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# Molluscicidal activity of *Melothria pendula* leaf extract 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 considered unpractical because of their toxic effect on non-target organisms, hence this study on innovative, plant-based extract to control golden apple snails. Leaves of pipinong gubat, 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) got the highest mortality rate, 90% and 97% among other concentrations used in 24 hours and 48 hours, respectively. The control group, bayluscide, attained 100% mortality both in 24 hours and 48 hours. Statistical analysis revealed that the LC50 and LC90 values of 48 hours of exposure showed an increased toxicity level against the snails than of 24 hours of exposure. Moreover, there is a significant difference between the effects of different concentrations and days of exposure on the mortality rate of the snail. Results indicated that Melothria pendula extract is a novel, environment- friendly molluscicide to control Pomacea canaliculata.

**Keywords:** *molluscicide, golden apple snails, pipinong gubat, leaf extract*

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## Introduction

The Golden Apple Snail (*Pomacea canaliculata*) is one of the most fatal invasive species in the Philippines, devastating lowland rice production (Picardal et al., 2018, Rosli et al., 2021). Introduced in 1982, Pomacea canaliculata initially supported low-income Filipino farmers but later on began devastating rice crops in northwest Luzon (Cowie, 2013). *Pomacea canaliculata* was one of three snails listed as one of the world's 100 worst invasive species by the World Conservation Union (IUCN) in 2000 (Joshi et al., 2017; Yang et al., 2018; Liu et al., 2018).

Kuhol, or the Golden Apple Snails in the Philippines, has gained popularity as an alternative to fish and meat (Niepes et al., 2023). However, P. canaliculata has been identified as a carrier of diseases and parasites crucial to human health (Chen et al., 2021; Yang et al., 2018). Numerous countries invested heavily in control methods of golden apple snails, 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 (Djeddour et al., 2021).

Furthermore, despite being recommended by the World Health Organization as a molluscicide, Niclosamide faces limitations due to its inability to dissolve in water and its negative impacts on fish and non-target organisms (Zheng et al., 2021; Dai et al., 2014). As a result, there was a need to create an approach for safe and efficient control of Golden Apple Snails (GAS) using Melothria pendula (Pipinong gubat), a natural plant uncultivated in the Philippines. Raju et al. (2021) highlighted the presence of tannins, saponins, and terpenoids in Melothria pendula leaves, crucial in regulating the snail population.

This study aimed to investigate the potential application of *Melothria pendula* as a molluscicide against *Pomacea canaliculata* (Golden Apple Snails). Specifically, this study sought to determine the effects of different concentrations and days of exposure against Golden Apple Snail in terms of mortality rate; the 50% and 90% Lethal Concentration of *Melothria pendula* to Golden Apple Snail; and the significant difference between the effects of different concentrations and days of exposure to the mortality of the snail.

## Methodology

***Collection of test organism and plant materials.*** The mature snail *Pomacea canaliculata* and *Melothria pendula* leaves were gathered from the town of Bansud, Oriental Mindoro. Snails with a weight greater than 5 grams and a height ranging from 25mm to 35 mm were subjected to the acclimatization process (Picardal et al., 2018). The snails were left to acclimatize for seven days before being used in the experiment, and only active golden snails were selected for the experiment (Massaguni & Latip, 2015). *Melothria pendula* 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).

***Ethanolic extraction.*** The collected leaves of *M. pendula* were thoroughly washed with distilled water to remove impurities (Dianito et al., 2022). The leaves were then subjected to air-drying at room temperature for two weeks (Raju et al., 2021). 100 grams of dried *Melothria pendula* leaves were pulverized using a mortar and pestle. The pulverized leaves were macerated with 100% ethanol in an air-tight container at room temperature for seven days with occasional stirring and shaking (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.

***Preparation of set-ups.*** *Melothria pendula* leaf extract was divided into proportions to form different experimental setups. The set-ups that were prepared have leaf extracts concentrations of T1: 5mL/L, T2: 10mL/L, T3: 15mL/L, and control group, bayluscide, T4: 0.200mg/L.

***Molluscicidal test.*** The molluscicidal activity was performed using the methods described by Abdullah et al. (2017). The experimental design was laid out using a Complete Randomized Design (CRD) with three replications. Paddy field water, sourced from the snails' habitat, was added to containers, providing a seven-centimeter depth. Ten test snails were placed in individual containers covered with netting cloth. 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, and placed in fresh distilled water for 24 hours for recovery, and then mortality rates were assessed.

***Mortality rate determination*.** Snails were considered dead when there was a change in the shell color and failure of the flesh portion to withdraw from the shell (Abdullah et al., 2017). The death of the snails was further ascertained by the complete opening of the operculum and when the head did not respond when pricked with a sharp needle (Prabharakan et al., 2017; Khalid., 2020).

***Statistical analysis.*** The LC50 value and LC90 value were calculated using statistical software SPSS version 29.01 (SPSS Inc.; Chicago, IL, USA) with a 95% confidence limit. A Two-Way ANOVA test with replication was also calculated using SPSS to determine the significant differences in variables.

## Findings

### *Effects of different concentrations in terms of mortality rate*

The response of *Pomacea canaliculata* to various concentrations of *M.pendula* leaf extract showed Treatment 3 (15mL/L) significantly affected *P. canaliculata*, resulting in a 90% mortality rate. Even at lower concentrations (5mL/L) and medium concentrations (10mL/L), mortality rates exceeded 50% among the ninety mature snails exposed to *M.pendula* leaf extract. Higher concentrations correlated with increased mortality, indicating the effectiveness of *M.pendula* as a molluscicide (Salem et al., 2017; Mandefro et al. 2017).

After 48 hours of exposure, Treatment 1 (5mL/L) and Treatment 2 (10mL/L) achieved 77% and 87% mortality rates, respectively. Treatment 3 (15mL/L) demonstrated significant molluscicidal activity, resulting in 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.

### *Effects of days of exposure in terms of mortality rate*

The time-based effects of *M. pendula* leaf extract on *P. canaliculata* snails were observed over 24 and 48 hours. Snail isolation within their shells began between 14 to 19 hours in Treatment 3 to Treatment 1 over 24 hours and between 16 to 23 hours over 48 hours, respectively. Moreover, snails exposed to bayluscide exhibited a quicker defensive response within 8 hours of exposure.

Furthermore, changes in shell color among snails exposed to different treatments occurred between 16 to 24 hours. Gradually, snails displayed reduced movement and responsiveness when prodded after 18 to 24 hours in 24-hour exposure and 28 to 35 hours in 48-hour exposure at declining treatment concentrations. Consequently, a 9.5% higher mortality rate was observed after 48 hours compared to 24 hours of exposure, indicating an escalated mortality rate with prolonged exposure to the extract (Noorshiwalati et al., 2020; Wang et al., 2022).

***50% and 90% Lethal Conentration of Melothria pendula to Golden Apple Snail***

The findings indicate that within 24 hours of exposure, a Lethal Concentration of 3.49mL/L caused a 50% mortality rate among the snail population, while an LC90 of 20.50mL/L resulted in a 90% mortality rate. However, over 48 hours, a reduced concentration of 2.26mL/L was sufficient to achieve a 50% mortality rate, with an LC90 of 10.040mL/L for a 90% mortality rate. This concluded that 48 hours of exposure with lower LC50 and LC90 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 (Malana & Salvador, 2020; Shen et al., 2018).

***Significant differences between the effects of different concentration and days of exposure to the mortality of the snails***

Based on the result, there is a significant difference between the concentrations used and days of exposure to the mortality of the snails with a significant level of 0.000 and 0.032 respectively ($p<0.05$). This means that as the concentration gets higher and days of exposure get longer, the higher the rate of mortality will be. However, a value of 0.466 was gathered between the interaction effect of concentration and time exposure showing no significant interaction effect as it exceeds the value ($p<0.05$). It can be said that the influence of concentration and time exposure does not depend on each other.

## Conclusion

The result of the study revealed that increasing concentrations and longer exposure durations of *Melothria pendula* leaf extracts led to higher mortality rates of Golden Apple

Snails. Additionally, the 50% and 90% Lethal Concentrations of *Melothria pendula* to Golden Apple Snail were more toxic at 48 hours of exposure. Results show that the lower the lethal concentration becomes, the more toxic the extract is. There is a significant difference between the effects of different concentrations and days of exposure on the mortality of the snail. However, there is no significant difference between the interaction effect of different concentrations and days of exposure. Furthermore, it is recommended to incorporate treatments exceeding 15ml/L and explore prolonged exposure durations for testing.

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