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Research Article

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Molluscicidal Effect of Eco-Friendly Agricultural Substances for Controlling Golden Apple Snails (*Pomacea canaliculata*, Lamarck)

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Abstract

The golden apple snail (*Pomacea canaliculata*) has been utilized as a natural and eco-friendly control of weeds in rice paddy fields. However, *P. canaliculata* can damage other crops. In this study, the effectiveness of plant extracts from various natural sources that are reportedly effective against pests in the control of *P. canaliculata* was investigated. The four plant extracts were effective against *P. canaliculata* and ranked in descending order as green tea seed (*Camellia sinensis*) > root of red spider lily (*Lycoris radiata*) > leaves of tobacco (*Nicotiana tabacum*) > root of sophora (*Sophora flavescens*). The mortality rate of *P. canaliculata* was increased using 200 to 2000 mg/kg of green tea seed powder. However, shrubby sophora root extract did not significantly increase the mortality rate. The LC_{50} and LC_{90} of green tea seed, tobacco leaves, shrubby sophora root, and red spider lily root were 900 and 2800 mg/L, 956 and 2320 mg/L, 2162 and 5325 mg/L, and 512 and 1054 mg/kg, respectively. The LC_{50} and LC_{90} of ground powder of *C. sinensis*, *N. tabacum*, *S. flavescens*

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and *L. radiata* were 248 and 646 mg/L, 403 and 733 mg/L, 409 and 905 mg/L, and 493 and 1141 mg/L, respectively. The findings indicate the remarkable control potency of green tea seeds against the golden apple snail. An organic material incorporating the four plant powders may help control green apple snail in an ecosystem-friendly manner.

Key words: Golden apple snail, Green tea (*Camellia sinensis*), Red spider lily (*Lycoris radiata*), Shrubby sophora (*Sophora flavescens*), Tobacco leaves (*Nicotiana tabacum*)

Introduction

Golden apple snails were introduced to Asian countries as a source of high-protein food and agricultural income in 1980s including Korea in 1983. The species of Golden apple snails introduced in Korea is *Pomacea canaliculata*, which is native to South America and has significant differences in appearance, host ranges and reproduction compared to the native snails in Korea [1,2]. This has begun to be used as a weed control agent in eco-friendly rice cultivation by utilizing their feeding habits since 1992 [3,4]. The weed management was highly effective, with a 96% control immediately after treatment and a sustained 98% control rate observed over the subsequent 7 days [5,6]. However, due to climate change, golden apple snails are now able to overwinter in Korea, causing damage to crops in the southern region [6]. Their ability to withstand various environmental stressors makes them a significant pest in waterlogged cultivated areas, attributed to their intrusive nature, adaptive mechanisms, and high survivability and serve as bioindicators of pollution. Consequently, effective strategies are essential to control their population. A lots of studies have been made on physiological and ecological research on overwintering and crop damage caused by golden apple snails in the field of eco-friendly agriculture [7-10]. The various studies focused on the physiological and ecological aspects of preventing overwintering and crop damage caused by GAS in the context of eco-friendly agriculture [11]. In 2015, Panda et al. [12] reported the mortality rate was correlated positively with the extract concentrations as the mortality of snail increased with the increase of extract concentration In this review, they explored as well the management of apple snails for ecotoxicity without compromising the environment and other inhabitants as examined their role as semi-sessile invasive ecotoxic markers and indicated that snails accumulate various substances, including 0.19-0.21% biphenyl ethers, 1.65% copper sulfate with a 26.7% mortality rate, 1.53-29.7% ivermectin, 43% polybrominated biphenyl ethers, 59% triphenylphosphine oxide, and 8-100% of various heavy metals and nanoparticles [12]. In 2017, Prabhakaran et al. [13] suggested that a combination of molluscicidal compounds from different plant extracts, acting synergistically, could serve as a viable alternative, and tested against *Pomacea maculata* to explore this approach, ethanolic extracts from six distinct plants (neem, tobacco, nerium, pongamia, zinger and piper). Among the various combinations were investigated, a binary extract (1:1) of nerium and tobacco (LC_{90} 177.71 mg/L, 48 h), as well as two triherbal extract formulations (1:1:1) of (nerium + tobacco + piper) and (nerium + tobacco + neem), demonstrated high efficacy. The LC_{90} values for these formulations were 180.35 and 191.52 mg/L, respectively, under laboratory conditions. The results indicate that selectively combining potent molluscicidal herbal extracts proves effective in managing *P. maculata* in laboratory [13]. In 2020, Yoon et al. [14] investigated that *Camellia sinensis* L. seed extracts have antimicrobial and antitumor activities as well as showed antifungal activities against Candida albicans IFO 1594 and *Cryptococcus neoformans*. Antifungal activity of seed extract was not decreased by heating at 80 and 100°C for 30 min or at 121°C for 15 min, indicating heat stability of seed component [14]. This paper examines the current stage of GAS invasion and its implications for global rice production. This review also includes an in-depth discussion of the various biological based strategies in pest management and the recent technological breakthroughs in entomopathogenic nematodes and entomopathogenic fungi as molluscicides and integrated pest management [15,16]. The Korean Ministry of Environment has designated GAS as a grade 2 organism for legal ecosystem management since 2007, leading to ongoing debates on GAS regulation. GAS is recognized as a rice pest, causing significant harm to rice crops in countries such as the Philippines, Taiwan, Southeast Asia, and Japan [17]. The International Union for Conservation of Nature (IUCN) classifies them as one of the world's top 100 invasive alien species. Unlike native snails that reproduce as larvae, golden apple snails reproduce through eggs, contributing to their pest status in original tropical regions [18]. The *P. canaliculata*, has been employed as a natural and environmentally friendly approach to manage weed growth in rice cultivation [19]. Despite its success in rice farming, *P. canaliculata* has the potential to inflict harm on other crops. Therefore, it is necessary is to develop a secure and effective organic agricultural material utilizing natural plant extracts as a control agent for the golden apple snail. It is to create a product that is not only eco-friendly but also highly efficient, suitable for application in organic farming practices. In order to find an environmentally friendly organic agricultural substance that is both safe and highly efficient, an experiment has been conducted to test the effectiveness of various organic farming materials in controlling golden apple snails. These materials included green tea seed, dried tobacco leaves, yambean leaves, red spider lily root, mustard greens, shcubby sophora root, neem extract, pyrethrum flower extract. This study aims to not only find natural plant extracts as an organic control agent against golden apple snails (GAS) to prevent the escape of them into ecosystem but also to develop a product that is both eco-friendly and highly effective to control GAS, which can be utilized in organic farming.

This approach will provide a safe alternative for organic farming practices through develop an eco-friendly organic agricultural material that is safe for use as well as highly effective by utilizing plant extracts from nature as an organic control agent for golden apple snails which can be used in organic farming. Furthermore, golden apple snails stand out as one of the most resilient freshwater invasive species, causing considerable economic damage to vegetation, particularly in rice fields. The mortality of GAS may be subjected to various concentration and solvent extracts indicated that neem seed crude extracts significantly killed the GAS.

Materials and Methods

Collection of Snails (*Pomacea canaliculata*, Lamarck)

The golden apple snails had been collected from a rice paddy field infested with snails at Gokseong, Jeonnam, Republic of Korea. Additionally, the snails for the molluscicidal test were purchased from "Dolsil snail village" at Gokseong, Jeonnam, Republic of Korea. They have been identified based on their conchological characteristics as *P. canaliculata* [20,21]. The snails were measured by a vernier caliper and used ranging in shell length from 10 to 20 mm (Fig. 1). These snails were acclimated in a 40 L laboratory aquarium tank (80 × 50 × 30 cm) filled with dechlorinated tap water at room temperature (22 ± 2°C). The snails were provided with carp food containing menthol two to three times a week, adjusted based on their consumption to prevent bacterial growth and water contamination.

Fig. 1. Gold apple snail (*Pomacea canaliculata*) eating rice field weeds.

Collection of Plant Materials

A total of 5 kg green tea seed (*C. sinensis*), tobacco leaves (*Nicotiana tabacum*), shrubby sophora root (*Sophora flavescens*) had been purchased from Garamwon, Wholeleaf tobacco and Talk talk commerce, the Republic of Korea. And also, 2 kg of fresh brown mustard (*Brassica juncea*), 2 kg of red spider lily (*Lycoris radiata*) and 2 kg of yam bean (*Pachyrhizus erosus*) were collected from the local farm beside Jeonnam, Republic of Korea (Fig. 2). The all plant samples were thoroughly cleaned with running tap water and dried under the shade for three weeks at room temperature (22 ± 2°C). The green tea seeds, tobacco leaves and shrubby sophora root were chopped into small pieces and dried under shade for four weeks. The dried substances were ground into a coarse powder using a food blender and then stored in airtight containers. These powdered samples were utilized to evaluate snail mortality under laboratory conditions. The specific plant materials assessed for their molluscicidal properties are outlined in Table 1.

Preparation of Plant Extracts

The 100 g of finely ground natural material were placed into non-woven fabric, securely tie it, and put into an extractor (Daewoong DW-890, Korea) contain-

Fig. 2. Plants used to assess molluscicidal properties for the golden apple snails. A, Green tea plant (*Camellia sinensis*); B, Tobacco (*Nicotiana tabacum*); C, Shrubby sophora (*Sophora flavescens*); D, Red spider lily (*Lycoris radiata*).

Table 1. Major plant materials evaluated for molluscicidal properties

Substances	Scientific name	Family	Parts used
Green tea	*Camellia sinensis* L. Kuntze	Theaceae	Seeds
Tobacco	*Nicotiana tabacum*	Solanaceae	Leaves
Shrubby sophora	*Sophora flavescens*	Fabaceae	Roots
Red spider lily	*Lycoris radiata* (L'Hér.) Herb.	Amaryllidaceae	Roots

ing 2 L of distilled water at 70°C for 24 hours to extract the green tea seed, tobacco leaves, shrubby sophora root and red spider lily root. After naturally drying, store the resulting solution in the refrigerator. Utilize eight different types of natural substances for this process. Subsequently, pour 5 L of tap water into square cages measuring 30 by 40 by 25 cm.

Formulation Preparation of the Organic Agricultural Materials using Plant Extracts and Powder

We prepared “Uleong-moa A” that was composed with tea seed extract (35%), shrubby sophora extract (10%), kaolin (54%) and xanthan gum (1%). Uleong-moa B is consisted of tea seed powder (30%), tobacco leaf powder (10%), sophora root powder (10%), red spider lily root powder (10%), kaolin (39%), xanthan gum (1%) and water (40%). This granule formulation is in progress to apply for announcement of the list of organic materials. And also, we formulated “Uleong-moa B” through mixture of tea seed powder (theasaponin, 30%), tobacco leaf powder (nicotin, 10%), shrubby sophora root powder (mathrin, 10%), red spider lily root powder (lycorine, 10%), kaolin (39%), xanthan gum (1%) and water (40%). The “Uleong-gagsi” granule was purchased from Hanmaeum BioIndustry Co., Jeonnam in the republic of Korea.

Evaluation of the Molluscicidal Potency of Plant Extracts and Powder

The 20 snails were applied in each cage, categorized by size (over 25 mm, 15-20 mm, under 10 mm). After that, the 50 ml (1%) of the raw solution was added for each of the potential molluscicidal substances and assess for mortality after 24 hours. The concentration of the treated material was measured using a sugar meter (ATAGO PAL-1) to validate the effectiveness of the treatment. The assessment of the molluscicidal effectiveness of plant extracts against juvenile snails of *P. canaliculata* (Lamarck) followed the guidelines by the World Health Organization (WHO) [14]. Various concentrations of individual plant extracts were prepared for bioassay against the snails. A stock solution of each crude plant extract was prepared by dissolving 1 g of each extract in 1000 mL of distilled water, resulting in a 0.1% concentration (1000 mg/L). From this stock solution, a 0.005% (50 mg/L) test solution was derived through tenfold dilution with dechlorinated tap water. Similarly, concentrations ranging from 100 to 5000 mg/L were prepared from the 5% stock solution. Plastic trays containing 20 uninfected juvenile snails (10 to 20 mm shell length) were exposed to 1000 mL of different concentrations of plant extract test solutions separately, with each extract test solution having eight different concentrations and three replicates of 20 snails. Control were conducted using dechlorinated tap water alone. A total of 378 snails were used for evaluating each plant extract. All molluscicidal assessments were conducted at room temperature (22 ± 2°C) under normal diurnal lighting (16L:8D), with plastic trays covered individually by fine plastic mesh to prevent snails from escaping. Snails exposed to varying plant extract concentrations were observed at 24 h and 48 h. After 48 h, the plant extract suspension was decanted, snails were rinsed twice with dechlorinated tap water, transferred to a new container filled with dechlorinated tap water, and observed for an additional 24 h, constituting the recovery period. Mortality was determined by removing dead snails and considering them deceased if they showed no movement, had retracted into their shells, or were hanging out of the shells. Death confirmation included the complete opening of the operculum and no response from the head when pricked with a sharp needle. Snail mortality was recorded for both exposure and recovery periods. LC_{50} and LC_{90} values, along with their 95% confidence intervals (95% CI), were calculated through probit analysis using Minitab (Version 17.1.0, Minitab LLC, State College, PA, USA) [15]. The mortality data for snails exposed to different concentrations of plant extracts were subjected to one-way analysis of variance (ANOVA), and mean comparisons were conducted using the least significant difference (LSD) test at a significance level of $p<0.05$. Sigma Plot (version 12.5 from Systat Software Inc.) was employed to explore snail mortality tendency using polynomial linear analysis and quadratic curve. The experiment was conducted over a period of 8 months, from May to December 2022.

Result and Discussion

Molluscicidal Activity of Four Plant Extracts and Ground Powder

The study assessed the molluscicidal activities of four plant extracts against *P. canaliculata* at various concentrations (ranging from 100 to 5000 mg/L). Control groups treated with water exhibited neither behavioral symptoms nor fatalities, suggesting that factors other than plant components were not responsible for the observed changes in snail behavior and mortality. An analysis of the molluscicidal effects of the four plant extracts on *P. canaliculata* revealed a clear linear relationship between the concentrations of the plant powder and snail mortality (Fig. 3).

This observation aligns with the results reported by Chauhan and Singh [16] and EI-Din et al. [18], where higher concentrations of plant extracts led to increased mortality rates among different pest snails. Taguiling documented a similar positive correlation between the concentration of plant extracts (*Sandoricum vidalii*, *Harpulia arborea*, and *Parkia* sp.) and the mortality of *P. canaliculata* [18]. Likewise, a positive correlation between the neem seed crude extract concentrations and the mortality of *P. canaliculata* was observed [8,19]. The mortality rate was correlated positively with the extract concentrations as the mortality of snail increased with the increase of extract concentration. In an experiment on the molluscicidal effect of the golden apple snail using solvents, there is no effect on the killing of golden apple snail (GAS), and at concentrations above 2%, there is an effect of the solvent itself at concentrations of less than 1% of methanol and ethanol. There was no significant difference in GAS size between under 10 mm and 10-25 mm. Table 2 showed that the tea seed, sophora root, tobacco leaves and red spider lily root have molluscicidal effect for controlling the GAS. The result indicated the four plant extract were effective against GAS in the order of green tea seed > red spider lily root > tobacco leaves > sophora root. As shown in the Table 2, the mortality rate of the GAS increased when the treated amount of powder of green tea seed was increased from 200 to 2000 mg/kg. However, the mortality rate did not increase significantly in the case of shrubby sophora root extract. Ob-

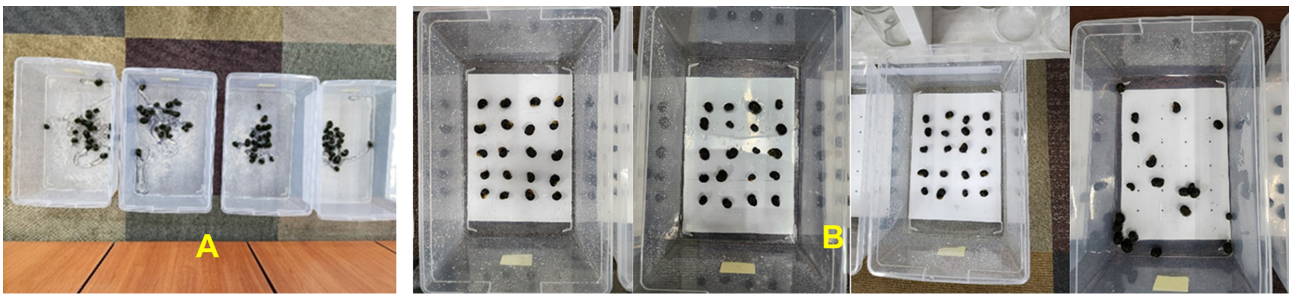

Fig. 3. Efficacy test of natural substances to suppress the golden apple snails (GAS). A, GAS being adapted to indoor conditions using the living box (22 cm × 35 cm × 15 cm); B, GAS being exposed to active dried plant powder after one day.

Table 2. Molluscicidal activity of four plant extracts on *P. canaliculata*

Concentration (mg/L)	Mortality of *P. canaliculata* (%) ±SD*			
	Green tea seed	Shrubby sophora root	Tobacco leaves	Red spider lily root
100	11.7±2.9	11.7±2.9	13.3±2.9	1.0±1.0
200	25.0±5.0	13.3±2.9	15.0±0.0	3.3±1.1
500	20.0±5.0	13.3±2.9	16.7±2.9	7.0±0.7
1,000	25.0±5.0	21.7±2.9	19.0±1.0	6.7±2.2
2,000	73.3±2.9	26.7±2.9	25.0±5.0	65.0±3.3
5,000	86.7±10.4	23.3±7.6	23.3±7.6	61.0±20.7
10,000	96.7±7.6	28.3±5.8	33.3±15.3	74.0±2.9
20,000	100±0.0	26.7±5.8	65.0±13.2	77.3±1.8
50,000	100.0±5.0	30.0±5.0	81.7±5.8	77.2±2.5

* Standard deviation (three replicates, n=20)

taining details on the extraction process for commercially available shrubby sophora extract is necessary. Consequently, this result signified that the molluscicidal properties of crude extracts from green tea seeds in the context of golden apple snail control [8]. Similarly, a direct relationship was identified between the concentrations of green tea seed crude extract and the mortality of *P. canaliculata*, as illustrated in Fig. 3. As shown in the Table 3, the LC_{50} and LC_{90} of plant extract of green tea seed, tobacco leaves shrubby sophora root and red spider lily root were 900 and 2800 mg/L, 956 and 2320 mg/L, 2162 and 5325 mg/L, 512 and 1054 mg/kg, respectively (Table 3). Likely, the LC_{50} and LC_{90} of ground powder of *C. sinensis*, *N. tabacum*, *S. flavescens* and *L. radiata* were 248 and 646 mg/L, 403 and 733 mg/L, 409 and 905 mg/L, and 493 and 1141 mg/L, respectively (Table 4). The results implicate that the molluscicidal activity of the GAS by the plant-grounded powder significantly increased to compare with the activity by the plant extract. However, there was not significantly difference in molluscicidal rate of GAS by using the plant extract and powder of red spider lily. Meanwhile, these results indicate that there are limits to extract the molluscicidal ingredients, theasaponins from green tea seeds, nicotine from tobacco leaf, mathrin from shrubby sophora root, and lycorine from red spider lily root for controlling the GAS from plants with hot water.

As a result, the grounded powder of green tea seed were more sensitive to the GAS (*P. canaliculata*) as evidenced by its lower LC_{50} (248 mg/L) and LC_{90} (646 mg/L) values, suggesting higher potency (Table 4) comparing to the aforementioned four plants, *Zingiber officinale* and *Paullinia pinnata* recorded LC_{50} and LC_{90} values of 485.48 and 767.63 mg/L, and 512.62 and 804.49 mg/L, respectively, against the snails (*P. maculata*) [13]. The findings imply that snails demonstrated greater sensitivity to *C. sinensis* compared to extracts from *N. tabacum*, *S. flavescens*, and *L. radiata*, as indicated by lower LC_{50} and LC_{90} values reflecting increased potency. Ultimately, the crude ethanol extract of *C. sinensis* proved to be the most effective, with an LC_{90} value of 341.57 mg/L at 48 hours. On the other hand, Yoon et al. [14] observed that the an-

Table 3. Summary of probit analysis on the mortality data of four different plant extracted with 70°C water against *P. canaliculata*

Plant name	Chi square	LC_{50}	Fiducial limits (mg/L)		LC_{90}	Fiducial limits (mg/L)	
			LL	UL		LL	UL
C. sinensis	186.168	900	769	1060	2800	2464	3256
N. tabacum	17.186	956	630	2192	2320	1494	5511
S. flavescens	21.013	2162	1900	2483	5325	4628	6038
L. radiata	566.938	512	462	569	1054	956	1174

*LC; Lethal concentration. LL, UL; lower and upper confidence limit. Degree of freedom (df) = 9 for each concentration of plant extracts tested.

Table 4. Summary of probit analysis on the mortality data of ground powder of four different plants against *P. canaliculata*

Plant name	Chi square	LC_{50}	Fiducial limits (mg/L)		LC_{90}	Fiducial limits (mg/L)	
			LL	UL		LL	UL
C. sinensis	646.022	248	437	1209	646	533	804
N. tabacum	38.916	403	366	446	733	668	816
S. flavescens	128.136	409	365	459	905	817	1018
L. radiata	475.126	493	439	554	1141	1032	1277

*LC; Lethal concentration. LL, UL; lower and upper confidence limit. Degree of freedom (df) = 9 for each concentration of plant extracts tested.

tifungal activity of seed extract remained unaffected even after exposure to heat at 80 and 100°C for 30 minutes or at 121°C for 15 minutes, indicating the heat stability of the seed component.

The Fig. 4 demonstrates the comparison of the molluscicidal rate of three plant extracts, the granule formulation of the plant combination ("Uleng-moa granule") and a commercial product ("Uleong-gagsi granule) at different concentrations against *P. canaliculata*. This study signifies that the organic material derived from a mixture of three plant extracts such as green tea seeds, shrubby sophora root and tobacco leaves, was highly effective on controlling golden apple snails (Fig. 4).

Likely, as shown in the Fig. 5, the molluscicidal rates of three plant grounded powders, the granule formulation of the plant grounded powder combination and a commercial product were compared different concentrations against *P. canaliculata*. This study demonstrates that the organic material derived from a mixture of four crushed plant powders is highly effective in controlling golden apple snails (Fig. 5).

On the other hand, the results of probit analysis for the mortality data of individual plant extracts on snails at LC_{50} and LC_{90}, along with confidence intervals, are summarized in Tables 3 and 4. Chi-square tests were employed for the probit analysis using the Pearson Goodness-Fit test, revealing a noteworthy molluscicidal efficacy (p<0.001) for *C. sinensis*, consistent with its LC_{90} value [22]. A similar pattern was observed with tobacco and shrubby sophora. Heterogeneity values below 1 indicated a substantial impact of all plant extracts on snail mortality, corroborating the findings of Massaguni and Latip [23]. In comparative analysis, tea seed extract exhibited the highest effectiveness, followed by tobacco, red spider lily, and shrubby sophora extracts (Table 3).

Molluscicidal Activity of Duple and Tri Green Tea Seed Combination

Combining different plant species with effective

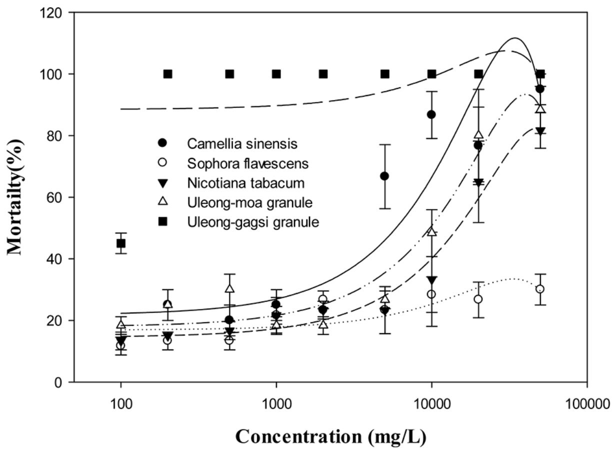

Fig. 4. Comparison of molluscicidal rate of three plant extracts, the granule formulation of the plant combination and a commercial product at different concentrations on *P. canaliculata*.

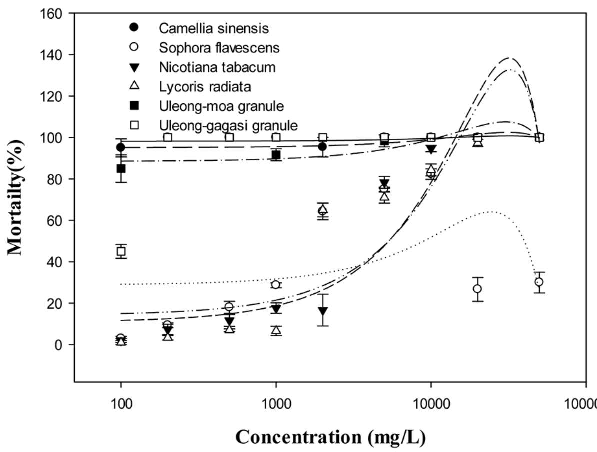

Fig. 5. Comparison of molluscicidal rate of four plant powder, the granule formulation of the plant-powder combination and a commercial product at different concentrations on *P. canaliculata*.

Table 5. Comparison of molluscicidal rate (%) of duple and tri green tea seed (*C. sinensis*) combination (grounded powder) against *P. canaliculata*

Concentration (mg/L)	Mortality of *P. canaliculata* (%)± SD*			
	Tea seeds + Shrubby sophora root	Tea seeds + Tobacco leaves	Tea seeds + Red spider lily root	Tea seeds + Shrubby sophora root + Tobacco leaves
100	16.0±0.0	23.7±3.8	29.3±4.0	44.0±0.7
200	16.3±1.5	24.3±4.0	32.7±2.5	52.7±2.4
500	17.3±2.5	34.5±5.7	41.3±5.5	62.3±5.1
1,000	26.7±4.5	35.7±8.0	44.3±11.0	64.7±1.8
2,000	29.3±4.0	43.0±4.4	55.3±9.5	66.0±7.3
5,000	46.7±5.1	51.3±10.1	70.3±5.9	100.0±0.0
10,000	60.0±6.2	70.3±6.8	80.3±3.8	100.0±0.0
20,000	75.7±5.1	100.0±0.0	100.0±0.0	100.0±0.0
50,000	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

* Standard deviation (three replicates, n=20)

molluscicidal properties is advantageous for the development of molluscicides. In the present study, among the four plant extracts evaluated individually as well as in combinations, *C. sinensis*, *N. tabacum*, *L. radiata*, *S. sophora* were found to be effective on the snails in laboratory conditions (Table 5). When these four plant extracts were tested individually, the LC_{90} values ranged from 1054 to 5325 mg/L, whereas, in their triple combinations significant reductions in the LC_{90} values (246.8 to 352.8 mg/L) were recorded (Table 6). It is evident that the triple combinations of plant extracts resulted in approximately 40% reductions in LC_{90} values based on the mortality of *P. canaliculata* as well as demonstrating the synergistic effect among these plant extracts. Therefore, combining different plant species with effective molluscicidal properties is advantageous for further reducing the lethal concentrations against snails. The molluscicidal activity of green tea seed, tobacco, sophora and red spider lily combinations of plant extracts against *P. canaliculata* is shown in Table 6. The results indicate that the combination of four plant extracts showed the average 44 % of mortality rate with the lowest LC_{50} and LC_{90} (69.4 and 289.1 mg/L, respectively) even at a lower concentration of 100 mg /L (Table 6).

Table 7 presented that the two kind of granule product by green tea seeds with three plant extracts have molluscicidal effectiveness against the golden apple snails. As shown in the Table 7, the LC_{50} and LC_{90} of organic materials made from a mixture of four plant powder ("Uleong-moa B") and four plant extracts (Uleong-moa A) were 54.7 and 107.1 mg/L, 1565.6 and 4216.4 mg/kg, respectively. The results showed that organic materials made from a mixture of four plant powders were effective in controlling the golden apple snails. The outcomes of this research align with Taguiling's findings [24], which highlighted significant variations in the effectiveness of plant extracts against GAS based on species combinations and dosages. Additionally, the differences in the molluscicidal effectiveness of plant extracts can be linked to three primary factors: species tolerance, concentrations utilized, and the presence of phytochemical constituents [25,26].

Interestingly, the study found that the mortality

Table 6. Comparison of LC values of of duple and tri green tea seed (*C. sinensis*) combination of plant powder against *P. canaliculata*

LC (mg/L) 48 h	Tea seeds + Shrubby sophora root (LL-UL)	Tea seeds + Tobacco leaves (LL-UL)	Tea seeds + Red spider lily root (LL-UL)	Tea seeds + Shrubby sophora root + Tobacco leaves (LL-UL)
LC_{50}	964.4 (850.8-1102.5)	495.9 (429.2-573.9)	317.8 (263.0-368.5)	69.4 (51.5-87.5)
LC_{90}	2309.5 (2046.7-2662.3)	1356.2 (1197.6-1570.2)	1078.3 (944.0-1263.6)	289.1 (246.8-352.8)

*LC; Lethal concentration. LL, UL; lower and upper confidence limit. Degree of freedom (df) = 9 for each concentration of plant extracts tested.

Table 7. Probit analysis on the mortality data of two type of formulation granule on *P. canaliculata*

Formulation	Chi square	LC_{50}	Fiducial limits (mg/L)		LC_{90}	Fiducial limits (mg/L)	
			LL	UL		LL	UL
Uleong-moa A[1)]	45.7	1565.6	1362.4	1808.6	4216.4	3730.0	4863.9
Uleong-moa B[2)]	377.68	54.7	89.9	31.6	107.1	87.7	135.4

*LC; Lethal concentration. LL, UL; lower and upper confidence limit. Degree of freedom (df) = 9 for each concentration of plant extracts tested
[1)]Tea seed extract (Theasaponin, 35%) + Shcubby sophora extract (Mathrin, 10%) + Kaolin (54%) + Xanthan Gum (1%).
[2)]Tea seed powder (Theasaponin, 30%) + Tobacco leaf powder (Nicotin, 10%) + Sophora root powder (Mathrin, 10%) + Red spider lily root powder (Lycorine, 10%) + Kaolin (39%) + Xanthan Gum (1%) + water (40%).

rate of GAS was maintained even at a low concentration of 0.01% in water treated with commercially available "Uleong-gagsi" granule and Uleong-moa" granule that was developed through this study, while other plant extracts and dried products showed a significant decrease in mortality rate at higher concentrations (Figs. 3 and 4).

Commercially available organic material extracts were also tested, and shrubby sophora was found to be the most effective, followed by Neem and Pyrethrum flower extract (not shown in this paper). This study also highlights the potency of green tea seeds in controlling GAS, with eight saponin glycosides identified as effective in killing the snails [27,28]. However, it was noted that the mortality rate of Shrubby sophora did not significantly increase with direct treatment with 0.1% powder or 0.1% methanol extract, which differed from the results of the extract test that showed 100% mortality rate at 0.1% treatment. This discrepancy indicates a need for more information on the extraction method of shrubby sophora extract in the commercial market. Overall, we maybe conclude that the tea seed powder is the most effective in controlling GAS, and other plant extracts with methanol extractable substances are also effective. In the case of commercially available organic material extracts, the control effect of GAS was investigated in the order of Shrubby sophora > Neem > Pyrethrum flower extract (not shown in this paper). The tea seed powder is the most effective in controlling apple snail, and for other plant extracts, it was confirmed that methanol extractable substances are effective in controlling apple snail. Interestingly, in an effort to search for natural products to control golden apple snails, we have found the green tea (*C. sinensis*) seeds is very potent to the golden apple snails (*P. canaliculata*). Even at a low concentration of 0.01% in water, commercially available 'snail brides' and green tea seed powder effectively sustain the mortality rate of Golden apple snails (GAS), whereas other plant extracts and dried products exhibit a significant decline in the mortality rate at concentrations ranging from 0.1% to 0.5% (Fig. 3). It is known that there are major eight saponin glycosides in the green tea seed. Saponins are natural compounds found in various plants, known to be used for medicinal purposes [24]. The eight saponin glycosides have been detected in the seed extract powder, they were (1) Theasaponin E7, (2) Theasaponin E1, (3) 3-O-xylosyl-(1→2)-arabinosyl-(1→3)-[galactosyl-(1→2)]-glucuronosyl-theasapogenol E16,22-di-O-acetyl-21-O-tiglic acid, (4) Theasaponin E8, (5) Assamsaponin B, (6) Theasaponin E4, (7) Theasaponin E9, and (8) Theasaponin E5 [29-34]. Especially, the theasaponins have been known to possess very strong molluscicidal activity against the *P. canaliculata*. These results suggest the theasaponins might be effective candidate component as biocontrol agent against golden apple snails. However, further investigation may be required to use these materials as commercial eco-friendly organic agricultural materials to control the golden apple snails in rice-growing environments. [35-38]. It may be necessary to examine the snails in further detail to utilize these materials as a commercial eco-friendly organic agricultural material for the purpose of controlling the apple snails (*Pomacea canaliculata*) in rice growing environment.

Conclusion

We found that the four plant extract were effective against GAS in the order of green tea seed > red spider lily root > tobacco leaves > shrubby sophora root as well as the mortality rate of the GAS increased while the green tea seed was applied from 200 to 2000 mg/kg as type of the tea seed powder. However, the mortality rate did not increase significantly in the case of shrubby sophora root extract.

In this case, the LC_{50} and LC_{90} of green tea seed (*C. sinensis*), tobacco leaves (*N. tabacum*), shrubby sophora root (*S. flavescens*) and red spider lily root (*L. radiata*) were 900 and 2800 mg/L, 956 and 2320 mg/L, 2162 and 5232 mg/L, 512 and 1054 mg/kg, respectively.

Additionally, the LC_{50} and LC_{90} of *C. sinensis*, *N. tabacum*, *S. flavescens* and *L. radiata* ground powder were 248 and 646 mg/L, 403 and 733 mg/L, 409 and 905 mg/L, and 493 and 1145 mg/L, respectively. The results implies that the green tea (*C. sinensis*) seeds exhibit remarkable potency against the golden apple snails (*P. canaliculata*). It may be necessary to develop an organic materials with a mixture of four plant powders for the purpose of preventing the escape of GAS into the ecosystem.

Note

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