Original Article

Molluscicidal Activity Of Cashew (Anarcadium Occidentale Linn.) Apple Against Golden Apple Snail (Pomacea Canaliculata Lamarck)

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Abstract

Background: The vast number of golden apple snail (*Pomacea canaliculata* Lamarck) in the Philippines is one of the serious problems of rice producers. Synthetic pesticides were utilized by farmers because of its effectiveness. However, the use of chemicals result to environmental pollution, eradication of non-target organisms and unsafe products due to the toxicity of applied chemicals. This study introduce the key and response to this environmental problems through extraction of cashew apple (*Anarcadium occidentale* Linn.) as an alternative molluscicides which is environmentally friendly and effective pesticide against golden apple snail. The development of the natural molluscicide would bring a healthier environment, safe to human and results to the increased food production that addresses the Sustainable Goal Development such as Life on Land (SDG 15) and Zero Hunger (SDG 2).
Methods: The golden apple snails were collected at the pond, nurtured, raised and allowed to lay eggs. The hatched eggs were nurtured until they reached the adult stage. The cashew apple were collected and extracted using manual juicer. There were six treatments with three replications. The golden apple snails were subjected to the different treatments and observed within eight hours. The percentage mortality in each treatment were computed.
Results: After eight (8) hours of exposure of the golden apple snails to the different treatments, the pure extract of cashew apple (T1) obtained 93.3% mortality which is comparable with the commercially available synthetic molluscicide (T6-positive control).
Conclusions: The results indicated that the extract of cashew apple is a potential molluscicide that may improve the food safety (rice) and protect the health of every Filipino farmers.

Keywords*:* cashew apple (*Anarcadium occidentale*), golden apple snail (*Pomacea canaliculata*), molluscicide, extraction, mortality

1. INTRODUCTION

For the health of every Filipino, environment protection and food (rice) safety, the organic farming is becoming popular and the demand of organically produced farm products is increasing around the world. However, organic crop producer are confronted by the problem of controlling usual pest in the crops which tempt them to use chemical and toxic pesticides. Therefore, there is a need to explore alternative pesticides (molluscicides, herbicides, insecticides) that may not be harmful or has less detrimental effects compared to the synthetic chemical pesticides (Taguiling, 2015). The golden apple snail (*Pomacea canaliculata* Lamarck) was introduced from Luzon around 1983 and into the Asturias Farm in Cebu in 1984 directly from Argentina or Taiwan for commercial production (Mochida, 1991). *Pomacea* culture was recommended to farmers through a livelihood project of the Philippine Government up to 1988. However, its infestations were soon reported to increase in Visayas and Mindanao making it one of the major pests throughout the country (Penaredondo et al., 2015).

The vast number of golden apple snail (*Pomacea canaliculata* Lamarck) in the Philippines is one of the serious problems of in rice production. According to Taguiling (2015), the proliferation of golden apple snail (*Pomacea canaliculata* Lamarck) is invading almost all rice fields in the lowlands including rice terraces in the uplands. In the planted area of the Philippines, the estimated yield tosses owing to this pest ranged from 1% to 40%, causing a massive production loss. Out of the 3 million hectares, 1.2 to 1.6 million hectares are infested with golden apple snail (*P. canaliculata*) on the rice fields of the Philippines. It was reported that 212 million pesos was spent. Since then, rice area infested with this pest has been increasing until it became a national menace (Madjos et al., 2015).

Nowadays, commercially available synthetic pesticides were utilized by farmers because of its effectiveness. The ability of marketable molluscicides only took for 2-3 days to golden apple snail (*P. canaliculata*) after the application of the synthetic molluscicide. However, such pesticides contain compounds that are lethal to non-destructive native snail species

(*Vivipara costata*). Triphenyltin compounds were the first pesticides used by farmers to eradicate golden apple snail (*P. canaliculata*) and those compounds were banned in 1993, because of its adverse effect in human health and environment. Also, endolsufan and organochlorine, registered as insecticides and proven effective to kill golden apple snail (*P. canaliculata*) and were banned by Fertilizer and Pesticide Authority (FPA). Unfortunately, there are four (4) registered chemicals as molluscicides in the list of FPA, namely: niclosamide, metaldehyde, izazophos and copper hydrosulfate. Farmers noticed that niclosamide was the most effective molluscicides available in the market (Cagauan & Joshi, 2003). The utilization of synthetic pesticides valued for its effectiveness and suitability but it also create certain problems including environmental degradation, health hazards to farmers and phytotoxicity and toxicity to non- target organisms. They can also accelerate development of resistant pests to specific pesticide chemicals (Massaguni et al, 2012).

Organic Agriculture Act of 2010 declared 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. It is suggested by Taguiling (2015) that it is timely to use not only organic fertilizers but also organic pesticides. Also, Cagauan et al. (2003) stated that several pest control and management are grouped as chemical, biological, cultural, botanical, mechanical and manual. Handpicking and application of chemical pesticides are the most broadly practiced method used by farmers to control golden apple snail (*P. canaliculata*).

Cashew (*Anacardium occidentale*) belongs to the order Sapindales, family Anacardiaceae and genus *Anacardium***.** This tropical tree native to northeastern Brazil and presently cultivated in many other countries (Tedong et al., 2010). The biological properties of cashew (*A. occidentale*) nut shell liquid are larvicidal, molluscicidal, anti-fungal, anti-microbial were also reported (Parasa et al., 2011). A study conducted by Arunlertaree et al. (2003) showed that the extraction of cashew (*A. occidentale*) nut shell on local liquor and ethanol had strong molluscicidal activity. Between these two different concentrations, the ethanolic cashew nut shell extract was more toxic to both small and medium sized golden apple snails (*P. canaliculata*) than the local liquor cashew (*A. occidentale*) nut shell crude extract.

The cashew apple is a part of the cashew fruit that is rarely eaten. More often, they are left behind and are usually thrown away by the cashew nut producers and may contribute to waste problems. Utilizing this wastes as a beneficial source of biopesticide against golden apple snail (*P. canaliculata*) that is safe to the human health and non-target organisms would be of great importance. Hence, this study will determine the molluscicidal activity of cashew apple against golden apple snail *(P. canaliculata)* through the process of extraction. In the study of (Aiswarya et al., 2011) it was revealed that the ethanolic extract of cashew (*A. occidentale*) apple contains phytochemicals such as steroids, glycosides, tannins, phenols and triterpenoids. The cashew apple also subjected to aqueous extract which also shows the presence of steroids, glycosides, carbohydrates, flavonoids, tannins, phenols and saponins. Santos et al. (2014) stated that some of the phytochemicals present contributing to the molluscicidal properties are tannins, saponins, terpenoids, steroids, and flavonoids. Al-sarar et al. (2012) also concludes that cardiac glycosides are very promising candidate compounds that could be used to control land snails. This study aims to determine the molluscicidal activity of cashew (*Anarcadium occidentale*) apple against golden apple snail (*P. canaliculata*).

2. MATERIALS AND METHODS

2.1 Golden apple snail (*P. canaliculata*) and Cashew apple (*A. occidetale*)

Matured and healthy golden apple snails (*Pomacea caniculata*) were collected randomly in selected ponds. Authentication of the snail samples was done. They were raised in an enclosed simulated pond and were not subjected to any chemicals. These golden apple snails (*P. caniculata*) were allowed to lay eggs. The eggs were nurtured until they reached adult stage. The adult (52 days old) golden apple snails, which are 30-40 mm in size were properly collected. These were used to test the molluscicidal activity of cashew (*A. occidentale*) apple extracts.

The cashew (*A. occidentale*) apple were washed with water to remove dust and unnecessary materials. The cashew apple (*A. occidentale*) were soaked in distilled water and drained afterwards. Moreover, the fresh juice of the cashew (*A. occidentale*) apples were squeezed out using a manual juicer. The juice was filtered to remove the unwanted materials. The fresh extracts were used immediately against golden apple snail (*P. canaliculata*) or were kept in the refrigerator to maintain its freshness.

2.2 The Treatments

In this study, six (6) treatments with three (3) replicates were used to test the toxicity. Treatments 1, 2, 3 and 4 consisted of cashew (*A. occidentale*) apple extracts at four different concentrations. The pure plant extract was considered as 100% extract (Treatment 1). Meanwhile, 75 % (Treatment 2), 50% (Treatment 3) and 25% (Treatment 4) were also prepared by diluting the pure extract with distilled water. A negative (Treatment 5) control and positive (Treatment 6) were also used and were containing of distilled water and Niclosamide, respectively.

2.3 Toxicity Tests

2.3.1 Preliminary Toxicity Tests
There were 180 adult golden apple snails (*P. canaliculata*) used for the six (6) treatments in three (3) replication during the preliminary toxicity tests. Thirty (30) golden apple snails were used in each treatment, ten (10) snails in every pan. The snails were exposed to the different treatments by placing twenty (20) mL of the treatment solutions in each representative pans. This was done to find out the number of hours the positive control (niclosamide) resulted to 100% mortality rate.

2.3.2 Final Toxicity Tests
There were 720 adult golden apple snails (*P. canaliculata*) used in the six (6) different treatments with three (3) replications. One hundred twenty (120) golden apple snails (*P. canaliculata*) were used in each treatments. The prepared solutions of different concentrations were placed in separate pans containing 20 ml of the extract for the toxicity test. Ten golden apple snails (*P. canaliculata*) were placed in each pan.

Observation was done every two (2) hours-interval. The percentage mortality in each treatment every two (2) hours observation were computed. Destructive sampling were used in the study, there were four (4) sets of samples in each treatment with three (3) replicates. Representative pans for each sampling time in every treatment were removed after determining if the snail was still alive or already dead. The sampling was done after two (2) hours, four (4) hours, six (6) hours and eight (8) hours.

Dead golden apple snails (*P. canaliculata*) were determined by pushing the snail's operculum. If the snails did not resist, then it was already dead. If it pulled back and closed up, then it was still alive. The operculum remained shut when the snail was alive, but it remained open when the snail was already dead (LaFollete, 2011).

Using the formula below, the percentage mortality of the golden apple snail (*P. canaliculata*) were computed as:

$$\% \text{ Mortality} = \frac{\text{no. of dead snails}}{\text{total no. snails used}} \times 100$$

2.4 Experimental Design and Statistical Analysis
The experimental design used in this study is Completely Randomized Design (CRD). There were six (6) concentration treatments and three (3) replicates for each samples. The molluscicidal activity of cashew (*Anarcadium occidentale*) apple extract against golden apple snail (*Pomacea canaliculata*) were performed at the Laboratory of Environmental Science. Analysis of variance and post-hoc analysis specifically Tukey's HSD were used to know the different effects of the different treatments on the mortality of the golden apple snails.

3. RESULTS AND DISCUSSION

3.1 Collection of the Golden Apple Snails (*Pomacea canaliculata*)
Matured golden apple snails (*P. canaliculata*) were placed in a simulated pond, raised for 52 days and maintained until they reached their adult stage. After 52 days, a total of 900 golden apple snails (*P. canaliculata*) were collected. There were 180 snails used in the preliminary toxicity test and 720 snails in the final toxicity test. The toxicity tests were performed at the Laboratory of Environmental Science, College of Arts and Sciences, Central Luzon State University.

3.2 Collection and Extraction of Cashew (*Anarcadium occidentale*) Apple
A total of three fourth (3/4) kilogram fresh cashew (*A. occidentale*) apples were brought to the laboratory, washed with running water, soaked in distilled water then drained afterwards to remove the unwanted materials. A manual juicer was used for the extraction of cashew (*A. occidentale*) apples and approximately 500 mL cashew (*A. occidentale*) apple extract was obtained. The extracted cashew (*A. occidentale*) apple have strong sweet smell, yellowish in color and have astringent taste with biting sensation in tongue and throat. According to Adou et al. (2012), the cashew (*A. occidentale*) apple juice contains minerals, three to six times more vitamin C than orange and ten times more than pineapple juice.

3.3 Effect of Different Treatments on Golden Apple Snails (*P. canaliculata*) During the Preliminary Toxicity Test
Analysis of variance (ANOVA) shows that there is a significant difference among treatments in the preliminary observation at 5% level of significance. This means that the cashew apple extracts together with the control treatments affects the mortality rate of the golden apple snails (*P. canaliculata*).

Table 1 shows the mortality of the golden apple snails (*P. canaliculata*) exposed to different treatments within six (6) hours. It was found out that after six hours of exposure to niclosamide (T6), all golden apple snails were already dead (100% mortality). This result is comparable with T1 which is consist of pure extract of the cashew apple (*A. occidentale*).

Table 1. Mortality of golden apple snail (*P. canaliculata*) after 6 hours during preliminary toxicity tests

Treatments	Mean	Percent (%) Mortality
T1 (100%)	8.67^{cd}	86.66
T2 (75%)	6.33^{bc}	63.33
T3 (50%)	4.33^{b}	43.33
T4 (25%)	4.00^{b}	40.00
T5 (Negative)	0.00^{a}	0.00
T6 (Positive)	10.00^{d}	100.00

Note: Means with the same letters are not significantly different

The table also shows that treatment 1 is not significantly different to treatments 2 and 6. It implies that treatment 1 gave the same effects on the mortality rate with that of treatments 2 and 6.
Among the six (6) treatments, treatments 6 have the highest percentage mortality of golden apple snails (*P. canaliculata*) since it is the positive control (Niclosamide). Treatment 1 (100%) cashew apple extract shows the second highest percentage mortality (86.7%) of snails. Furthermore, comparison among means in treatments 2, 3 and 4 shows that they are comparable with each other. Lastly, treatment 5 shows that there is no dead snails since it is the negative control (distilled water).

3.4 Final Toxicity Tests

The final toxicity tests were done within eight (8) hours and observed in two-hour interval. A total of 720 golden apple snails (*P. canaliculata*) were used for the final toxicity test. There were six (6) treatments with three (3) replicates. Four (4) destructive samplings were done in each treatments.

3.5 Molluscicidal Activity of Cashew (*A. occidentale*) Apple against Golden Apple Snails (*P. canaliculata*)

Figure 1 illustrates the percentage mortality of golden apple snails exposed to different treatments. It is evident that the percentage mortality of golden apple snails in all treatments except for the negative control (T5) are increasing in two hour interval within eight (8) hours.

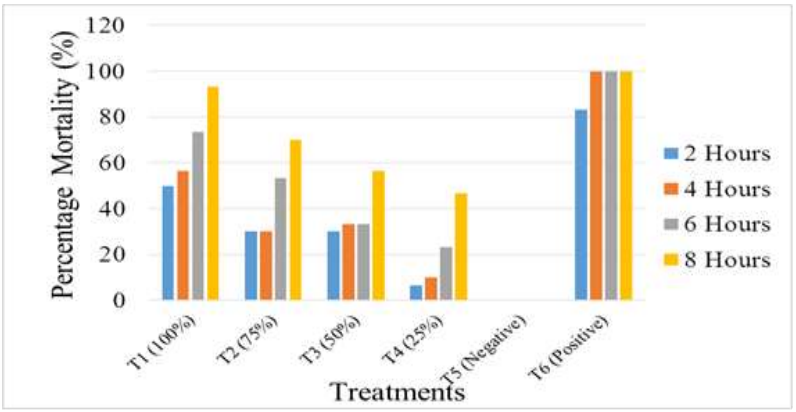

Figure 1. Mortality of Golden apple snails (*P. canaliculata*) exposed to the different treatments

Treatment 5 (Negative control) didn't show any mortality on golden apple snails (*P. canaliculata*). It is also noticeable that the mortality of the golden apple snails exposed to treatment 6 (Positive control) is high on the first two (2) hours and it continuously gained 100% mortality from four hours up to eight hours.
It can also be noted that among the four treatments consisting of the cashew apple extracts, T1 (100% extract) gave the highest percentage mortality.

3.6 Effect of Cashew Apple Extracts on Golden Apple Snails after Two Hours

The analysis of variance shows significant results after two (2) hours of observation at 5% level of significance. It indicates that the treatments significantly affect the mortality of golden apple snails (*P. canaliculata*). Table 2 shows the mean and percentage mortality of golden apple snails (*P. canaliculata*) of each treatment after two (2) hours.

Table 2. Mortality of golden apple snails (*P. canaliculata*) after 2 hours.

Treatments	**Mean**	**Percent (%) Mortality**
T1 (100%)	5.00^{c}	50.00
T2 (75%)	3.00^{bc}	30.00
T 3 (50%)	3.00^{bc}	30.00
T4 (25%)	0.67^{ab}	6.67
T5 (Negative)	0.00^{a}	0.00
T6 (Positive)	8.33^{d}	83.33

Note: Means with the same letters are not significantly different

Statistical analysis on the six (6) different treatments indicates that treatment 6 is significantly different to the other treatments. While treatments 1, 2 and 3 illustrates that they are not significantly different, it infers that treatment 1 gave the same effects on the mortality of golden apple snails (*P. canaliculata*) as to that of treatments 2 and 3. However, treatments 2 and 3 also gave similar effects to the mortality rate given by T4. Lastly, treatments 4 is not significantly different to treatments 5 (negative control). It implies that they give the same effects on the mortality rate of golden apple snails (*P. canaliculata*). Thus, T4 gave the least effect on the mortality of the golden apple snails after two hours of exposure.

On the first two hours of the observation, treatment 1 shows significant effects on the golden apple snails (*P. canaliculata*). Half of the number of snails in each replicates died after exposure to 100% pure cashew (*A. occidentale*) apple extract. This result is comparable with T2 and T3. Moreover, the effect of treatment 6 (Positive control) is very evident, majority (83.33%) of the golden apple snails (*P. canaliculata*) died on the first two hours of the observation.

3.7 Effect of Cashew Apple Extracts on Golden Apple Snails after Four Hours

Analysis of variance exhibited significant results after four (4) hours of exposure to different treatments at 5% level of significance. Table 3 shows the mean and percentage mortality of golden apple snails (*P. canaliculata*) exposed to different treatments after four (4) hours.

Table 3. Mortality of golden apple snails (*P. canaliculata*) after 4 hours

Treatments	Mean	Percent (%) Mortality
T1 (100%)	5.67^{c}	56.66
T2 (75%)	3.00^{b}	30.00
T3 (50%)	3.33^{b}	33.33
T4 (25%)	1.00^{a}	10.00
T5 (Negative)	0.00^{a}	0.00
T6 (Positive)	10.00^{d}	100.00

Note: Means with the same letters are not significantly different

Statistical analysis shows that after four (4) hours of exposure, treatment 6 is significantly different to the five other treatments. Likewise, the effect of treatment 1 is also significantly different to other treatments. Meanwhile, treatments 2 and 3, and treatments 4 and 5, are respectively not significantly different with each other.

As shown in Table 3, all golden apple snails (*P. canaliculata*) died after four (4) hours of exposure to the treatment 6 (Positive Control). Among the different concentration of cashew (*A. occidentale*) apple extracts, treatment 1 (100%) have the highest percentage mortality (56.7%) of golden apple snails (*P. canaliculata*). This is followed by treatment 2 and 3 with 53.3% and 26.7% mortality, respectively. Moreover, there were no dead golden apple snails (*P. canaliculata*) found on treatment 5 (negative control). Statistically, this result is not significant with treatment 4 (25% extract).

3.8 Effect of Cashew Apple Extracts on Golden Apple Snails after Six Hours

The analysis of variance of the different treatments shows significant results at 5% of significance. This implies that the different treatments affects the mortality rate of golden apple snails (*P. canaliculata*) after six (6) hours exposure to the cashew apple extracts.

Table 4. Mortality of golden apple snails (*P. canaliculata)* after 6 hours.

Treatments	Mean	Percent (%) Mortality
T1 (100%)	7.33^{d}	73.33
T2 (75%)	5.33^{c}	53.33
T3 (50%)	3.33^{bc}	33.33
T4 (25%)	2.33^{b}	23.33
T5 (Negative)	0.00^{a}	0.00
T6 (Positive)	10.00^{e}	100.00

Note: Means with the same letters are not significantly different

Treatment 6, 1 and 5 are significantly different with each other denoting that they have different effects to the mortality of golden apple snails (*P. canaliculata*). Treatments 2 and 3 are significantly the same denoting for having similar effects on the golden apple snails. Moreover, treatment 3 also gave similar effect with treatment 4.

All snails are already dead in treatment 6 (positive control). In treatment 1, the effect of cashew (*A. occidentale*) apple extract is very evident, majority of the golden apple snails (*P. canaliculata*) died after exposure. Treatment 1 obtained the highest percentage mortality (73.3%) among the four (4) different concentrations of cashew (*A. occidentale*) apple extracts. Treatment 2 (75% extract), obtained 53.3% mortality of golden apple snails (*P. canaliculata*) but significantly comparable with treatment 3 (50% extract).

3.9 Effect of Cashew Apple Extracts on Golden Apple Snails after Eight Hours

The analysis of variance of different concentrations observe after eight (8) hours shows significant result at 5% level of significance. It infers that the different concentrations affect the mortality of golden apple snails (*P. canaliculata*).
After eight (8) hours of exposure, treatments 1, 2, 3, and 4 shows an increase in the percentage mortality of golden apple snails (*P. canaliculata*). In treatment 5, no golden apple snails (*P. canaliculata*) died and in treatment 6 all the golden apple snails (*P. canaliculata*) died because of Niclosamide.

Table 5. Mortality of golden apple snails (*P. canaliculata)* after 8 hours

Treatments	Mean	Percent (%) Mortality
T1 (100%)	9.33^{d}	93.33
T2 (75%)	7.00^{c}	70.00
T3 (50%)	5.67^{b}	56.67
T4 (25%)	4.67^{b}	46.67
T5 (Negative)	0.00^{a}	0.00
T6 (Positive)	10.00^{d}	100.00

Note: Means with the same letters are not significantly different

Table 5 shows that treatment 6 (positive control) and treatment 1 (100% extract) are significantly comparable. This indicates that 100% cashew (*A. occidentale*) apple extract and positive control that kill all the snails have the same effects to the mortality of golden apple snails (*P. canaliculata*). Meanwhile treatment 2 (75% extract) is significantly different, whereas treatments 3 and 4 give similar effects to the mortality of golden apple snails (*P. canaliculata*).
At this time of observation, the golden apple snails (*P. canaliculata*) exposed to treatment 1 (100% extract) obtained 93.3% mortality, but is insignificant with the positive control. Treatment 2 was observed with a 70% mortality of golden apple snails (*P. canaliculata*) after eight (8) hours of exposure.

After eight hours of exposure to treatments 3 and 4, 56.7% and 46.7% mortality was observed, respectively. This results shows that low concentrations of the cashew apple extract may also give a significant molluscicidal activity against the golden apple snails at longer period of exposure.

4. CONCLUSION

The cashew (*A. occidentale*) apple was a potential and alternative for commercial molluscicide. The pure crude extract of the cashew apple gave the highest GAS mortality after eight hours of exposure. Moreover, lower concentrations of the cashew (*A. occidentale*) apple extracts also shows a promising molluscicidal activity but in longer exposure. The natural molluscicide is an environmentally friendly and less expensive and applying this natural molluscicide could improve the food safety (rice) and protect the health of every Filipino farmers.

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