The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

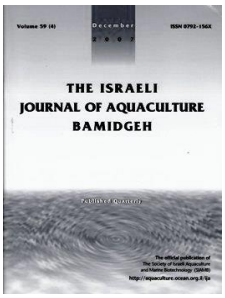
Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawai'i at Mānoa Library
&
University of Hawai'i at Mānoa Aquaculture Program
in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_67.2015.1177, 6 pages



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

Francis Nuestro Baleta,* Marites M. Ramos-Castro, May Anne S. Canceran

Department of Natural Sciences and Mathematics, College of Arts and Sciences, Isabela State University, Echague, Isabela, Philippines

(Received 24.12.14, Accepted 17.2.15)

Key words: Castor bean, molluscicide, piscicide, golden apple snail, mosquito fish

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.

* Corresponding author. e-mail: fnbaleta19@yahoo.com

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 Phillippines 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 molluscicidal and piscicidal 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 *Gambussia 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 *Gambussia 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 1. Lethal concentration (LC_{50} and LC_{100}) values (ml/L) of the fresh and dried bean extracts of *R.communis* against small and large *P. canaliculata* at different exposure times.

Time elapsed (h)	*Lethal concentrations*	*Small snails*		*Large snails*	
		Fresh beans	*Dried beans*	*Fresh beans*	*Dried beans*
24	LC_{50}	51.17	44.87	39.28	47.05
	LC_{100}	105.89	91.75	96.21	124.02
48	LC_{50}	27.85	24.10	24.82	14.92
	LC_{100}	79.55	61.11	66.66	74.87
72	LC_{50}	10.69	3.95	1.74	1.04
	LC_{100}	36.89	41.07	3.58	2.60
96	LC_{50}	7.54	1.04	1.04	1.04
	LC_{100}	15.86	2.60	2.60	2.60

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 (28.71hr) was significantly lower compared to small snails (67.50hr). No significant differences were found between dried (39.64hr) and fresh beans (58.19hr) (Table 2).

Table 2. LT_{50} of castor beans to Golden apple snail with interaction on their means.

Parameter	*Size*[1]		*Types*[2]		*S * T*[3] *Interaction (Pr>F)*	*M.S.E*
	S	**L**	**D**	**F**		
LT_{50}	67.50[a]	28.71[b]	39.64[a]	58.19[a]	0.16	$8x10^3$

Under same main factor, means (S.E) with dfferent superscript are significantly different ($p \leq 0.05$).
[1]*Size: S-small; L-large* [2]*Types: D-dried; F-fresh* [3]*Interaction between size and types*

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.

Time elapsed (h)	*LC_{50}*		*LC_{100}*	
	Fresh beans	*Dried beans*	*Fresh beans*	*Dried beans*
24	0.88	0.35	2.08	1.71
48	0.31	0.46	0.65	0.92
72	0.23	0.23	0.49	0.49
96	0.18	0.18	0.42	0.42

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.

Parameter *Treatment*[1] *(ml^{-1})*	*0.3*	*0.5*	*12.5*	*2.5*	*3.0*	*4.0*	*5.0*
LT_{50}[2]	4.11[a]	3.39[ab]	2.60[abc]	1.84[bc]	1.43[bc]	1.06[c]	0.96[c]

The same main factor with different superscripts are significantly different ($p \leq 0.05$)
[1]*Treatment: 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 innoxa* root extract (Ayuba and Ofojekwu, 2002), *O. niloticus* exposed to different concentrations of cassava effluent (Wade et al., 2002), and *Heteroclarias* hybrid fingerlings exposed to water extract of bark of *Thevetia peruviana* (Oti, 2003), the use of *T. tetraptera* and *S. occidentale* on *C. gariepinus* (Fafioye, 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.

Acknowledgements

The authors would like to thank the Provincial Institute of Fisheries, Isabela State University Roxas Campus, Roxas, Isabela, through the Chairman Prof. Isagani P. Angeles, Jr., for providing the facilities for this research.

References

Anderson B, 1993. The Phillippine Snail Disaster. *The Ecologist*, 23: 70-72.

Asraf M., Ayub M,. Sajjad T., Elahi N., Ali I. and Z. Ahmed, 2010. Replacement of Rotenone by locally grown herbal extracts. *Int J Agricult Biol*, 12: 77-80.

Ayuba V. and P. Ofojekwu, 2002. Acute toxicity of the root of Jimson's weed, Datura innoxia to the African catfish, *Clarias gariepinus* fingerlings. *J Aquacult Sci*, 17(2): 131-133.

Cheng T. and J. Sullivan, 1977. Alterations in the osmoregulation of the pulmonate gastropod *Biomphalaria glabrata* due to copper. *J Invertebr Pathol*, 29: 101-104.

Clark T. and C. Appleton, 1996. The physiological effects of aqueous suspensions of plant molluscicides on *Helisoma duryi* (Gastropoda: Planorbidae). *J Mollus Stud*, 62: 459-476.

Cruz-Lacierda E. R., 1992. Toxicity of rotenone to milkfish, *Chanos chanos*, and tilapia (*Oreochromis mossambicus*). In: Shariff M, Subasinghe R. and J. R. Arthur (eds.) *Dis Asian Aquacult* Fish Health Section, Asian Fisheries Society, Manila. 419-423 pp.

Dela Cruz, M. S., Joshi R. C. and E. C. Martin, 2000. Potential effects of commercial molluscicides used in controlling golden apple snail on the native snail, *Vivipara costata* (Qouy and Gaimard). *Philippine Entomologist*, 14 (2): 149-157.

Essawy A., Abdelmeguied N., Radwan M., Hamed S. and A. Hegazy, 2009. Neuropathological effect of carbamate molluscicides on the land snail *Eobania vermiculata*. *Cell Biol Toxicol*, 25: 275-290.

Fafioye O. O., 2005. Plants with piscicidal activities in Southwestern Nigeria. Turkish *J Fish Aquat Sci* 5: 91-97.

Halwart M., 1994. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. *Int J Pest Manage*, 40:199-206.

Harry H. W., Cumbie B. and J. Martinez De Jesus, 1957. Studies on the quality of freshwaters of Puerto Rico relative to the occurrence of *Australorbis glabratus*. *Am J Trop Med Hyg*, 6: 313-322.

Jaiswal P. and D. Singh, 2008. Molluscicidal activity of Carica papaya and Areca catechu against the freshwater snail *Lymnaea acuminata*. *Vet Parasitol*, 152: 264-270.

Kristoff G., Guerrero N. and A. Cochon, 2010. Inhibition of cholinesterases and carboxylesterases of two invertebrate species, *Biomphalaria glabrata* and *Lumbriculus variegates* by the carbamate pesticide carbaryl. *Aquatic Toxicol*, 96:115-123.

Litsinger J. A. and D. B. Estano, 1993. Management of the Golden Apple Snail *P. Canaliculata* (Lamarck) in rice. *Crop Protection*, 12: 363-370.

Matienzo L. H., 1984. Wilson Ang's big foot snails. *Greenfields*, 14: 24-29.

Osterberg R., 1987. Physiology and Pharmacology of Copper. In: Webb G (ed.), *The toxicology of Molluscicides* Pergamon Press, Oxford, 13-34 pp.

Oti E. E., 2003. Acute toxicity of water extracts of bark of the *Thevetia peruviana* to the African freshwater catfish "Heteroclarias" hybrid fingerling. *J Fisher Tech*., 2, 124-130.

Palis, F. V., Macatula R. F. and L. Browning, 1996. Niclosamide, an effective molluscicide for the golden apple snail (*Pomacea canaliculata* Lamarck) control in Philippine rice production systems. *British Crop Protection Council Symposium Proceedings,* 66: 213-230.

Salawu O. and A. Odaibo, 2011. The molluscicidal effects of *Hyptis suaveolens* on different stages of *Bulinus globosus* in the laboratory. *African J Biotechnol* 10(50):10241-10247.

Shekhawat N. and R. Vijayvergia, 2010. Molluscicidal activity of some Indian medicinal plants against the snail *Lymnaea acuminata* and in the control of Fascioliasis. *J Herbal Med Toxicol* 4:109–12.

Tantawy A. A., 2002. Effects of sublethal concentrations of *Atriplex halimus* (Chenopediaceae) on *Biomphalaria alexandrina*, the snail vector of *Schistosoma mansoni* in Egypt. *J Egypt Soc. Parasitol* 32: 297-305.

Teo S. S., 2001. Evaluation of different duck varieties for the control of the golden apple snail (*Pomacea canaliculata*) in transplanted and direct seeded rice. *Crop Protection.* 20: 599-604

Teo S. S., 2003. Aestivation behavoir of the Golden Apple Snail (*Pomacea canaliculata)* under glasshouse conditions. *Sabah Agricult Res J*, 1:75-80.

Von B. T., Baernstein H. and B. Mehlman, 1950. Studies on the anaerobic metabolism and aerobic carbohydrate consumption of some freshwater snails. *Biol Bull* 98: 266-276

Wade J. W., Omoregie, E. and Ezenwaka, 2002. Toxicity of cassava (*Manihot esculenta* CRANTS) effluent on the Nile Catfish *Clarias gariepinus* (l) under laboratory condition. *J Aquat Sci* 17: 2-34.