

Chinese Spinach (*Amaranthus viridis* Linn.) as Natural Molluscicide Against the Hatchability of Golden Apple Snail (*Pomacea canaliculata* Lamarck) Eggs

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Abstract

Background: Golden apple snail (*P. canaliculata*) is an invasive pest which destroyed the wide range of agricultural crops, especially rice. It has been found to be a major pest resulting to losses in rice production. To control this pest easily farmers used synthetic molluscicides which can lead to several problems such as environmental contamination and health hazards. In view of this, some plants have been investigated for their intrinsic molluscicidal properties in an attempt to find an alternative pesticide which is not only safe and environmental friendly but also readily available and affordable. The development of the natural molluscicide would bring a healthier environment, safe to human and results to the increased food production. Hence, the study addresses the SDG 15, Life on Land and SDG 2, Zero Hunger. This study aims to determine the molluscicidal activity of chinese spinach (*A. viridis*) leaf extracts against the hatchability of *P. canaliculata* eggs.

Methods: Plant samples were prepared and extracted using expression extraction method. Different concentrations of molluscicides using the extracts of *A. viridis* were prepared. Four experimental treatments based on the *A. viridis* extract concentration (i.e. 25%, 50%, 75%, and 100%), one negative control (distilled water), and one positive control (commercially available synthetic molluscicide) were used in the study.

Results: Results showed that the leaf extracts of *A. viridis* altered the normal development *P. canaliculata* eggs.

Conclusion: The results demonstrate that *A. viridis* is a potential alternative pesticide that inhibit the hatchability of the golden apple snail and which is not only safe and environment friendly but also readily available and affordable that will surely benefit farmers.

Keywords: *Pomacea canaliculata* Lamarck, *Amaranthus viridis* Linn., anti-hatching, molluscicidal effect

1. INTRODUCTION

Pomacea canaliculata Lamarck is a well-known golden apple snail, which is originally from South America. It was introduced in Asia through the pet trade and as a potential food source for humans (Musman, 2010). It is known by an array of common names such as: golden miracle snail, golden snail, jumbo snail and Argentinian apple snail. It is also termed as *bisocol* in Ilocano and *golden kuhol* in Tagalog. The term “golden” refers not to their color, but to the amount of money the snail farmhands could make raising them. However, golden apple snail (GAS) is its frequently used common name. It is listed as one of the World’s Worst 2 Invasive Alien Species (IAS) as its invasion ability is based on unique morphological and biological characteristics that support their capacity to survive in adverse environmental conditions and still reproduce fast (Joshi, 2005).

Early 1980’s when the intention of its introduction in Asia has been considered for use as an aquaculture species that provide dietary high protein supplement for local consumption and as an income for the rural poor (Massaguni & Latip, 2012). The initial introduction is thought to have been from Argentina to Taiwan, but by 1982, the golden apple snail had been introduced from Taiwan to the Philippines and continued to China (1985), Korea (1986), Sarawak and Peninsular Malaysia, Malaysia (1987), Java and Sumatra, Indonesia (1989), Thailand (1989), Vietnam (1989) Hong Kong (1991), Laos (1992), and Cambodia (1995). Unfortunately, the low market value due to the unexpectedly poor consumer reception resulted in the elimination of its existence in Taiwan (Tangkoonboribun, 2009). Nevertheless, many snail-farming projects were abandoned and the golden apple snail escapes into irrigation ditches and the natural waterways, and subsequently it invaded the rice fields (Massaguni & Latip, 2012). Thus, the golden apple snail has been reported as major pest of paddy rice in all of these countries and the damage is clearly sufficiently serious to warrant major concern.

The rapid invasion of *P. canaliculata* across Asia during the last decades has been terrifying. The snails have been causing damage to rice production in Indonesia and other countries (Musman, 2010). In the Philippines, the proliferation of golden

apple snail (*Pomacea canaliculata* L.) invading almost all rice fields in the lowlands including rice terraces in the uplands is one of the serious problems of rice producers (Taguiling, 2015).

Pomacea canaliculata L. (golden apple snail) is an invasive pest that seriously affects rice cultivation causing one of serious agriculture problems in many Asian countries (Phuagphong, Nawanopparatsakul, Kitcharoe, & Chinpaisal, 2015). It was also reported by Massaguni & Latip (2011) that it could cause severe damage on food crop especially paddy, which is a staple food for more than 60% of the world population. As a result, farmers mostly rely on chemical and synthetic molluscicides to overcome this problem which could give negative impact on the environment. Nowadays, the dependency on synthetic chemicals has prompted the large scale synthesis of newer chemicals. Even though these synthetic pesticides valued for effectiveness and convenience but it also pose certain problems including phytotoxicity and toxicity to the non-target organisms, environmental degradation and health hazards to farmers (Girish & Shankaran, 2008).

Due to the adverse effects of the synthetic pesticides, the Organic Agriculture Act was developed and promoted in 2010. The Act declares to promote, propagate, develop further and implement the practice of organic agriculture in the Philippines that will cumulatively condition and enrich the fertility of the soil, increase farm productivity; reduce pollution and destruction of the environment, prevent the depletion of natural resources, further protect the health of farmers, consumers and the general public, and save program for the promotion of community-based organic agriculture systems which include, among others, farmer produced purely organic fertilizers such as compost, pesticides and other farm inputs, together with a nationwide educational and promotional campaign for their use and processing, as well as the adoption of organic agricultural system as a viable alternative shall be undertaken.

Concern with the negative impact of synthetic pesticide on environment and human health, numerous studies investigating botanical pesticides have been carried out in order to find alternative methods of pest control. In addition, the increasing amount of studies on plant-pest chemical interactions in the last few decades has unveiled the potential of utilizing botanical pesticides in the form of secondary plant metabolites as pest control agents. (Massaguni & Latip, 2015). Moreover, pesticides of plant origin are widely used because of high selective toxicity, biodegradable, safe for human and low cost (Singh et. al., 2004).

Chinese Spinach (*Amaranthus viridis* Linn.) belongs to family Amaranthaceae. It is possibly of Asian origin but now a cosmopolitan weed in the tropical and subtropical regions of the world, also ubiquitous faraway to temperate regions such as Europe, North America, Asia and Australia. In equatorial Africa it is also a profound and common weed. It is occasionally cultivated in Nigeria, Gabon and DR Congo (Ferdous et. al., 2015). In the Philippines the *A. viridis* L. is commonly known as “kolitis” and it can be eaten as a vegetable. Numerous studies reported that the *A. viridis* L. contains several compounds like amino acids, lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine etc. In search of new activities chemical entities, phytochemical screening of the extract from leaves of *A. viridis* L. indicates the presence of biologically active constituent: saponins, tannins and phenols, flavonoids, alkaloids, cardiac glycoside, steroid and triterpenoids. The plant was also found to have some chemical constituent that exhibits potent anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral actions. The purpose of this study is to develop environmentally friendly and safe molluscicide using the leaves of kolitis (*Amaranthus viridis* Linn.). Hence, this study to evaluate the molluscicidal activity of the kolitis leaf extracts against the eggs of golden apple snail (*Pomacea canaliculata* L.).

This study aimed to determine the molluscicidal activity of *Amaranthus viridis* Linn. extracts against the hatchability of golden apple snail (*Pomacea canaliculata* L.) eggs.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of the *Amaranthus viridis* Linn.

Fresh leaves of Chinese spinach (*Amaranthus viridis* Linn.) were collected and were submitted to an expert for its proper identification. The leaves were washed with running water to remove unwanted materials. It was then rinsed with distilled water. Fresh leaves were cut into small pieces using scissor. The juice was then squeezed out using mechanical juicer and the residue were also squeezed out using nylon cloth. The juice was filtered to obtain its pure extract. The plant extract was kept in the refrigerator to maintain its freshness. Different concentrations (75%, 50% and 25%) were prepared by diluting required amount from the pure plant extract (100%) using distilled water as solvent.

2.2 Collection and Preparation of GAS Eggs

Matured golden apple snails (*Pomacea canaliculata* L.) whose sizes are 30-40mm were randomly collected in rice fields and were placed in a simulated natural habitats planted with rice and twigs. The place was covered with net to prevent the snails from escaping. The snails were nurtured by proper care and proper feeding to ensure the health of the snails. The snails were allowed to lay eggs. Plants or twigs attached with eggs were carefully lifted and transferred into separate container and set on the top above water level. The newly hatched egg clusters were used to test the molluscicidal activity against the hatchability of the golden apple snail eggs at different treatments.

2.3 The Treatments

There were six (6) treatments used in the study with three (3) replicates each. Treatment 1 was the negative control using distilled water. Treatments 2, 3, 4 and 5 consisted of the plant extracts at four different concentrations. The pure (100%) extract was considered as Treatment 2, whereas 75%, 50% and 25% were prepared by diluting the pure extract with distilled water and served as solutions for treatments 3, 4 and 5, respectively. A positive control (Treatment 6) was also included using commercially available molluscicide.

2.4 Initial Counting of Hatched Eggs Per Cluster

Ten (10) clusters of golden apple snail eggs were placed in separate containers. After 15 days, the number of hatched eggs per cluster were determined by counting the number of newly hatched golden apple snails. The mean range of hatched eggs were computed and it was the basis on computing the percentage range on the hatchability of eggs.

2.5 Molluscicidal Activity Test

Egg clusters of the golden apple snails were used to different treatments, with three replicates each. These cluster of eggs were exposed to six (6) different treatments by spray method. The molluscicidal activity against the hatchability of the golden apple snail eggs were done by spraying 1 ml of the molluscicide to the cluster of eggs. The results of the experiment was done within the hatching period which usually last within 15 days (Rondon & Callo, 1991). Observations on the number of hatched eggs were done every day for 15 days after the application of the different treatment solutions.

2.6 Pathway of Experimental Procedure

The experimental design was Completely Randomized Design (CRD) with six treatments and three replicates each. The study was performed in the laboratory to test the molluscicidal activity of the *Amaranthus viridis* Linn. leaf extracts at different concentrations against golden apple snail eggs.

Analysis of variance (ANOVA) was conducted to determine if there was a significant difference among the treatments. To compare and to know if the treatments were significant to the initial count done in the study, Dunnett (2-sided) Multiple Comparisons was conducted.

3. RESULTS AND DISCUSSION

3.1 Initial Count of Hatched Eggs Per Cluster

Ten (10) clusters of golden apple snail eggs were used to determine the initial count of hatched eggs per cluster in the laboratory. The eggs were initially bright pink in color. The color remained for two days but became light pink on the third and fourth day. On the fifth day, some eggs already turned white. The white colored eggs increased as days passed until they hatched. Out of 922 eggs in ten clusters, there were 602 newly hatched golden apple snails observed within 15 days. The mean percentage of hatchability was 65.29%.

This result conforms with the results of Demetillo et al. (2015) where 68.46% hatchability of golden apple snail eggs was observed in the laboratory. Moreover, the result of this study was found to be lesser than what was reported by Massugani & Latip (2015) that the hatchability rate of the the water (Tanaka et al., 1999).

3.2 Molluscicidal Activity Against the Hatchability of Golden Apple Snail Eggs

Table 1 shows the description of the *P. canaliculata* (GAS) egg clusters at different treatment within 15th days of observations. Initially, the egg cluster samples were bright pink in color. The color changed after the different treatment solutions (treatments 2, 3, 4 and 5) were sprayed. The eggs clusters in treatments 2, 3, 4 and 5 turned green which was also the color of the treatment solutions. Meanwhile, the egg clusters in treatments 1 and 6 remained bright pink.

There were no color change that occurred in the egg clusters on the 2nd and 3rd day but on the 4th day some of the bright pink eggs turned into light pink eggs. On the 5th day, the egg shells gradually faded and turned into white. On the 6th to fifteenth day, the color of the egg shells continuously faded into white, but some remained light pink and some remained green.

Figure 1 shows the number of hatched eggs at different treatments within the 15th days of observation. It was observed that the eggs in the negative control (T1) started to hatch on the 7th day of the samples in the laboratory where a mean of 23.33 newly hatched golden apple snails was obtained. The shell of the newly hatched snails were about 1.5-2 mm in size characterized of having light brown with reddish spots from embryonic shell.

Table 1. Description of the GAS egg clusters at different treatment within 15th days.

Day	T1	T2	T3	T4	T5	T6
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	(negative control)	(100% extract)	(75% extract)	(50% extract)	(25% extract)	(positive control)
0	bright pink	bright pink	bright pink	bright pink	bright pink	bright pink
1	bright pink	dark green	slightly light green	green	light green	bright pink
2	bright pink	dark green	slightly light green	green	light green	bright pink
3	light pink	dark green	slightly light green	green	light green	light pink
4	light pink	dark green	slightly light green	green	light green	light pink
5	white (some)	dark green	slightly light green	green	white (some)	light pink
6	white (some)	dark green	slightly light green	green	white (some)	light pink
7	white (almost)	dark green	slightly light green	green	white (some)	white (some)
8	white (almost)	white (some)	slightly light green	green	white (some)	white (some)
9	white (almost)	white (some)	white (some)	green	white (some)	white (some)
10	white (almost)	white (some)	white (some)	green	white (some)	white (some)
11	white (almost)	white (some)	white (some)	white (some)	white (some)	white (some)
12	white (almost)	white (some)	white (some)	white (some)	white (some)	white (some)
13	white (almost)	white (some)	white (some)	white (some)	white (some)	white (some)
14	white (almost)	white (some)	white (some)	white (some)	white (some)	white (some)
15	white (almost)	white (some)	white (some)	white (some)	white (some)	white (some)

The golden apple snail eggs in this negative control (T1) continuously hatched and produced more snails up to the 12th day. There were no more snails added during the 13th to 15th day of observation. Thus, there were 45.66 accumulated mean of the hatched eggs up to the 15th day.

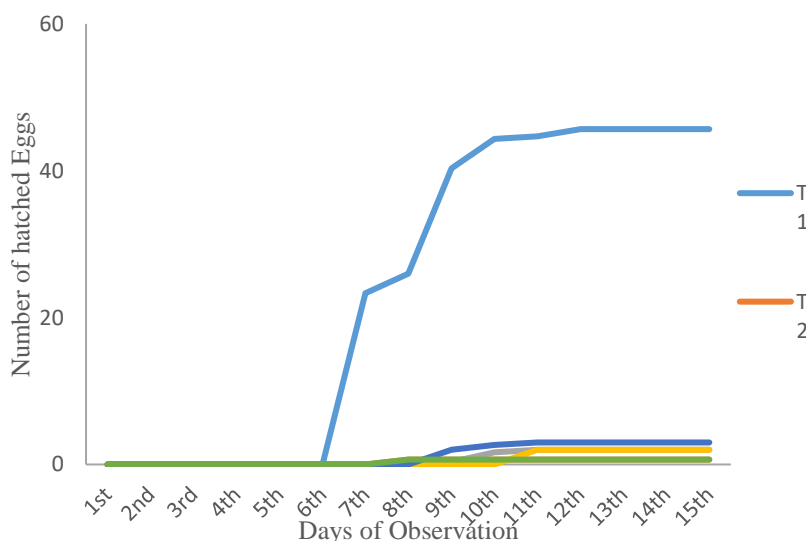


Figure 1 The number of hatched eggs per treatment within 15 days

On the 8th day, newly hatched golden apple snails were observed in treatments 2 and 6. It is only in the 9th day that treatments 3 and 5 produced newly hatched snails. On the 10th and 11th day, there were only few snails that hatched in treatments 3, 4 and 5. On the 12th day onwards, there were no recorded hatched eggs in these treatments. Results show that the hatchability of the golden apple snail egg samples were obstructed or disturbed by the application of the Chinese spinach plant extracts at different concentrations.

Results also show that the golden apple snail eggs started to hatch on its 7th day and stopped hatching on its 13th day in the laboratory where the room temperature ranged from 31°C to 35°C. The age of the egg clusters used in toxicity test were two days old. Thus, the hatching period of the egg samples actually started on its 9th day and ended on its 15th day. This conforms with the result of Rondon & Callo (1991) where an egg cluster with 25-500 eggs hatches in 8-15 days. The newly hatched snails shell is light brown with reddish spots from embryonic shell to umbilicus. Their shells harden in 2 days and the hatchlings crawl when they reach 2-5 mm in size (Dela Cruz et al., 2000).

A snail begins life with hundreds of other bright pink eggs that are laid on the edge of the water. The snail reproduces rapidly, depending on temperature (Estebenet & Martin 2002). In addition, as stated by Yin et al. (2000) hatching occurs in 15-28 days or 8-15 days, at temperatures of 20-24°C and 28-32°C, respectively. Temperature is a factor that can affect the hatchability of the egg masses, the higher the temperature, the rapid the incubation time is.

3.3 Effect of the Treatments to the Hatchability of the Eggs

Figure 2 shows that treatment 1 (negative control) had the highest number of hatched eggs. This treatment consisted of water, thus there was high rate of hatchability observed. It can also be seen that treatments 2, 3, 4, 5 and 6 also produced snails but very few.

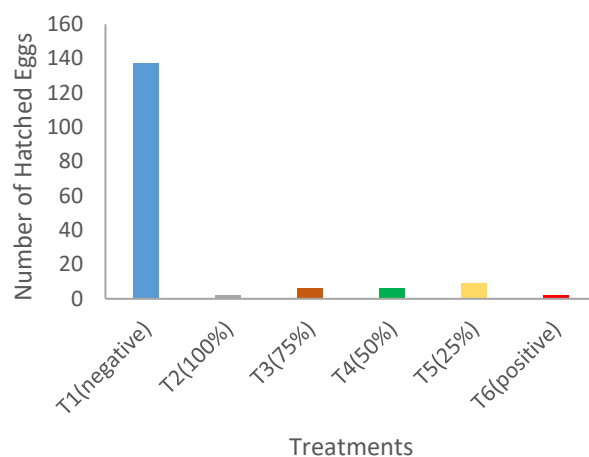


Figure 2. Number of hatched eggs per treatments

At 5% level of significance, analysis of variance shows that there was significant differences among the treatments. This implied that the treatments had different effects on the hatchability of golden apple snail eggs. Dunnett test (2-sided) Multiple Comparison showed that treatment 1 is not significant with the initial count, while treatments 2, 3, 4, 5 and 6 are significantly different to the initial count.

Table 2. Percentage means of unhatched and hatched *P. canaliculata* eggs at different treatments.

Treatments	Unhatched Eggs (%)	Hatched Eggs (%)
Initial count	35.11 ^a	64.89 ^a
Treatment 1	50.58 ^a	49.42 ^a
Treatment 2	99.28 ^b	0.72 ^b
Treatment 3	97.84 ^b	2.16 ^b
Treatment 4	97.84 ^b	2.16 ^b
Treatment 5	96.75 ^b	3.25 ^b
Treatment 6	99.28 ^b	0.72 ^b

Note: Means of the same letter are not significantly different

Table 2 shows that all the treatments exhibited successful hatching. Treatment 1 (negative control) exhibited the highest percentage in terms of hatched eggs (49.42%) which was not significant with the initial count of the hatched eggs. Furthermore, treatments 2, 3, 4, 5 and 6 which were all not significantly different from each other obtained the highest percentage of unhatched eggs. The treatments consisted of plant extract (treatments 2, 3, 4 and 5) and the positive control (treatment 6) consisted of commercially synthetic molluscicide. Results also showed that the ability of the plant extracts to inhibit the hatchability of the golden apple snail eggs was significant with the commercially synthetic molluscicide (Treatment 6). Thus, the treatments consisting of the plant extract significantly altered the normal development of *P. canaliculata* eggs.

4. CONCLUSION

Plant extracts of *Amaranthus viridis* caused alterations on the development of *P. canaliculata* by greater anti-hatching ability of its eggs. Preventing the snails to hatch is an excellent way to prevent the increasing number of golden apple snails. The results demonstrated that *A. viridis* is a potential alternative pesticide which is not only safe and environment friendly but also readily available and affordable that will surely benefit farmers.

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