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The efficacy of *Melothria pendula* leaf extract as Novel Molluscicide against *Pomacea canaliculata*

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Abstract

The control of the invasive species Golden Apple Snail, *Pomacea canaliculata*, plays a crucial role in preventing the continuing destruction of lowland rice production in the Philippines. However, some molluscicides are unpractical because of their toxic effect on non-target organisms; hence, this study is on the innovative, plant-based extract used to control golden apple snails. Leaves of wild cucumber, *Melothria pendula*, were collected, air-dried, pulverized, and macerated in ethanol. The extracts were subjected to a water bath at 80 ºC for four hours. Active snails were immersed in solutions of 5mL/L, 10mL/L, and 15mL/L of *Melothria pendula* leaf extract for 24h and 48h. The snail mortality was determined, and LC50 & LC90 values were calculated. Treatment 3 (15mL/L) had the highest mortality rate, 90% and 97%, among other concentrations used in 24 and 48 hours, respectively. The control group, bayluscide, attained 100% mortality both in 24 hours and 48 hours. Analysis revealed that the LC50 and LC90 values of 48 hours of exposure, 2.26 mL/L and 10.04 mL/L, respectively, showed an increased toxicity level against the snails than that of 24 hours of exposure. Significant differences between the effects of different concentrations on the mortality rate of the snail and days of exposure were noted with p values of 0.000 and 0.032 ($p<0.05$). Results indicated that *Melothria pendula* extract is a novel, environment-friendly molluscicide to control *Pomacea canaliculata*.

Keywords: *Molluscicide, Golden Apple Snails, wild cucumber, leaf extract*

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1. Introduction

Pomacea canaliculata is one of the most fatal non-indigenous species in the Philippines, devastating lowland rice production (Picardal et al., 2018). Introduced in 1982, *Pomacea canaliculata* initially supported low-income Filipino farmers but, by 1986, began devastating rice crops in northwest Luzon. Its rapid spread threatens the country's rice production and food security. In 2000, the International Union for Conservation of Nature (IUCN) ranked *Pomacea canaliculata* among the top 100 most harmful species worldwide, along with two other types of snails (Joshi et al., 2017). Kuhol, or the Golden Apple Snails (GAS) in the Philippines, has gained popularity as an alternative to fish and meat. However, *P. canaliculata* has been identified as a carrier of diseases and parasites crucial to human health (Chen et al., 2021). Moreover, *P. canaliculata* was later discovered by Hu et al. (2018) as a vital intermediate host for *Angiostrongylus cantonensis*, the rodent lungworm, which can infect humans and lead to angiostrongyliasis, a condition characterized by eosinophilic meningitis. It was also reported by Joshi et al. (2017) that consumption of undercooked infected snails and clams can lead to Echinostomiasis infection.

According to Nurjanah et al. (2019), the GAS can produce up to 20 groups of 500 eggs each, every time, and can hatch after fourteen days from its release. The poisonous eggs hardly have any predators, once hatched they can have approximately six months to two years of survival. Temperature and the recurrence of seasons significantly impact how long the snail's life cycle lasts. As the temperature increases, the longer the life expectancy of GAS is; that is why in tropical countries like the Philippines, snail production is very rampant. Once matures, *P. canaliculata* consumes 7 to 24 rice seedlings every day and attacks or destroys the early stems and leaves of plants (Noorshilawati et al., 2020)

For over three decades since the GAS invasion, rice farmers' financial losses have been enormous, mostly as a result of the productivity loss and the expense of trying to contain the voracious snails. Niclosamide, the sole commercial molluscicide endorsed by the World Health Organization, was reported in 1993 to be effective due to its high efficacy, low toxicity to mammals, and no pesticide residual problems (Zheng et al., 2021). Niclosamide, on the other hand, is poorly soluble in water and toxic to fish and other non-target biota, causing snails to rise. Furthermore, farmers' health was affected by the extensive use of hazardous pesticides, and food and nutrition security were compromised due to production losses. Numerous countries invested heavily in control methods, varying in effectiveness and cost. While some succeeded at a price, others saw ongoing devastation to wetland crops and potential environmental harm from the snails. With this, there is a need to create an approach for safe and efficient control of GAS using *Melothria pendula*, commonly known as pipinong gubat in Filipino, and wild cucumber in English, a creeping vine uncultivated in the Philippines.

In tropical climates just like the Philippines, the slender vine *M. pendula* grows as a short-lived perennial plant; however, its uses are not well utilized. While limited scientific research has been done to confirm wild cucumber's medicinal properties, a few of *M. pendula's* historical medical uses include treating diabetes and anemia as well as acting as an anti-inflammatory. There are records indicating that *"pipinong gubat"* has been used to treat wounds, burns, and snake bites (Jay, 2022).

Raju et al. (2021) highlighted the presence of tannins, saponins, and terpenoids in *M. pendula* leaves, crucial in regulating the snail population. The presence of saponins is an important molluscicidal compound that disrupts cellular permeability and osmoregulatory functions by interactions with membrane sterols of the cell and can cause hemolytic activities in the snails (Beressa et al., 2020) Additionally, terpenoids act as acetylcholinesterase inhibitors resulting in neurotoxicity in snails according to Mandefro et al. (2018). Meanwhile, tannins demonstrate a direct toxic impact that leads to the mortality rate of *P. canaliculata* (Noorshilawati et al., 2020).

This study addressed the United Nations' Sustainable Development Goals on Responsible Consumption and Production and Good Health and Well-Being. It aimed to investigate the potential use of *Melothria pendula* as a molluscicide against *Pomacea canaliculata.* Specifically, this study sought to determine the effects of different concentrations and days of exposure against GAS in terms of mortality rate; the 50% and 90% lethal concentration of *M. pendula* to GAS; and the significant difference between the effects of different concentrations and days of exposure to the mortality of the snail.

2. Literature Review

2.1 Golden Apple Snails (Pomacea canaliculata)

As explained by Liu et al. (2018), *P. canaliculata*, often known as the Golden Apple Snail, is known for its high-stress tolerance, quick growth, high reproductive rates, and adaptation to many environments. Yoshida et al. (2016) revealed that even though no food was given during the juvenile stage, the snails reached mature sizes in less than two months. Similarly, Hollis (2022) reported that GAS lived around one to two years in tropical climates making snails reproduce throughout their lifetime and not hibernate because of warm temperatures.

The shell of the GAS is spherical, typically measuring between 40-60 mm in height and 45-75 mm in width, but it can reach lengths of up to 150 mm. It may display hues of yellow, green, or brown and comprises five to six whorls divided by a deeply marked suture. The operculum of *P. canaliculata* is relatively thick and concentric, with a color spectrum ranging from light to dark brown. While closely related to other canaliculata species, distinctions can be made based on egg coloration, shell dimensions, angle of suture indentation, and aperture shape (Hoswalde & Kondapalli, 2013). Additionally, *P. canaliculata* spends most of its time in water making it hard to detect by farmers, however, its egg is more visible because snails lay just above the waterline. According to Yang et al. (2018), a gravid female snail adult can lay as many as 25-1200 eggs per week with 80% hatchability. Once adult, they consume new and emerging rice plants by cutting the rice steam at the base, thus destroying the entire plant.

In the Philippines, it was observed that *P. canaliculata* continued to attack rice plants in irrigated paddies. According to Hoswalde and Kondapalli (2013), farmers first reported the activity of the snails as a major pest of newly transplanted rice seedlings in 1986 at Isabela Region II when about 300 hectares of rice harm were heavily damaged. Currently, for over three decades, GAS has affected all irrigated rice in the Philippines and can cause 85% crop damage. On the other hand, pipinong gubat, in the Cucurbitaceae family, has related species like *Cucumis sativus* that have been used as a snail attractant (Cordova et al., 2020), and *Citrullus lanatus* as a larvicidal agent (Saad et al., 2021). However, no studies have explored the molluscicidal potential of the *Melothria* genus.

2.2 Pipinong Gubat (Melothria pendula)

M. pendula, commonly referred to as wild cucumber, is a vine that climbs using slender, smooth stems and spiraling tendrils. It possesses toothed leaves and tiny, yellow flowers with five petals. In some other countries, its fruits and leaves are used as food and beverage either raw or cooked. It is also used to make sauces that are combined with onions and chili. In addition, its fruit contains vitamins that are used to heal several ailments, including gonorrhea, heart palpitations, anemia, and even witchcraft-related impotence. The whole plant is used to cure wounds, kidney stones, stomach problems, and snake bites (Guerrero-Torres et al., 2022).

In the Philippines, *M. pendula* is abundant in *Tagalog* areas where it is used as food and as a treatment for various diseases. Research findings suggest that using ethanolic leaf extract and mashed fruit at 75% and 100% concentrations from *M. Pendula* significantly reduces blood sugar levels in diabetic mice. Moreover, methanolic extracts from eighteen plants, among them *Melothria pendula*, showed noteworthy antiviral activity against Herpes Simplex 1, one of the most common infections in the world. Furthermore, results from studies on the synthesis of silver nanoparticles using an aqueous leaf extract of *M. Pendula* showed efficacy against *Staphylococcus aureus* and *Escherichia coli* (Stuart, 2023).

2.3 Phytochemical Analysis of Melothria pendula

The investigation conducted by Raju et al. (2021) on the *Melothria pendula* revealed a high concentration of saponins, alkaloids, quinones, anthraquinones, steroids, xanthoproteins, terpenoids, and tannins in phytochemical analysis. Among these crucial phytochemical constituents, it is concluded that the mortality of snails is caused by tannins, terpenoids, and saponins. While the wild cucumber plant offers some information and effectiveness in medicinal use, it has not been explored on a wider scale. For instance, although *Melothria pendula* contains essential phytochemical constituents for molluscicidal activity, no studies have been conducted on this aspect.

Tannins. Tannins function as potential therapeutic agents, free radical scavengers, and metal-binding agents, while also affecting enzyme activity and lipid peroxidation processes (Singh & Kumar, 2020). When it comes to its molluscicidal activity, it was believed that tannins are endogenous growth inhibitors for several types of pests

(Noorshilawati et al., 2020). Additionally, Vu et al. (2017) also revealed that tannins play an important role as bactericides. Most notably, the presence of tannins in phytochemical screening indicates a direct toxic effect, which contributes to the high mortality rate of GAS.

Terpenoids. According to Singh and Sharma (2015), terpenoids serve an important part in plant defense systems against both biotic and abiotic stressors, as well as signaling chemicals that attract insects and mollusks. It was also discovered by Chawech et al. (2017) that molluscicidal activity was correlated with the presence of terpenoids. The toxicity of terpenoids acts as an antifeedant and growth disruptor (Noorshilawati et al., 2020). Similarly, terpenoids act as acetylcholinesterase inhibitors resulting in altering the normal activity of the nervous system of snails (Mandefro et al., 2018).

Saponins. Saponins are an essential component of plants that causes hemolytic activity toward the snail (Beressa et al., 2020). Thus, it was revealed that saponins destroys the red blood cells of the snail leading to the release of hemoglobin. In the study conducted by Noorshilawati et al. (2020), saponin disrupts the snail's eating and growth, making it molluscicidal. Additionally, Souza et al. (2013) found that saponins cause increased toxicity in snails by being reabsorbed into their hepatic cells, resulting to death. Finally, saponins reduce the surface tension of water and inhibit *P. canaliculata's* respiration.

Among the three important phytochemical components of *Melothria pendula*, saponins exhibit strong molluscicidal activity against snails. In fact, in the study conducted by Mendes et al. (2018), saponins work by lysing the snails' cell membranes and pushing their contents out of them. Hence, according to Rangel et al. (2023), snails are vulnerable to niclosamide as well as biochemical substances such as glycosides, flavonoids, and saponins.

2.4 Determination of Snails' Mortality

Various factors are used to assess the mortality of GAS. According to Abdullah et al. (2017), indicators of snail death include mucus secretion, a change in shell color, the inability of the flesh section to retract within the shell, and the body protruding from the shell. This is aligned with the findings of Prabhakaran et al. (2017) that snails were considered dead if they exhibited no movement and either had fully withdrawn into their shells or were hanging outside of them. Additionally, the death of the snails can be further verified by the complete opening of the operculum. Upon such, if the head did not respond when prickled with sharp metal or needle, the snails can be considered dead.

2.5 Molluscicidal Activity of Plant Extracts

Because of its potent molluscicidal action against a variety of snail species, niclosamide is the only molluscicide that the World Health Organization recommends. Though it works well to control snails like GAS, it has certain drawbacks. According to He et al. (2017), niclosamide is ecologically destructive and highly toxic to non-target organisms such as marine organisms. It was supported by the World Health Organization, which states that niclosamide was costly, difficult to dissolve in both organic solvents and water, and very hazardous to other aquatic animals and plants. Thus, the search for local plants with molluscicidal properties has gained increasing attention as researchers look for more effective alternatives to niclosamide.

The study conducted by Abdel-Haleem (2013) showed that extracts of numerous plant species were found to have molluscicidal activities against various types of snails, causing digestive gland injury. This was supported by several studies showing plant mollusciciding as an important strategy in controlling snail hosts (El-Sherbini et al., 2010; Oyeyemi, 2021; Abdel-Haleem, 2013). Furthermore, the utilization of plants with molluscicidal characteristics is a much simpler, cheaper, and safer option than other commercial products. For instance, ground cherry plant extract demonstrated a repellent effect against the golden snail (Malana & Salvador, 2020). The root extract has been shown to possess stronger molluscicidal activity than other extracts and is the most suitable for biological applications since it provides a potentially easy, affordable, and ecologically safe molluscicidal agent.

3. Methodology

3.1. Collection of Test Organism and Plant Materials

The matured snails *P. canaliculata* were collected from the paddy fields in Bansud, Oriental Mindoro in the Philippines. The acclimation process was carried out on snails weighing more than 5 grams and measuring between 25mm and 35mm in height. The snails were washed, acclimatized in distilled water for seven days while fed cabbage leaves, and covered with netting. Seven hundred and twenty (720) snails were selected for the experiment, divided into groups of 10 per container, with three replications, and, were exposed to *M. pendula* leaf extract for 24 and 48 hours (Picardal et al. 2018). On the other hand, *M. pendula* leaves were also harvested in Bansud, Oriental Mindoro, wherein only leaves collected on the same day which were green in color, healthy, mature, and located near the stem were chosen for the extraction procedure (Raju et al., 2021).

3.2. Ethanolic Extraction

The collected leaves of *M. pendula* were thoroughly washed with distilled water to remove pollutants. The leaves were then subjected to air drying at room temperature for two weeks (Raju et al., 2021). The 100 grams of dried *M. pendula* leaves were pulverized using mortar and pestle. The pulverized leaves were macerated with 100% Ethanol contained within a sealed container at room temperature for seven days with periodic shaking and stirring (Comia et al., 2018). The extracts were then filtered using cheesecloth. Residual ethanol in the solution was evaporated by immersing the solution in a water bath for four hours at 80^{o}C.

3.3. Preparation of Set-Ups

M. pendula leaf extract was divided into proportions to form different experimental setups. The set-ups that were prepared had leaf extracts concentrations of T1: 5mL of extract/1L of distilled water, T2: 10mL of extract/1L of distilled water, T3: 15mL of extract/1L of distilled water which were the positive control, and control group, bayluscide, T4: 0.200mg of powdered bayluscide/1L of distilled water (Tchounwou et al., 1991). Each container contains 10 snails with a total of 720 matured snails that were used in the experiment

3.4. Molluscicidal Test

The molluscicidal activity was determined using the procedures described by Abdullah et al. (2017). The experimental design was laid out using a Complete Randomized Design (CRD) with three replications. Three setups for 24h and three setups for 48 h were prepared. Paddy field water, sourced from the snail habitat, was added to containers, providing a seven-centimeter depth. Then, ten test snails were placed in the containers covered with netting cloth allowing for adaptation and to prevent escapes. After a 30-minute acclimation period, 100 mL of the treatment was added. Snails were observed for 24 and 48 hours, after which the plant extract was removed. Snails were rinsed twice, and placed in 100 mL fresh distilled water for 24 hours for recovery, and then mortality rates were assessed (Abdullah et al., 2017).

3.5. Mortality Rate Determination

Snails were regarded dead when the shell color changed and the flesh section failed to withdraw from the shell (Abdullah et al., 2017). The mortality of the snails was confirmed by the full expansion of the operculum and the lack of response from the head when pricked with a sharp needle (Prabhakaran et al., 2017).

3.6. Lethal Concentration of Plant Extracts

The LC50 values and LC90 values were used as indicators to assess the toxicity of *Melothria pendula* leaf extract to GAS in the given time duration. LC values were often expressed as LC50 or LC90, representing the concentration at which 50% or 90% of the test organisms die, respectively. The LC50 value and LC90 value were calculated using statistical software SPSS version 29.01 with a 95% confidence limit.

3.7. Statistical Analysis

A two-way ANOVA test with replication was calculated using SPSS to determine the significant differences in variables. Standard deviation was also calculated to determine the consistency and reliability of the data.

4. Findings and Discussion

4.1 Effects of Different Concentrations in terms of Mortality Rate

Table 1

Mortality Rate P. canaliculata against different concentrations of M. pendula leaf extract and bayluscide after 24 hours

	24 HOURS			
	T1	**T2**	**T3**	**Control**
R1	6	7	8	10
R2	7	6	10	10
R3	6	8	9	10
Total	19	21	27	30
SD	4.71	8.16	8.16	0
Percentage of Mortality (%)	**63%**	**70%**	**90%**	**100%**

Table 1 presents the response shown by *Pomacea canaliculata* against different concentrations of *M. pendula* leaf extract and the control group (bayluscide) after 24 hours of exposure and 24 hours of the recovery period. The Treatment 3 (15mL/L) significantly affected *P. canaliculata*, resulting to a 90% mortality rate. Even at lower concentrations (5mL/L) and medium concentrations (10mL/L), mortality rates exceeded 50% among the ninety (90) mature snails exposed to *M. pendula* leaf extract with percentage mortality of 63% and 70%, respectively. Meanwhile, the control group established a 100% mortality rate during 24 hours of exposure. Higher concentrations correlated with increased mortality, indicating the effectiveness of *M. pendula* as a molluscicide. Based on these findings, it can be drawn that *M. pendula* leaf extract contains phytochemical elements such as terpenoids, tannins, and saponins, which allow it to retain natural control over mollusks. Plants, according to Thakur et al. (2019), was the most reliable approach for managing snail overpopulation because of their availability, low cost, rapid biodegradability, and mollusks' reduced statistical possibilities of obtaining resistance. This relates to the study conducted by Noorshilawati et al. (2020) wherein *Ipomoea batatas* leaf extracts, a plant with alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids, managed to kill more than 50% of the snails even at the lowest concentration with 24 hours of exposure.

As for the control group, which shows a mortality rate of 100% even with a low concentration of 0.200mg/L, means that its toxicity against *P. canaliculata* was high. Because of its comparatively high toxicity to *P. canaliculata* snails, bayluscide was chosen as the preferred molluscicide by the World Health Organization for managing these snails. Rather than reducing the snails' acid-soluble sulphydryl content, the mechanism of action of bayluscide was to interfere with their osmoregulatory system. It has been noted that bayluscide, which contains niclosamide, is hazardous to non-target creatures even while it completely kills the targeted snails (Zheng et al., 2021).

This concludes that using niclosamide and other commercial molluscicides with niclosamide were toxic enough to kill 100% of the snail population as normally reported by related studies, however, plant molluscicides were also a promising molluscicide that is non-toxic to targeted organisms. It was noted in the study that a higher concentration of *M. pendula* used as a molluscicide, the higher is the rate of mortality.

Table

Mortality Rate P. canaliculata against different concentrations of M. pendula leaf extract and bayluscide after 48 hours

	48 HOURS			
	T1	**T2**	**T3**	**Control**
R1	8	9	10	10
R2	9	7	10	10
R3	6	10	9	10
Total	23	26	29	30
SD	12.47	12.47	4.17	0
Percentage of Mortality (%)	**77%**	**87%**	**97%**	**100%**

Table 2 shows *P. canaliculata's* response to different concentrations of *M. pendula* leaf extract and the control group (bayluscide). After 48 hours of exposure, Treatment 1 (5mL/L) and Treatment 2 (10mL/L) achieved 77% and 87% mortality rates. Treatment 3 (15mL/L) demonstrated significant molluscicidal activity, resulting to a 97% mortality rate. Only 13% of the mature snail population survived exposure to *M. pendula* leaf extract. Notably, similar to the 24-hour exposure, Bayluscide led to a 100% mortality rate after 48 hours. Apparently, the same result was observed within 24 hours of exposure, that the higher the concentration applied, the higher the mortality rate.

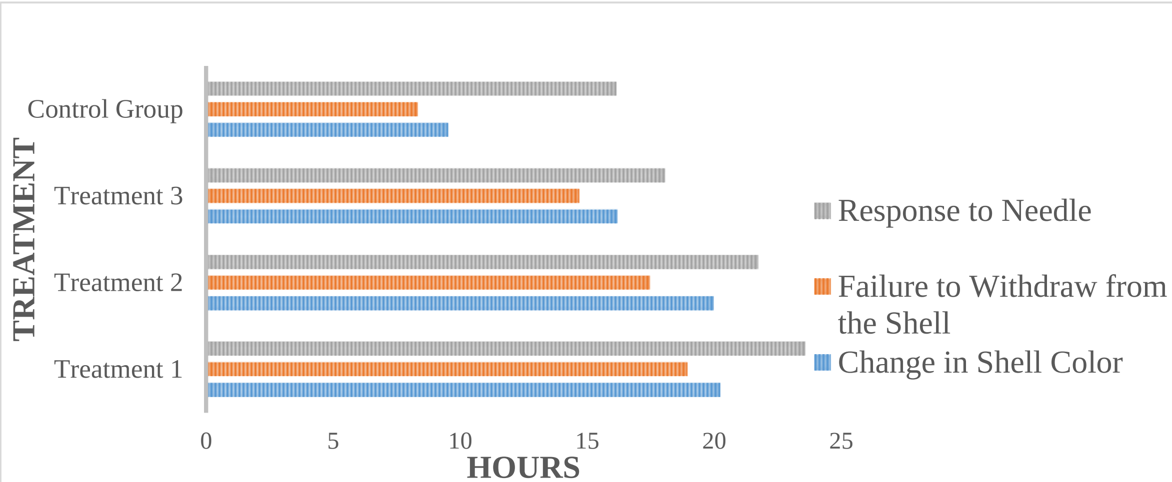
Relative to the molluscicidal results of *M. pendula* leaf extract in 48 hours (Mandefro et al. (2018), it can be drawn that both treatments became toxic to the snail as the concentration increased. Using *M. pendula* leaf extract, the 77% mortality rate of the lowest concentration increased to 97% at the highest concentration used. It is parallel to the mortality rate gathered using *Achyranthes aspera* wherein from 13% mortality rate of the lowest concentration, it increased to 100% at the highest concentration during 48 hours of exposure. The results were in agreement with Noorshilawati et al. (2020) and Abdullah et al. (2017) who discovered that certain plant extracts have molluscicidal capabilities. However, these depend on concentration and duration; the higher the concentration and time exposure, the higher the expected mortality rate.

4.2 Effects of Different Days of Exposure in terms of Mortality Rate

The time-dependent action of *M. pendula* leaf extract on *P. canaliculata* snails were depicted in figure 1 and figure 2. The lethal effect of different concentrations was observed for 24h and 48h.

Figure 1

Snails' behavioral response to extract after 24 hours



The *P. canaliculata* snails exposed to *M. pendula* leaf extract for 24 hours had a promising mortality rate of 63%, 70%, and 90% in Treatment 1 to Treatment 3, respectively. The color shift of the shell and the flesh portion's inability to separate from the shell served as indicators of the snails' mortality rate (Abdullah et al., 2017). From the observed response, snails' isolation to shell started from 14 hours in Treatment 3 until 19 hours in Treatment 1. However, snails exposed to bayluscide showed a more immediate response as a way to protect themselves from the extract after 8 hours of exposure. Additionally, snails exposed to different treatments started to change their shell color in a 16 to 20-hour duration period. In the control group, the snails started to change shell color after 11 hours. Sharp needles were used to prick the flesh parts of the snails to determine their cause of death as described in the methods used by Prabhakaran et al. (2017). Snails fail to show movement and response when pricked after 18 to 24 hours in ascending treatment concentrations and 16 hours in the control experimental set-up.

According to Ye et al. (2018), toxic extracts caused the snail to perform a noticeable behavioral response as a protection. As the snails were gradually exposed to higher concentration, the shorter time it takes for *P. canaliculata* to cease moving and close the operculum rapidly. It was also noted that the behavior was due to snails' increased resistance. The snails' immobility was caused by the combined activities of phytochemicals like tannins and saponins. (Vehovszky et al., 2019). Similarly, direct tissue destruction was made from exposure to the extracts which resulted in decreased tissue protein and hemolymph causing the snail to remain on its shell. Meanwhile, Kolawale (2021) stated that snails do not change shell color on their own, and external factors such as being exposed to a controlled environment and treatment cause discoloration in the shell. Color loss in the snails' shell was a sign of deterioration as exposed to a toxic concentration. The longer the snails were exposed to the extract, the shell color lightens and gradually deteriorates. Esquilla et al. (2021) stated that the tannins in the plant extract changed the color of the snail shell. Tannins, known for their astringent properties, can bind to various organic compounds and minerals within the snail's tissue, resulting in a yellowish tint on the shell. With this, test snail discoloration, immobilization, and no response to needle pricking indicated that the snail weakened and gradually died due to exposure to *M. pendula* leaf extracts.

Figure 2

Snails' behavioral response to extract after 48 hours

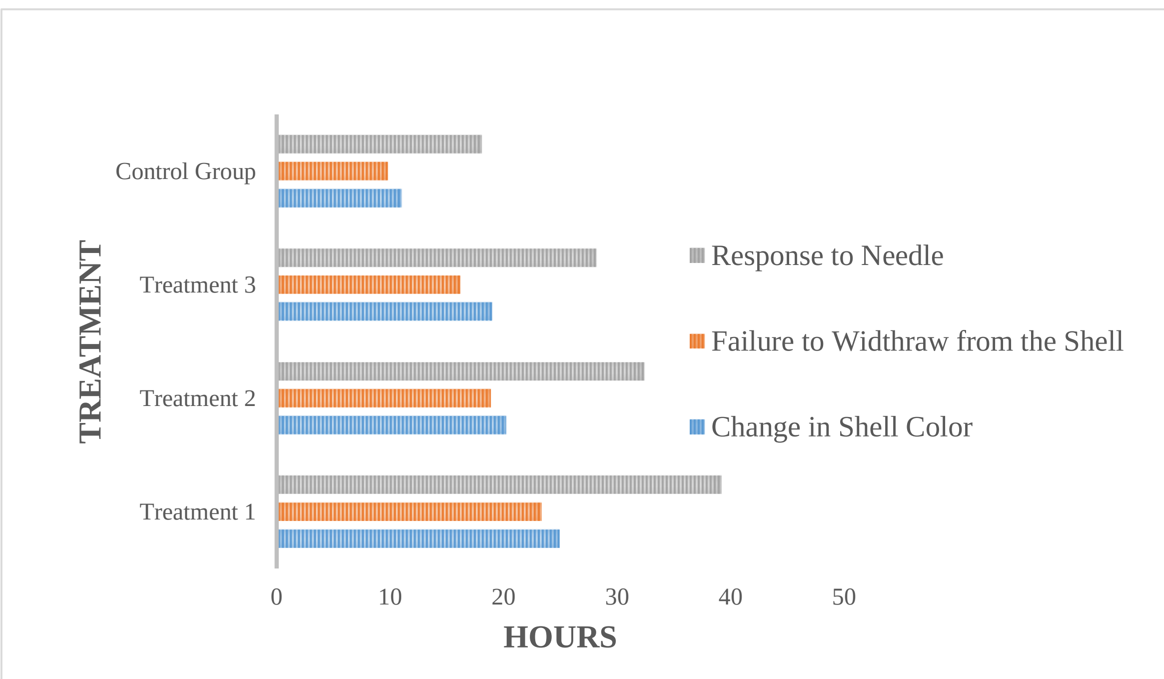


Figure 2 depicts the snails' response to *M. pendula* leaf extract after 48 hours of exposure. Based on the result, the flesh portion of the snail refused to be withdrawn from the shell after 16 hours to 23 hours, as seen from treatment 3 to treatment 1, respectively. Moreover, 18 to 24 hours of exposure was needed for the snail to change its color in 48 hours of exposure. This amount of time was comparable to the snail's time response after a day of exposure. It took the snail, regardless of the exposure length, between 14 and 23 hours to become motionless within its shell and 16 to 24 hours to change its color from dark red-brown to lighter yellow-brown. This is more of a result based on varying treatments and not of the time exposure. After 28 to 35 hours of exposure, the death of the snail was determined by opening the snail's operculum and recording the snail's response when pricked with a needle resulting in a 9.5% higher mortality rate compared to 24 hours of exposure. This means that the longer the time of exposure of the snails to the extract, the higher the mortality rate becomes.

As noticed from tables 1 and 2, the mortality rate of Treatment 3 in 24 hours which is 90% comes close to the mortality rate of Treatment 2 in 48 hours which is 87%. It only means that as the time exposure to *M. pendula* leaf extract increased, the more toxic it became to the snails. For higher days of exposure, it is observed that the mortality rate increased even with a low concentration of extract.

It was observed that the rate of mortality rate increased as the time exposure of *Melothria pendula* leaf extract increased. This is evident when compared to the study conducted by Wang et al. (2022) where extended exposure time increased the total snail mortality. This was also similar to the study of Noorshilawati et al. (2020) wherein it has been demonstrated that the number of dead snails increases with an extended length of exposure to treatments. From a 30% mortality rate during 24 hours of exposure to a 100% mortality rate in 48 hours at 10, 000 ppm or 10mL/L of *Ipomoea batatas* leaf extracts. Moreover, it was recorded in the study of Mandefro et al. (2018) that snails' mortality in mid-concentration of 50-75ppm or 0.05-0.075mL/L increased with prolonged exposure time. According to the study, from a 58% mortality rate in 24 hours, it increased to 61% after 48 hours. Similarly, in connection to the result of the mortality rate in terms of days of exposure, a study conducted by Malana and Salvador (2020) depicted a 48-hour result of mortality with treatment one (1) and treatment (2) of *Physalis minima Linn.* to achieve a molluscicidal effect comparable to that of the commercially available molluscicide than that of 24 hours of exposure. This goes the same with 48 hours result of the study having an 87% mortality rate, a promising number comparable to the effect of bayluscide.

Figure 3

*Pomacea canaliculata Reaction to Melothria pendula leaf extracts***:** *a.) Active snails before treatment exposure, b.) Frothy foam around the snails and snail's egg, and c.) P.canaliculata mucus secretion*

It was depicted in figure 3(a) that *P. canaliculata* snails were active before their exposure to different treatments and paddy field water was cleared from snail secretes. However, it was noticed that the snails at lower treatments lay eggs after 24 hours, and as concentration increased, the production of eggs lowered to none. It relates to the study of Sisa et al. (2016), which demonstrated that increasing the concentration of the molluscicide decreased the reproduction of the snails. It was also noticed that the snails tried to escape and crawl out of the container after the treatments were poured out. According to Malana and Salvador (2020), treatments from extracts eventually irritate the snails. This is *P. canaliculata's* defensive action to avoid coming into touch with treated water.

In figure 3(b), frothy foam around the snails was noticed. According to Dronet (2017), the production of frothy foam around the snail is its way of protecting itself from aggression. From figure 3(b) to figure 3(c), secretion of mucus increased from 24 hours to 48 hours. Abdullah et al. (2017) stated that *P. canaliculata's* secretion of mucus in response to the leaf extract was a means to minimize their contact with the molluscicides.

The presence of active compounds, such as saponins, hinders the breathing process of GAS by impeding the diffusion of oxygen through their gills. This obstruction is facilitated by the secretion of mucus. Furthermore, the toxicity induced by saponins affects the snails' cell membranes and reduces surface tension, preventing them from crawling out. In the study conducted by Picardal et al. (2018), it was noticed that slugs and snails tend to produce large amounts of mucus wherein the excessive mucus secretion can induce dehydration, which can lead to the snails' death.

Meanwhile, the presence of terpenoids has caused the dilation of *P. canaliculata* muscle which was rooted in slow movement of crawling out. This observation is comparable to the results of Ruma and Sanchez (2016) wherein the presence of terpenoids caused the closure of the operculum, dilation of muscles, and secretion of mucus. Lastly, the presence of tannins acts as repellent, and antifeedants, and prevents insect growth regulatory activities which also causes the snail to be irritated by the extract (Rosli et al., 2021). With this, the extract from *Melothria pendula* leaf extract induces reactions in GAS, impacting their mortality rate.

4.3 50% and 90% Lethal Concentration of Melothria pendula to Golden Apple Snail

Table 3

LC50 and LC90 results for 24 hours and 48 hours

		95% Confidence Level	
HOURS	**Probit equation Y = a+bx**	**LC50**	**LC90**
24 Hours	Y=-0.903x+1.666	3.486mL/L	20.500mL/L
48 Hours	Y=-0.698x+1.976	2.255mL/L	10.040mL/L

M. pendula leaf extract shows a 74% mortality rate after a day of exposure and 87% after 48 hours of exposure. Findings in table 3 show that within 24 hours of exposure, the Lethal Concentration of 3.49mL/L killed 50% or half of the snails’ population with LC90 results of 20.50mL/L of concentration killing 90% of the population. On the other hand, the result in 48 hours, shows that it only needs a lowered concentration of 2.26mL/L to kill 50% of the population compared to 24 hours. The Lethal Concentration of 10.040mL/L for killing 90% of the snail’s population in 48 hours is almost half the value of concentration needed from 24 hours.

It is noted that LC50 and LC90 are inversely proportional to toxicity. A concentration with lower LC50 and LC90 values was more toxic than the concentration with higher LC50 and LC90 values. This indicates that 48 hours of exposure having a lower 50% and 90% Lethal Concentration compared to 24 hours of exposure is more toxic to snails and can kill mollusks with more lethality. It can also be said that the toxicity of the leaf extract is directly proportional to the time of exposure. The longer the snails were immersed in each treatment, the more toxic the extracts became.

The result of the analysis relates to the study of Shen et al. (2018) wherein from two concentrations of 48 hours and 72 hours, 72 hours got a lower concentration of 0.30mg/L S. canadensis extract compared to the LC50 value of 0. 57mg/L on 48 hours of exposure. The findings of Malana and Salvador (2020) also revealed that an increase in the concentration of the extract induced an increase in the mortality rate of the snails at the same exposure period, while an increase in the length of exposure led to the concentration of the extracts becoming more toxic. Moreover, Abdullah et al. (2017) concluded that a concentration of 1611 ppm was needed to kill the 50% population of the snail with *E. rheedii* bark extract which was just a little higher than its lowest concentration of 1000 ppm meanwhile the extract LC90 value of 4266 ppm was closer to the second lowest concentration used which was 5000 ppm. Both LC50 and LC90 values were much lower than the determined highest concentration used of 20000 ppm.

4.4 Determining Significant Differences between the Effects of Different Concentrations and Days of Exposure to the Mortality of the Snail.

Table 4

Levene's test of equality of error variance

		Levene Statistic	df1	df2	Sig.
Mortality	Based on Mean	2.584	7	16	.055
	Based on Median	1.143	7	16	.386
	Based on the Median and with adjusted df	1.143	7	9.091	.415
	Based on trimmed mean	2.476	7	16	.063

Note: Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
Dependent variable: Mortality
b. Design: Intercept + Concentration + Exposure + Concentration * Exposure

Table 4 displays the results of Levene's Test for Equality of Error Variance, which was employed to examine whether the variables exhibit equivalent variances or homogeneity of variance. The results established that the mean, median, median with adjusted degrees of freedom, and trimmed mean exhibit p-values exceeding 0.05, indicating statistical significance, namely: 0.055, 0.386, 0.415, and 0.063, respectively. As the p-value for Levene's test exceeds 0.05 ($p>0.05$), the homogeneity assumption of variance was met. Therefore, the assumptions for ANOVA were also met.

Table 5

Two-Way ANOVA test with Replication

Tests of Between-Subjects Effects								
Dependent Variable: Mortality								
Source	**Type III Sum of Squares**	**f**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**	**Noncent. Parameter**	**Observed Power[b]**
Corrected Model	41.292[a]		5.899	6.435	**001**	.738	45.045	.990
Intercept	1751.042		1751.042	1910.227	**000**	.992	1910.227	1.000
Concentration	33.792		11.264	12.288	**000**	.697	36.864	.997
Exposure	5.042		5.042	5.500	**032**	.256	5.500	.596
Concentration * Exposure	2.458		.819	.894	**466**	.144	2.682	.202
Error	14.667	6	.917					
Total	1807.000	4						
Corrected Total	55.958	3						

Notes: R Squared = .738 (Adjusted R Squared = .623)
b. Computed using alpha = .05

Table 5 depicts the between-subject effects test, which uses concentration and period of exposure as independent variables and mortality as the sole dependent variable. The statistical results indicate a noteworthy distinction between the concentrations employed and the mortality of the snails, with a significant level of *0.000* ($p<0.05$). This suggests that as the concentration increases, so does the mortality rate. This relates to the study of Prabhakaran et al. (2017) that an increase in the concentration of plant extracts resulted in high mortality rates of *Pomacea maculate*). Furthermore, a notable variance was observed between the duration of exposure and the mortality of *P. canaliculata*, with a significance level of *0.032* ($p<0.05$). This was reflected in the result presented in table 4, which denotes that the higher the time exposure of the snails to *M. pendula* leaf extracts, the higher the mortality rate becomes. This also relates to the study of Mandefro et al. (2018) emphasizing that snail mortality increased with prolonged exposure time. Nevertheless, a value of *0.466* was obtained for the interaction effect of concentration and duration of exposure. The results indicate the absence of a significant interaction effect between concentration and duration of exposure since it exceeds the significant value of 0.05. It can be inferred that the influence of concentration used does not depend on the time exposure. As the mortality rate was determined by the concentration applied, the duration of exposure for the snails to the extracts holds no relevance. It can also be said that the influence of time exposure does not depend on the concentration. Since both variables do not influence each other, the specific concentration utilized was inconsequential in determining the snail mortality rate based on exposure duration.

Table 6

Descriptive statistics (standard deviation for 24h and 48h)

Dependent Variable: Mortality

Concentration	Exposure	Mean	Std. Deviation	N
Control	24 hrs	10.0000	.00000	3
	48	10.0000	.00000	3
	Total	10.0000	**.00000**	6
5ml/L	24 hrs	6.3333	.57735	3
	48	7.6667	1.52753	3
	Total	7.0000	**1.26491**	6
10ml/L	24 hrs	7.0000	1.00000	3
	48	8.6667	1.52753	3
	Total	7.8333	**1.47196**	6
15ml/L	24 hrs	9.0000	1.00000	3
	48	9.6667	.57735	3
	Total	9.3333	**.81650**	6
Total	24 hrs	8.0833	1.67649	12
	48	9.0000	1.34840	12
	Total	8.5417	**1.55980**	24

Table 6 shows the result of 24 hours and 48 hours of exposure to each concentration where Treatment 3 (15 mL/L) attained the lowest standard deviation of 0.81650, which determines the accuracy and consistency of the data that are clustered tightly around the mean. However, this was followed by Treatment 1 (5 mL/L) with a higher standard deviation of 1.26491 and Treatment 2 (10mL/L) with the highest standard deviation of 1.47196 among the three. This means that data points are spread further away from the mean. This proves that Treatment 3 demonstrated the highest molluscicidal effectiveness against GAS with a mean of 9.3333. Additionally, in the sum of the concentrations it was revealed that 48 hours of exposure got a lower standard deviation of 1.34840 than 24 hours of exposure with 1.67649 standard deviation. It indicates that the longer the time exposure, the higher the mortality rate. The control group, Bayluscide, meanwhile attained a 0.000 SD with a total mean of 10.

5. Conclusion

The study found that the effects of different concentrations and days of exposure on GAS mortality rate were directly proportional. Therefore, the higher the concentration and longer the days of exposure of *M. pendula* leaf extracts that were used, the higher the mortality rate of *P. canaliculata.* Additionally, the 50% and 90% Lethal Concentrations of *M. pendula* to GAS were more toxic at 48 hours of exposure. Results show that the lower the lethal concentration becomes, the more toxic the extract is. While there is a significant difference between the effects of different concentrations and days of exposure on the mortality of the snail, there are no notable differences in the interaction effect of different concentrations and exposure durations. As *Melothria pendula* leaf extract, a plant-based molluscicide, is more biodegradable, environment friendly, and less harmful to organisms other than their intended targets, it can reduce chemical pollution and have a positive environmental impact. By being more selective and focusing primarily on mollusks, they preserve beneficial species and save biodiversity. Additionally, they help avoid resistance development in pests due to their various mechanisms of action. Hence, it is recommended to incorporate treatments exceeding 15mL/L and explore prolonged exposure durations for testing.

The limitation lies in the specificity of this study to GAS, implying that further exploration is necessary to ascertain whether *M. pendula* leaf extracts exhibit similar effects on other pest species. Future researchers should also focus on broader field trials, comparative studies, and integrated pest management strategies. Thus, this study's findings can serve as a foundation for future research aimed at investigating the efficacy of *M. pendula* leaf extracts in controlling rice field pests.

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