for 8 hours at 60°C to 65°C using a water bath [17]. The extract was concentrated using a rotary evaporator with the water bath at 30°C to 40°C on constant pressure for solvent evaporation. The extracts were then stored at 18°C for two days.

Application of Extracts. The concentrated extracts were diluted with distilled water to obtain the desired concentrations of 0.25 mg/L, 2.50 mg/L, 25 mg/L. Ten fingerlings each were exposed to the said concentrations for 96 hours [18]. Water quality parameters and mortality were checked after 1, 3, 6, 12, 24, 48, and 96 hours of exposure [11].

Data Analysis. The Kruskal-Wallis test (H-test) was used in comparing the mortality of the *Chanos chanos* fingerlings at the given concentrations. Median Lethal Concentration (LC_{50}) was computed using Probit analysis.

Safety Procedure. The fingerlings were allowed to acclimatize to laboratory conditions for 48 hours and fed commercial fish pellets daily until a day before the experiment. Dead fingerlings were

immediately removed from the tanks while the remaining live fingerlings were euthanized after the 96-hour mark. Euthanasia was done by surrounding the container with the remaining live *Chanos chanos* fingerlings with ice [19, 20]. The dead fingerlings were then buried. Chemicals were handled and disposed accordingly to the Guidelines for the Safe handling and disposal of chemicals used in the illicit manufacture of drugs [21] with the MSDS of each chemical taken into consideration.

Results and Discussion. This study used *Pachyrhizus erosus* (yam bean) seed extracts to determine its effect and toxicity on *Chanos chanos* (milkfish) fingerlings. The fingerlings were exposed to 0.25 mg/L, 2.5 mg/L, and 25 mg/L concentrations of *Pachyrhizus erosus* seed extracts for 96 hours. The mortality and water quality parameters (temperature, dissolved oxygen, salinity and pH) were recorded and monitored at 0, 1, 3, 6, 12, 24, 48, and 96 hours. Kruskal-Wallis Test (H test) was used to determine if there is a significant difference between mortality at the given concentrations, and Probit analysis was used for the determination of LC_{50} values at different time exposure.

Mortality Rate. The mean mortality rates of the fingerlings vary at different time intervals and concentrations except for the control where no mortality was observed throughout the duration of the experiment. Additionally, the 25 mg/L concentrations showed a 100% mortality rate at 1-hour exposure to extracts.

Table 1. The mean and standard deviation of the mortality rate of *Chanos chanos* fingerlings at different time exposure.

Exposure (h)	Mortality rate (%)			
	*Control	**0.25 mg/L	**2.50 mg/L	**25.00 mg/L
1	0±0	0±0	35±15	100±0
3	0±0	35±5	100±0	100±0
6	0±0	80±10	100±0	100±0
12	0±0	100±0	100±0	100±0
24	0±0	100±0	100±0	100±0
48	0±0	100±0	100±0	100±0
96	0±0	100±0	100±0	100±0

*mean and standard deviation for 1 replicate
**mean and standard deviation for 2 replicates

Upon application of the highest concentration of extracts, the fingerlings immediately reacted with behavioral abnormalities, including erratic swimming and later on, loss of balance. The other fingerlings that were exposed to 0.25 mg/L and 2.5 mg/L of extracts, did not readily react upon application. However, after several minutes some of the fish start to show behavioral abnormalities similar to the set-up with the highest concentration. The recorded mortalities were directly proportional to the extract concentration and exposure period. Observations in the said investigations are indications that mortality of the fish may not only be due to impaired metabolism but could be due to nervous disorder as earlier reported by Akinwande et al. [22].

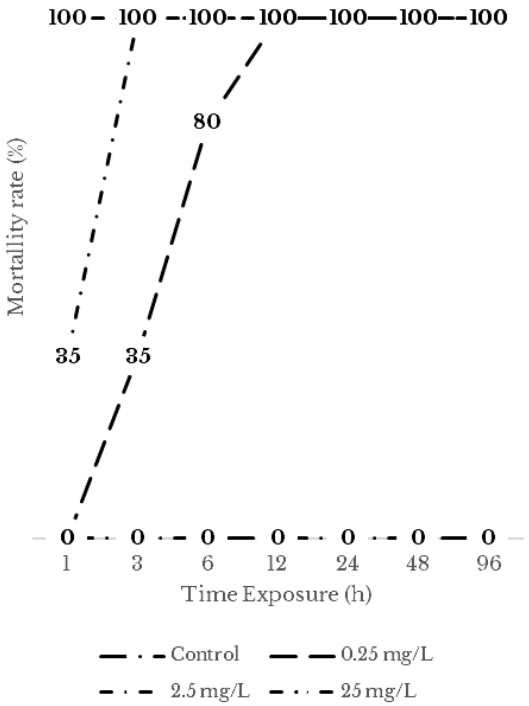

Figure 1. Mean mortality rates of *Chanos chanos* fingerlings at different concentration under different time-exposure.

According to Ade-Serrano [23] and Adewole et.al [24], fish treated with 25 mg/L react by rapid swimming, loss of balance and swimming at the surface. This behavior was reflected by increased opercular ventilation with increased concentration of extracts. Changes in the opercular movement of the fish also indicate physiological stress to the fish due to pollutants that cause decrease in oxygen uptake or transport [11].

The stressful breathing observed on the fish may be as a result of respiratory inhibition due to effect of rotinine, a fuming biocide, on the gills [22]. Narongchai et.al. [10] reported that other toxic compounds found in *Pachyrhizus erosus* seeds that may affect the behavior of fish are pachyrrhizine, pachyrrhizione, 12-(A)-hydropachy-rrhizone, dolinone, dehydro-pachyrrhizine, erosone, neodehydrorautenone, erosenone, erosenin, 12 -(A)-hydroxylinenone, Pachysaponin A&B as well as rotenone [1]. Furthermore, the respiratory inhibition may be the cause for the recorded mortalities which was shown to be significantly different ($p>0.05$).

Kruskal-Wallis Test. Test for Independent Sample Kruskal-Wallis test was used in Statistical Package for the Social Sciences (SPSS). Results were already

run through post-hoc by the program which provided the decision to reject the null hypothesis.

The H-test value, computed to be 0.021, was lower than α=0.05 (refer to Table 2). Therefore, there is enough evidence to reject the null hypothesis. It can be concluded that there is a significant difference in mortality rate at different concentrations of the seed extract.

Table 2. Hypothesis test summary with a significance level of 0.05.

Null Hypothesis	Test	Sig.	Decision
There is no significant difference in mortality rate at different concentrations	Independent Samples Kruskal-Wallis Test	.021	Reject the null hypothes-is

LC_{50}. The LC_{50} values were computed using SPSS Probit analysis. Upper and lower bound values under a 95% confidence interval did not show when inputted in SPSS.

Table 3. LC_{50} values of the extracts on *Chanos chanos* fingerlings.

Exposure Time (h)	LC_{50}	95% CI	
		Lower	Upper
1	2.03	~	~
3	0.25	~	~
6	0.06	~	~
12	ND	ND	ND
24	ND	ND	ND
48	ND	ND	ND
96	ND	ND	ND

~ -Could not be calculated due to high mortality
ND - No data due to 100% mortality

Results showed that the LC_{50} values of the experiment varied with time. It decreased as exposure time increased until the 6th hour mark. No data was collected by the 12th hour due to samples approaching 100% mortality, while the lower and upper bounds could not be calculated because of high mortality (refer to Table 3). The data coincides with other articles, since the longer the exposure of the subjects, the less concentration would be needed to kill half of the samples [11].

Error Analysis. Table 4 shows that mean temperature varied from 28.21°C to 28.44°C, dissolved oxygen from 5.71 mg/L to 6.24 mg/L, salinity from 34.76 parts per thousand (ppt) to 35.86, values that indicate brackishwater range, while pH ranged from 7.82 to 8.00 for all the concentrations. These values indicate normal environment conditions suitable for *Chanos chanos* fingerlings according to the standards used by Barker et al [16].

Normal and good conditions for fish should have maintained dissolved oxygen levels at a saturation of at least 5 mg/L with pH ranging normally between pH 6.5 to 9.0 and temperature range for growth is between 24°C to 32°C.

Table 4. The mean and standard deviation values of physicochemical parameters of the various concentration of *Pachyrhizus erosus* seed extracts on *Chanos chanos* fingerlings.

Parameters	*Cont-rol	**0.25 mg/L	**2.5 mg/L	**25 mg/L
Mean temperature (°C)	28.44 ±0.11	28.31 ±0.14	28.33 ±0.16	28.33 ±0.15
Mean Dissolved oxygen (mg/L)	06.23 ±0.20	05.71 ±0.44	06.24 ±0.35	05.74 ±0.51
Salinity (ppt)	35.86 ±0.9	34.76 ±0.62	34.86 ±1.01	35.05 ±1.02
pH	08.00 ±0.20	07.85 ±0.18	07.86 ±0.29	07.82 ±0.19

* mean and standard deviation for 1 replicate
** mean and standard deviation for 3 replicates

Although the monitored water quality parameters are found to be suitable and normal for the fingerlings, it was noted that at 48-hour exposure, there was a sudden change in the dissolved oxygen and pH values from 5.33 mg/L to 4.56 mg/L and 8.0 to 7.7, respectively in the tank containing 0.25 mg/L of extract. The values of the replicates were calculated and then subjected to the Q-test for outliers. The values were still found to be in the acceptable range and retained for use of the experiment.

Conclusion. The water quality values indicate normal conditions for *Chanos chanos* fingerlings. Therefore, water quality parameters did not attribute to the death of the *Chanos chanos* fingerlings. LC_{50} values at the three concentrations were 2.03 mg/L, 0.25 mg/L, and 0.06 mg/L at 1, 3, 6, and 12 hours respectively. No data was calculated in the later hours due to 100% mortality. This proposes a template for probable concentrations for future studies. There is also a significant difference in the mortality rate of the fingerlings when exposed to different concentration of *Pachyrhizus erosus* seed extracts.

Results suggest that *Pachyrhizus erosus* seeds extract can be a probable ingredient in creating piscicides. This study may be used in pond preparation to eradicate invasive species. It may serve as basis for future studies on the effects of *Pachyrhizus erosus* seed extracts on non-mammals.

Recommendations. It is recommended to increase the number of control setups in order for the data to be more precise. Larger sample sizes are also recommended for the samples to approach normality. This can make the gathered data more

accurate in regard to the population along with minimizing the errors that could occur during the span of the experiment. Regulated lab conditions during the conduct of the experiment are needed to avoid extraneous factors that could affect the data. Water quality must be monitored closely to ensure that the fingerlings are not significantly affected by factors other than the extracts.

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