



Research article

The efficiency of molluscicidal plant extracts for controlling of golden apple snails (*Pomacea canaliculata*)

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ABSTRACT

Six representative molluscicidal plants have undergone experimentation and study as potential means of controlling golden apple snail populations. The technique employed in this study involved static aquatic toxicology conducted over a 48-hour period. Throughout the process, the snails were exposed to molluscicidal plants, and their mortality rates were recorded and calculated to determine the median concentration (LC₅₀). The plant exhibiting the lowest LC₅₀ value was regarded as the most efficient in terms of molluscicidal effect. The LC₅₀ values (measured in parts per million, ppm) for the crude water extracts of molluscicidal plants are as follows: *Thevetia peruviana* (334.39), *Calotropis gigantea* (148.0), *Lantana camara* (391.13), *Camellia oleifera* (6.21), *Hyptis suaveolens* (888.31), and *Erythrophleum succirubrum* (399.69). The Department of Agriculture of Thailand (DAT) has traditionally recommended the use of niclosamide as a chemical molluscicide. However, when considering the potential benefits, a natural product like plant-based molluscicide emerges as a superior option. Using a natural plant molluscicide is not only safer and more cost-effective but also carries a lower environmental impact compared to chemical alternatives. niclosamide was employed as the positive control in our study, demonstrating an LC₅₀ value of 0.42 ppm. Consequently, the Tea oil plant (*C. oleifera*) exhibited the highest molluscicidal effect, as evidenced by its LC₅₀ value. In practical application scenarios, it is noteworthy that the combined molluscicidal efficiency of *C. oleifera* and niclosamide decreased by 50% after 4.4 days and 4.2 days, respectively. Furthermore, both compounds underwent complete decomposition within a mere 15-day period. This underscores the potential of *C. oleifera* as a viable and environmentally friendly alternative for golden apple snail control.

Introduction

Pomacea canaliculata Lamarck (Gastropoda: Ampullariidae), commonly known as the golden apple snail (Figure 1) They are freshwater snail that belonged to the

Ampullariidae family and was discovered in 1810. The golden apple snail (*Pomacea canaliculata*) is a freshwater snail, it had been introduced to Thailand in the 1980s and spread throughout the entire country.

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Recently, they were reported as one of the most severe rice pests in Thailand. These snails thrive in humid tropical and subtropical environments, with their original habitat located in Africa and South America. They were introduced to Thailand in the 1980s, primarily as aquatic pets or for aquaculture purposes. They have expanded throughout the entire country after they were released to the natural later on. Recently, golden apple snails were reported as one of the most severe rice pests in Thailand (Litsinger & Estano, 1993; Cowie et al., 2006). Other plants have their poison dissolved in the sap or latex, including *Calotropis gigantean*, *Nerium oleander*, *Plumeria rubra*, *Euphorbia tirucalli*, and *Thevetia peruviana*. It could result in skin rashes, allergies, blindness, tingling or numbness in the mouth, diarrhea, unconsciousness, and even death when humans touched or drank it (Phumthum et al., 2019). Other plants with a strong scent that acts as an insecticide or repellent include *Hyptis suaveolens*, *Catharanthus roseus*, and *Chromolaena odorata* (Urumarudappa et al.,

2019). The Department of Agriculture of Thailand (DAT) recommended 4 methods to control the snails, first, the mechanical method, second, the biological control method, the third pesticide control method, and the last integrating method. On the contrary, the use of chemical molluscicide is the most preferred method for Thai farmers to control golden apple snails in the rice field. However, using chemicals will create many effects on human health, the natural environment, and the ecological system (Maneepitak & Cochard, 2014). This method will leave some chemical residuals in soils and anthropogenic substances in the aquatic organism. Alternatively, if using the plant molluscicide method with a plant whose have the characteristic of a pesticide that can kill mollusk which is specifically for snail and slug. Good molluscicidal plants ought to have some properties: high toxicity, specific performance, easy to use or prepare, and leave no residual to the environment. If it is possible to find the capability of plant molluscicide, it might be the best alternative way to control golden apple snails.



Figure 1 *Pomacea canaliculata*.
Source: Lamarck (1828)

There were 6 plants used as a representative to study the result of the molluscicidal property to control golden apple snails. Some plants of this group contained very high toxicity in humans and animals such as *Erythrophleum succirubrum*

and *Lantana camara*. If a human eats these plants in a small amount they will become unconscious, have anoxia, and even die (Pengeleig, 2002). Other plants such as *Calotropis gigantean* and *Thevetia peruviana* have their poison dissolved in sap or latex.

When people touched or ingested, it could be caused by skin irritation, blindness, numbness or tingling in the mouth, allergy, and even death (Faculty of Pharmacy, Mahidol University, 2010). Some other plants such as *Hyptis suaveolens* can produce a strong smell that gives a repellent effect (Medthai, 2014). There are also some reports about the toxicology in snails such as *Camelia oleifera*. (Litsinger & Estano, 1993). Anthropogenic or chemical substances, as well as sediments, can degrade and erode aquatic ecosystems. However, there is some positive news as both chemical molluscicides and plant molluscicides can undergo processes such as evaporation, decomposition, or degradation through exposure to sunlight. These procedures include hydrolysis, oxidation, photolysis, and evaporation in the presence of sunlight in the environment. Many factors may be involved, the intensity of solar radiation, the water transparency, the water surface area, and the water's natural body. The photo-transformation is limited and dependent on the radiation in sunlight for 14 and of sunlight exposes to the aquatic system (Federal Register, 2012). The following processes were permitted: photolysis, hydrolysis, oxidation, and evaporation of the environment utilising sunlight. The amount of solar radiation, the water's transparency, its surface area, and its natural body are just a few of the variables that could be at play. The amount of photo-transformation is constrained and is reliant on how much sunshine radiation is exposed to the aquatic system (Jia et al., 2019). The crude water extraction of *Schefflera actinophylla* (200 ppm) and niclosamide (1 ppm) solution can be degraded by sunlight. Their molluscicidal efficiencies were decreased by 50% when left in sunlight for 14 and 9 and completely disintegrated within 20 and 15 days respectively (Wongsiri, 2002). Compatible with Rambhare's findings, who examined the

effects of chlorpyrifos on the snail *Lymnaea stagnalis*: increased mucus secretion, decreased respiration, and inhibition of rates of motility and external stimulation (Rambhare et al., 2020).

The Probit analysis is a statistical analysis program, it can be a method to determine the relative toxicity of substances and test organisms. The values of concentration and mortality rate are reported as a sigmoid curve. Then use the Probit analysis program to transform the sigmoid curve into a linear curve by using a regression equation. Finally, the dose and response testing will be reported as the sub-lethal, median, and lethal concentrations (Wangsomnuk, 1997; Wangsomnuk et al., 2000).

Material and methods

Experimental animal

Adult snails of *P. canaliculata* measuring up to 3 to 5 cm in size will be collected from the rice field habitats located at Amphoe Muang, Khon Kaen. Snails were transferred to the animal's house. Fifty snails were acclimatized to the laboratory condition and fed with soft vegetables such as Chinese cabbage, lettuce, morning glory, etc. After 6 weeks, they lay their eggs and hatch within 7 to 10 days. The juvenile snails were very tiny and their sizes are around 0.1 mm in diameter. After feeding them for 6 months or their shell measurement around 2.5 - 3 cm in diameter, they were transforming into the mature stage and ready for experiments. During intoxicated snails, there should be an observation of animals' behavior.

Plant molluscicide preparation

Plant molluscicide is a plant that has characteristics of a pesticide that can kill mollusk, especially snails and slugs. In this experiment, the representatives of 6 poisonous plants were studied for their molluscicidal properties to control Golden apple snails: *T. peruviana*, *C. gigantea*, *L.*

camara, *C. oleifera*, *H. suaveolens*, and *E. succirubrum* (Figure 2). Mature leaves and soft stems of plants were collected from the natural condition at Amphoe Muang, Khon Kaen. Then they were dried in a hot air oven at 60°C and ground to a fine powder by an electric blender. Sieving plant powders by

the standard net and keeping them in the desiccator. The testing leaf powders were weighed depending on their suspected concentration, then they were suspended in the water for each concentration for a few hours before use.

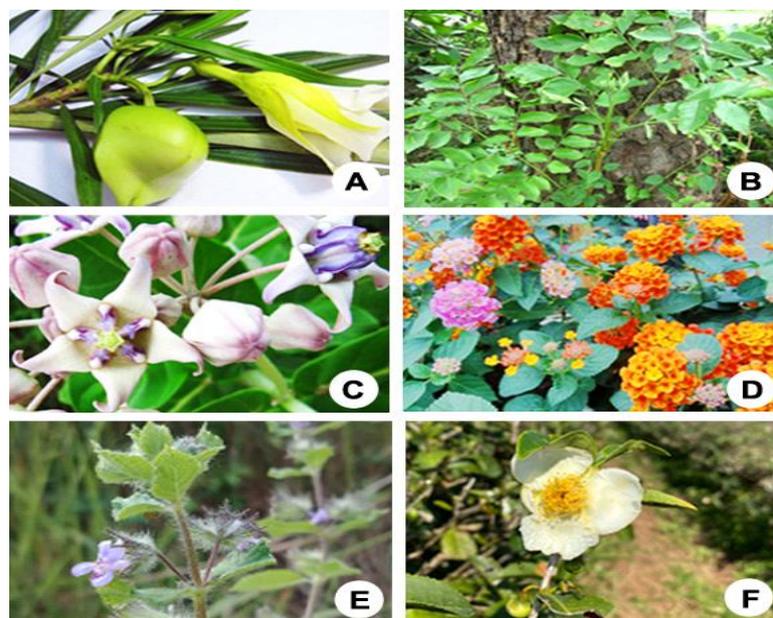


Figure 2 Six plant molluscicides; *Thevetia peruviana* (A), *Erythrophleum succirubrum* (B), *Calotropis gigantea* (C), *Lantana camara* (D), *Hyptis suaveolens* (E) and *Camellia oleifera* (F).

The toxicity test

This experiment was used the technique of static aquatic toxicology testing by using the acute toxicity test within 48 hours. The results will indicate the value of the sub-lethal concentration, the median lethal concentration, and the absolute lethal concentration. The snails were intoxicated with 5 liters in each dilution of plant extract at room temperature. Group of control and positive control were also tested in the same condition. After 48 hours, the treated snails were removed from the solutions, washed, and transferred to the new water for another 24 hours. Some snails might be recovered during this time. The number of dead and surviving snails was inspected and counted after (Jones & Lassiter, 2020). The snails were considered dead by their opened operculum and lost response when touching their foot (Balram, 2010). The preliminary screening: this test was used to widely find out the lethal and the

absolute lethal concentration of plant molluscicides within 48 hours. The definitive screening: this test was for finding out the definitive lethal concentration of plant molluscicides. The number of concentrations between sub-lethal and absolute lethal concentrations was defining distribution. For each concentration, 10 snails were treated in 5 replications then, their accumulated lethal rate was recorded. The positive control: niclosamide was used for the positive control group, and the snails were intoxicated as same as the method of plant molluscicides testing. The objective was to compare the molluscicidal efficiency of plant and chemical molluscicide. The control group: the snails were performed in pure water in the same condition as the experiment groups.

Photo-degrading test

The residual testing compared plant molluscicide and niclosamide when degraded by sunlight. The experiment was performed by preparing the lethal concentration solutions of *C.*

oleifera (30 ppm) and Niclosamide (1 ppm) in 5 liters per 8 treatments. Each treatment will be tested in 3 replications. Both solutions were radiated by sunlight for 8 times intervals i.e. 1, 3, 5, 7, 10, 15, 20, and 30 days. The 10 snails were intoxicated in each test; recorded their mortality rate for each time interval. The total accumulation of their mortality rate will be analyzed by the Probit analysis program at 95% confidence (Matsumura, 1982).

Statistical analysis

The mortality rate of the snails in each intoxicated group will be analyzed by Probit regression in the SPSS program at 95% confidence interval. The LC_{50} and LC_{90} values of each plant molluscicide will be calculated. The LC_{50} of all plant molluscicides will be compared. The plant which reveals the lowest LC_{50} value is considered to have the highest efficiency of molluscicidal effect. In addition, the correlation between lethal concentrations and mortality rates will be estimated from the slope function of a normal curve (Finny, 1952).

Results and Discussion

Experimental animal behavior

When snails were placed in the molluscicidal solution, they initially sank to the bottom of the chamber and then shut their operculum tightly to protect themselves from poisoning. After 15 minutes, however, they began to loosen up, crawling up to the surface of the water to extend their respiratory siphon and ingest air. They once more sank to the bottom, shut their operculum tightly, and secreted a huge amount of mucus. This is similar to Rambhare's earlier report on a study he conducted with the snail *Lymnaea stagnalis* in which chlorpyrifos stimulated mucus secretion, inhibited locomotion and external stimulation rates, and also decreased respiration (Jareankied et al, 2015). The snail died as a result of these copious and intense mucus secretions coming from almost every organ. Some of them had their operculum open at 48 hours and were dead, while the others had tightly closed operculum and might have survived if they had been moved to freshwater 24 hours earlier.

The toxicity

The mortality of each molluscicide was analyzed by the Probit analysis program at a 95% confidence interval. The results showed the value of sub-lethal concentration, median lethal concentration, and absolute lethal concentration (Table 1 and Figure 3 and 4).

The acute toxicity test within 48 hours of 6 crude water extracts of plant molluscicides with matured golden apple snail. All of the plants showed the molluscicidal effect. Considering the LC_{50} of each plant extract, *C. oleifera* revealed LC_{50} as 6.21 ppm. It was the lowest LC_{50} value among other plants in this experiment. Therefore *C. oleifera* was considered the highest-efficiency plant molluscicide in this group. Similarity to the report of Chunyapes revealed the absolute lethal concentration of *C. oleifera* to control *P. canaliculate* at 40 ppm (Litsinger & Estano, 1993). Comparison with, the LC_{50} of crude water extract of *C. oleifera* (6.21 ppm) showed higher efficiency than the LC_{50} of crude alcohol extract of *L. camara* (72.83 ppm) at 48 hours (Rambhare, 2020). When compared with the study by Jareankied et al. (2015) reported that the hot water or alcohol extraction of *Nicotina tabacum*, *Azadirachta indica*, *Sapindus emarginatus*, and *Durio zibithinus* can kill golden apple snails at the 500-2,000 ppm at 72 hours (Jareankied et al., 2015). Consequently, the water extract of *C. oleifera* revealed the highest molluscicidal effect than other plant extracts and easier preparation than alcohol or oil plant extraction. The study of the Niclosamide effect on matured golden apple snails showed the LC_{50} and absolute lethal concentrations as 0.42 and 1 ppm respectively. Compatible with the report of Chunyapes that study about niclosamide on *P. canaliculate* in the rice field revealed the LC_{50} at 24 hours as 0.62 ppm. (Litsinger & Estano, 1993). When considering LC_{50} of both experiments there show a slightly different; it might be depended on conditioning and surrounding of the experimental method. The rice field is another factor to determine the snail mortality such as high temperature, sunlight, soil absorption, approached time, etc. (Finny, 1952)

Table 1 The Lethal concentration of 6 molluscicidal plants and niclosamide

Common name (Thai)	Scientific name	Sub-lethal concentration (ppm)	Median concentration (ppm)	Lethal concentration (ppm)
Punchard	<i>Erythrophleum succirubrum</i>	50	399.69	1000
Rumploi	<i>Thevetia peruviana</i>	200	334.39	600
Ruk	<i>Calotropis gigantea</i>	10	148	500
Pakakong	<i>Lantana camara</i>	100	391.13	800
Manglukka	<i>Hyptis suaveolens</i>	500	888.31	1500
Cha	<i>Camellia oleifera</i>	4	6.21	30
Niclosamide		0.1	0.423	1.0

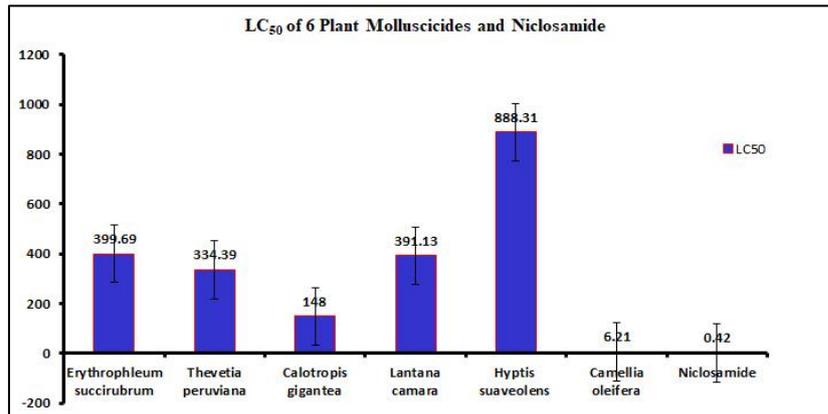


Figure 3 The Median concentration (LC₅₀) of 6 molluscicidal plants and niclosamide.

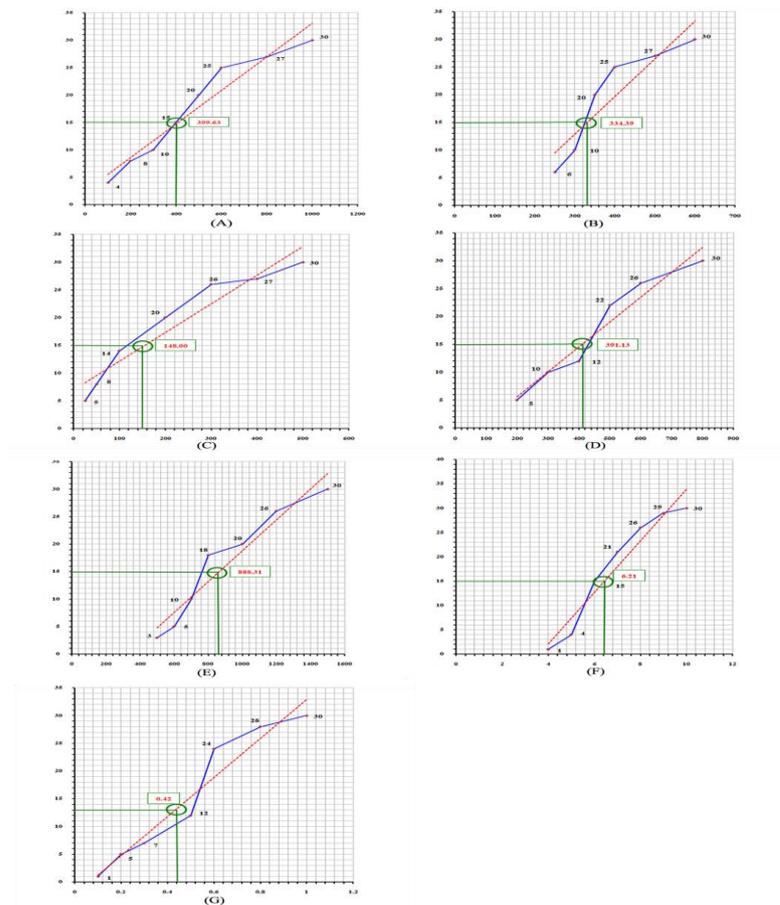


Figure 4 The Median concentration (LC₅₀) of *Erythrophleum succirubrum* (A), *Thevetia peruviana* (B), *Calotropis gigantea* (C), *Lantana camara* (D), *Hyptis suaveolens* (E), *Camellia oleifera* (F) and Niclosamide (G).

Photo-degradation

The photo-degradation by sunlight could be another method to be used as a representative for measuring the residual of molluscicidal substances. The mortality rate of snails was reduced directly proportional to the amount of time elapsing time (Wongsiri, 2002). *C. oleifera* at a concentration of 30 ppm and niclosamide at 1 ppm had a 50 % reduction in molluscicidal efficiency after 4.4 and 4.2 days, respectively, and completely degraded after 15 days (Figure 5). Compatible with the experiment of Chanyapes that used 3 molluscicidal plants and niclosamide to control golden apple snail in the rice field. They found that the efficiencies of molluscicides were directly proportional to the time decrease and most molluscicides were completely degraded in 20 days (Chanyapes et al., 2017). The results are consistent with The Committee on plant and animal pests reports that describe the slow decomposition of DDT in water by sunlight. As for the plant extracts, 90% of organic matter is decomposed by sunlight and 10 % is degraded by soil microorganisms. The LC₅₀ and LC₉₀ values of various chemical substances could change if it affected by physical factors such as sunlight, ultraviolet rays, winds, and temperature (Finny, 1952). Plant extractions are commonly known to affect the environment and nature. Finding information and experiments about the efficiency of plant molluscicides, the water extract of *C. oleifera* showed the highest capability to control

Pomacea canaliculata. The concentration that could completely eliminate snails was 10 ppm and the LC₅₀ is 6.21 ppm within 48 hours. This level of concentration has almost no effect impacted on farmers' health and almost no residual in the rice field's environment. Therefore, if the researchers promote their research or knowledge, it will give the greatest benefit to farmers, communities, and the country.

Consequently, conducting a photo-degradation study is of utmost importance as it can provide valuable insights into the half-life of plant or chemical molluscicides, which directly impact aquatic organisms, the environment, and farmers. Based on this dissertation, we hope that plant molluscicides can be further developed into an instant form, allowing farmers to use them as organic pesticides for effective control of the golden apple snail in the near future

Statistical analysis

The Probit analysis program was used for statistical analysis to determine the relative toxicity of substances and test organisms to get the data. Then transforms the data into the sigmoid curve and linear curve by using the regression equation in the excel program. The final step was to use the dose and response testing reported as the lethal concentration at each suspect level.

The results of the Probit analysis study of *C. oleifera* and Niclosamide were presented in Table 2.

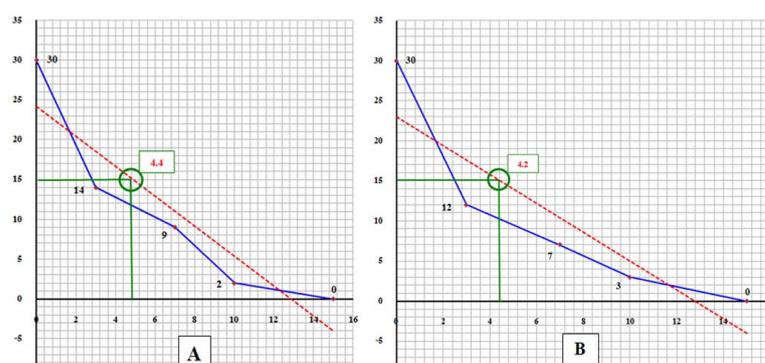


Figure 5 The photo-degrading testing of *Camellia oleifera* (A) and niclosamide (B).

Table 2 The lethal concentration in each expected value of *Camellia oleifera* and niclosamide

	Molluscicide	Sub-lethal Concentration	LC ₁₀	LC ₅₀	LC ₉₀	Lethal Concentration
4.1	<i>Camellia oleifera</i>	4	4.75	6.21	8.11	10
4.2	Niclosamide	0.1	0.17	0.42	0.82	1.0

Conclusion

The study aimed to evaluate the effectiveness of crude water extracts from plant molluscicides in controlling the golden apple snail using static aquatic toxicology within a 48-hour period. The results indicated that all six plant extracts exhibited molluscicidal effects against *Pomacea canaliculata* using the aforementioned technique. Among them, the crude water extract of *C. oleifera* demonstrated the highest efficacy as a plant molluscicide. The sub-lethal concentration was determined to be 4 ppm, the median-lethal concentration was 6.21 ppm, and the absolute-lethal concentration was 10 ppm. Niclosamide was used as the chemical molluscicide for the positive control. The acute toxicity test at 48 hours revealed a sub-lethal concentration of 0.10 ppm, a median-lethal concentration of 0.42 ppm, and an absolute-lethal concentration of 1.0 ppm. When assessing the combination of *C. oleifera* plant extract and niclosamide, their molluscicidal efficiency decreased by 50% after a period of 4 days and completely degraded within 15 days.

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Conflict of Interest: The authors declare no conflict of interest.

Samples Availability: Samples are available from authors.

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