Molluscicidal and Piscicidal Activities of Extracts of Castor (Ricinus communis) Bean for Aquaculture Management

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Abstract

The study assessed the molluscicidal and piscicidal activities of castor plant Ricinus communis using its fruit (dried and fresh) against large and small golden apple Snails (GAS) and mosquito fish. We focused on the laboratory determination of lethal concentrations LC$_{50}$ and LC$_{100}$ through a static bioassay test. Separate experiments were performed for GAS and mosquito fish and ten experimental animals where stocked in each experimental unit. Based on a 24-hour lethal concentration (LC$_{100}$), the toxicity for large GAS was 96.21 ml/L for fresh fruits, 124.02 ml/L for dried beans. For small GAS, the toxic concentrations were 91.75 ml/L for dried beans, and 105.89 ml/L for fresh fruits. For the 24-h LC$_{50}$, the toxicity to large GAS of the two extracts were 47.05 ml/L for dried beans, and 39.28 ml/L for fresh fruits, and for small GAS they were 44.87 ml/L for dried beans, and 51.17 ml/L for fresh fruit. The lethal concentration LC$_{100}$ for mosquito fish (Gambusia affinis Baird and Gerard) was 2.08 ml/L for fresh extract and 1.71 ml/L for dried extract, while LC$_{50}$ on the 24-hour basis was 0.88 ml/L for fresh extract, and 0.35 ml/L for dried extract.

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Introduction

The presence of unwanted organisms such as snails and mosquito fish in tilapia aquaculture ponds is a serious problem. These organisms adversely affect the cultured fish population by sharing food and habitat thereby causing losses to fish farmers. The method of eradication of these unwanted organisms in culture ponds, however, must be considered before introducing unwanted toxicants to the environment.

The golden apple snail, *Pomacea canaliculata* (Lamarck, 1822), is native to South America as well as to the Philippines. It was introduced from Argentina to Taiwan in 1980’s (Mochida, 1991) and was later widely distributed in Asia as a dietary protein supplement and source of income in rural areas (Matienzo, 1984; Anderson, 1993). However, due to its high level of ability to reproduce it became both a major pest in irrigated rice farms in Cagayan Valley, and later a national nuisance (Dela Cruz et al., 2000).

In the Philippines in 1989, approximately 400,000 ha. were infested. Of 3 million ha. of rice fields in the Philippines, 1.2-1.6 million ha. were infested with golden apple snail. In the Philippines in 1990, a significant amount of money was spent to control this pest. Pesticides were chosen arbitrarily, and applied indiscriminately causing environmental pollution. In addition the pesticides were a hazard to the general health of farmers (Anderson, 1993). Several control techniques including biological (Halwart, 1994; Teo, 2001), cultural (Teo, 2003), and chemical (Litsinger and Estano 1993; Palis et al., 1996), have since been developed.

Chemical control by molluscicides and piscicides has been carried out using different synthetic compounds (Tantawy, 2002; Essawy et al., 2009; Kristoff et al., 2010). However, the high cost of chemical toxicants, and the possibility of developing resistance to these products by non-target organisms has spurred the search for alternative use of plant extracts such as herbal molluscicides and piscicides.

Environmentally safe plant extracts are improved alternatives for these harmful synthetic toxicants. This study aims at evaluating the molluscidal and piscidical activities of *Ricinus communis* (using its fresh fruit and dried beans) to deal with the problems of the golden apple snail *Pomacea canaliculata*, and mosquito fish *Gambusia affinis*.

Materials and Methods

*Test snail.* Golden apple snails (GAS) *Pomacea canaliculata*, were obtained from the rice fields of Rang-ayan, Roxas, Isabela. The snails were sorted by size into two groups: small (1.85 g ± 0.56 SE), and large (5.34 g± 2.18 SE). The snails were acclimatized for 3 days prior to the bioassay.

*Test fish.* Mosquito fish *Gambusia affinis*, were collected from the Gideon Farm ponds at Matusalem, Roxas, Isabela. The experimental fish were graded using a number 24 fish grader, and acclimated for 3 days under laboratory conditions at the Provincial Institute of Fisheries.

*Test plant.* The beans of *Ricinus communis* were collected at Bantug, Roxas, Isabela. Processing of the plant materials was carried out immediately after collection of the beans to ensure freshness. Some beans were air-dried for 4 days and were later used for extraction of toxicants. Fresh plant material was weighed using a digital weighing scale and then processed in a food blender. Tap water was added to the beans before grinding. The ratio of plant material to the volume of freshwater was 1:1 or 100 g of beans was added to 100 ml of distilled water. The extract and solid plant materials were separated using cheesecloth.

*Test Concentrations.* Test concentrations used for the plant were predetermined using a range finding test based on the progressive bisection of intervals on a logarithmic scale. A range of concentrations of fresh and dried beans used to test both the snails and fish were 10, 20, 30, 40, 60 and 100 ml/L.
The final bioassay tests were carried out in a 50 L capacity plastic container at room temperature. There were three replicates of different concentrations of fresh and dried beans (0, 1.56, 3.12, 6.25, 12.5, 25, 50 ml/L) for snails, and (0, 0.3, 0.5, 1.25, 2.5, 3, 4, 5 ml/L) for fish.

**Bioassay.** The static bioassay procedure followed the standard methods prescribed by APHA, AWWA and WPCF (1980) with some modifications. The concentrations for each treatment were prepared in a container, and then the test organisms were introduced. Observations were made at 1, 2, 3, 6, 12, 24, 36, 48, 60, 72 and 96 hours for snail and fish mortality and behavior. Dead snails and fish were removed immediately. Separate experimental conditions and containers were used for both the fresh and dried beans in the snail and fish experiments.

**Statistics.** Lethal concentrations (LC$_{50}$ and LC$_{100}$) of fresh and dried beans were determined by plotting concentrations of the bean extract against snail and fish mortality every 24 h to 96 h. Interpolation between two concentrations where the mortality occurred was carried out.

Linear regression equations derived from trendline analysis on Microsoft Excel were used to estimate LC$_{50}$ and LC$_{100}$ of the fresh and dried bean extracts against *P. canaliculata* and *G. affinis*, and were also subjected to one-way analysis of variance (ANOVA).

### Results

Test snails stocked in higher concentrations of bean extracts exhibited retraction to permanent closure of the operculum when prodded. A few hours after stocking, almost all the snails settled at the bottom and did not rise from the bottom of the tanks. A thin film-like covering formed on top of the water in the tanks preventing oxygen from penetrating into the water therefore the snails died due to lack of oxygen. The toxicity of different preparations of *R. communis* bean (fresh and dried), against GAS (small and large), was time and dose dependent (Table 1).

<table>
<thead>
<tr>
<th>Time elapsed (h)</th>
<th>Lethal concentrations (ml/L)</th>
<th>Small snails</th>
<th>Large snails</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh beans</td>
<td>Dried beans</td>
<td>Fresh beans</td>
</tr>
<tr>
<td>24</td>
<td>LC$_{50}$</td>
<td>51.17</td>
<td>44.87</td>
</tr>
<tr>
<td></td>
<td>LC$_{100}$</td>
<td>105.89</td>
<td>91.75</td>
</tr>
<tr>
<td>48</td>
<td>LC$_{50}$</td>
<td>27.85</td>
<td>24.10</td>
</tr>
<tr>
<td></td>
<td>LC$_{100}$</td>
<td>79.55</td>
<td>61.11</td>
</tr>
<tr>
<td>72</td>
<td>LC$_{50}$</td>
<td>10.69</td>
<td>3.95</td>
</tr>
<tr>
<td></td>
<td>LC$_{100}$</td>
<td>36.89</td>
<td>41.07</td>
</tr>
<tr>
<td>96</td>
<td>LC$_{50}$</td>
<td>7.54</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>LC$_{100}$</td>
<td>15.86</td>
<td>2.60</td>
</tr>
</tbody>
</table>

The 24-h LC$_{50}$ of fresh bean aqueous extract against small and large GAS was 51.17 ml/L and 39.28 ml/L, respectively. Dried beans were also toxic to both small (44.87 ml/L) and large (47.05 ml/L) GAS. The extracts of both fresh fruit and dried beans were toxic (96-h LC$_{100}$: 2.60 ml/L) to large snails. There was a difference between the effect of fresh beans (96-h LC$_{100}$: 15.86 ml/L), and dried beans (2.60 ml/L), against small GAS. In two-way ANOVA, the effect on large snails (28.71 hr) was significantly lower compared to small snails (67.50 hr). No significant differences were found between dried (39.64 hr) and fresh beans (58.19 hr) (Table 2).
Shekhawat and Vijayvergia

dependent with decreasing concentration

water

snail bodies

be due to the uptake of the active compounds which progressively increase in

fish depending on the concentration

occurring lectin

1

The piscicidal activity of *R. communis* bean extract is more pronounced than on

GAS (Table 3).

Table 3. Lethal concentration (LC$_{50}$ and LC$_{100}$) values (ml/L) of the fresh and dried bean

extracts of *R. communis* against *G. affinis* at different exposure times.

<table>
<thead>
<tr>
<th>Time elapsed (h)</th>
<th>LC$_{50}$ Fresh beans</th>
<th>LC$_{50}$ Dried beans</th>
<th>LC$_{100}$ Fresh beans</th>
<th>LC$_{100}$ Dried beans</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.88</td>
<td>0.35</td>
<td>2.08</td>
<td>1.71</td>
</tr>
<tr>
<td>48</td>
<td>0.31</td>
<td>0.46</td>
<td>0.65</td>
<td>0.92</td>
</tr>
<tr>
<td>72</td>
<td>0.23</td>
<td>0.23</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>96</td>
<td>0.18</td>
<td>0.18</td>
<td>0.42</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Stocks of *G. affinis* exhibited erratic swimming behavior, rapid opercular

movement, and lost their balance leading to mortality. After 24-h exposure to

fresh bean extract (LC$_{50}$: 0.88 ml/L), the fish exhibited rapid opercular movement.

The same was true with dried beans (LC$_{50}$: 0.35 ml/L). For both fresh fruit and

dried bean extract (96-h LC$_{100}$: 0.42 ml/L), fish exhibited erratic swimming

behavior and settled at the bottom leading to mortality. In one-way ANOVA (Table

4), for the treatment 0.3 ml/L (4.11h) there was a significant difference between

treatments 2.5 ml/L (1.84h), while among treatments 0.5 (3.39h), 1.25 (2.60h),

2.25 (1.84h), and 3.0 (1.43h) there was no significant difference. A direct

comparison of lethal concentration values suggests that dried castor bean extract

has higher value of LC$_{50}$ and LC$_{100}$ than fresh bean extract.

Table 4. LT$_{50}$ of different concentrations of fresh and dried castor bean.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.3</th>
<th>0.5</th>
<th>12.5</th>
<th>2.5</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT$_{50}$</td>
<td>4.11</td>
<td>3.39</td>
<td>2.60</td>
<td>1.84</td>
<td>1.43</td>
<td>1.06</td>
<td>0.96</td>
</tr>
</tbody>
</table>

The same main factor with different superscripts are significantly different (p≤0.05).

1Treatment: Different concentration; 2 LT$_{50}$: Median lethal time

Discussion

The toxic effect of pressed *R. communis* seeds may be due to naturally

occurring lectin (ricin). It enters through the operculum of snails and gills of fish,

and interferes with their respiration. Later on, it may be transported by the blood

to other organs. This process may cause lethal or sub-lethal effects in snails or

fish depending on the concentration. Toxic effects of these botanical products may

be due to the uptake of the active compounds which progressively increase in the

snail bodies as exposure increases (Jaiswal et al., 2008).

This study revealed unusual behavior in snails when exposed to the toxicant.

With higher concentrations, the snails withdrew and did not emerge from the

water. The behavioral response of the test organisms was observed to be dose

dependent with decreasing concentrations. This observation is in agreement with

Shekhawat and Vijayvergia (2010), where snails were exposed to different

concentrations of ethanolic extract of *Eclipta alba* for different durations. The
snails also tried to escape from the solution by crawling out of the container and some were unable to attach to the substrate. Other stocks exhibited excessive production of mucus and inflammation of the body (Salawu and Odaibo, 2011). These effects were more noticeable with higher concentrations of the plant extracts. General distress syndromes observed resulted from exposure of the snails at various stages to the plant extract (Harry et al., 1957). Water imbalance caused by the introduction of the plant extract creates anaerobic conditions that boost snail inactivity and cause its extrusion from the shell (Von et al., 1950). This has been suggested as a possible cause of mortality in snails (Clark and Appleton, 1996). Similar occurrences have been reported when snails were exposed to copper (Cheng and Sullivan, 1977). Swelling of the body has been suggested to interfere with respiration and subsequent death by suffocation (Osterberg, 1987).

Various forms of abnormal behaviors were observed in G. affinis when exposed to different concentrations of fresh and dried R. communis bean extracts. These included erratic swimming behavior, rapid opercular movement, settling at the bottom, gasping or trying to escape from the toxicants. Some of these behavioral responses have been reported in C. chanos and O. mossombicus exposed to rotenone (Cruz-Lacierda, 1993), fingerlings of C. gariepinus exposed to Datura innoxia root extract (Ayuba and Ofojekwu, 2002), O. niloticus exposed to different concentrations of cassava effluent (Wade et al., 2002), and Heteroclyrias hybrid fingerlings exposed to water extract of bark of Thevetia peruviana (Oti, 2003), the use of T. tetraperta and S. occidentale on C. gariepinus (Faiyo, 2005), and leaf extracts of N. oleander, C. sativum, D. alba, Adenophyllum spp., N. tabacum and R. communis to trash fish (Asraf et al., 2010), respectively.

Emission of strong foul odors from exposure to the test solution for 96h may be attributed to oxygen depletion. Stressed breathing exhibited by the fish may result from respiratory impairment due to effect of toxicants on the gills. The inability of the gill surface to actively carry out gaseous exchange might be responsible for the recorded mortalities. The mechanism by which these bean extracts cause snail and fish death is not exactly known and will require further research.

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