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Bioactive Components of *Allium sativum* (L.) and *Tinospora crispa* (L.) as Organic Molluscicidal Agents

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Abstract

The golden apple snail *Pomacea canaliculata* (L) is one of the most pervasive pests in rice farms in the Philippines. Synthetic molluscicidal formulations are one of the most widely used pest control methods. However, this affected the non-target organisms and the environment adversely. Thus, there is a need to find a viable alternative in the form of a plant-based pesticide that is commonly available and less costly. The garlic *Allium sativum* (L.) and the makabuhay plant *Tinospora crispa* (L.) are widely known for medicinal purposes. According to studies, these plants' bioactive components can also be a potential pesticide for snails in lowland rice fields. Thus, this study sought to determine whether a singly or binary combination of these fermented plants' extracts could demonstrate the molluscicidal effect in P. *canaliculata*. The mortality rate of P. canaliculata after applying different extracts in varying concentrations within 24-hour exposure time was collected and tested using ANOVA. Among the various extracts and concentrations tested, 60 percent of the garlic and makabuhay individual plant fermented extract were the most effective, with the highest mortality recorded for golden apple snails.

Keywords

Bioactive components, Plant extract, Molluscicidal agents

INTRODUCTION

The Philippines is a rain-fed country with lowland and upland areas required for rice to grow well. Because of its rich ecology and abundant irrigation, the Philippines can expand rice yield and potentially aid the country's objective of self-sufficiency significantly (Fernandez, 2014). The Organic Agriculture Act of 2010, Republic Act No. 10068, was adopted to expand and improve organic agriculture methods in the Philippines. This will include a comprehensive program to promote community-based organic agriculture systems consisting of farmer-produced wholly organic fertilizers, pesticides, and other crop production. Also, a nationwide informational and marketing campaign to encourage their use and processing and sustainable production as a viable option (Alcala, 2011).

Organic and sustainable farming methods harmonizing with nature and not hurting other organisms have proved viable alternatives to costly agriculture systems. Farmers are already adopting these options to fertilize the soil, safeguard crops, and provide food for all (Badgley et al., 2007).

The Province of Laguna, located in the western section of the CALABARZON Region, has favorable characteristics for farming. However, several factors have been forcing the agricultural sector in previous years, including significant alterations in its landscape, land conversion, and the common problem of infestation by invasive species such as snails.

Golden apple snails *Pomacea canaliculata* (Lamarck, 1819) are considered the most invasive pest in rice farms of Southeast Asia (Prabhakaran et al., 2017). It is also regarded as one of the worst invasive species that adversely affects rice production in lowland areas in the Philippines (Lightfoot et al., 1993; Joshi & Cagauan, 2002; Picardal et al., 2018). According to the International Rice Research Institute (IRRI), it devours immaturely and develops rice plants. They severed the rice stem at its base, killing the entire plant. Irrigation channels, natural water distribution pathways, and flooding occurrences are where golden apple snails can spread. When there is no water, *P. canaliculata* can bury themselves in the earth and hibernate for six months. Snails may develop if water is reapplied to fields. P. canaliculata damages wet-seeded rice and transplanted rice up to 30 days old. After 30-40 days, the rice plant will be strong enough to survive the snail. *P. canaliculata* is a major threat to rice production and can entirely damage $1m^2$ of the field in one night if no control measures are adopted. This damage could result in a yield loss of more than 50% (IRRI, 2014).

Applying commercial and synthetic molluscicidal formulations has been widely considered as the method of choice for snail control (Prabhakaran, 2017). Pests were often treated with chemical pesticides, which could harm the environment and non-target organisms (Ali et al., 2019). For example, Bouman et al. (2002) detected dangerous pesticide residues in artesian wells near agricultural regions in numerous country sections, including some parts of Laguna on Luzon Island. The levels of Azin and butachlor in numerous wells exceeded the European Union (EU) safety limit of 0.1 g/L. Other pesticides (Carbofuran, DDT, Diazinon, Endosulfan, Endrin, MIPC, and Parathion) had maximum observed values that were likewise greater than the EU norm. In light of pesticides' negative effects on agricultural production and community health and the high cost of synthetic applications, studies have suggested that plant-derived pesticides, which are less expensive and less harmful to the environment than synthetic pesticides, should be pursued (WHO, 1986). This initiative used typical plant extracts to create organic pesticides for rice farm pests like snails.

The plant species included in this study, such as garlic and makabuhay, were identified based on the pesticide activity reported in prior studies. Garlic, *Allium sativum* (L.), has molluscicidal properties (Singh & Singh, 2008; Picardal et al., 2018; Garin et al., 2019; Lucero et al., 2020). Garlic is sometimes planted near roses or vegetables to keep insect pests away. The pungent garlic spray can be diluted to cover a larger area when used as an insect repellent. The odor could also disguise pests' scent to find their preferred host. The solution must be stronger to kill pests (Anwar et al., 2014).

Makabuhay *Tinospora crispa* (L.), on the other hand, was a locally used insecticide that has been linked to pesticide action in diverse pest populations in previous studies (Rejesus et al., 1987; Isa et al., 2013; Ahmad et al., 2016). It is a dioecious climbing vine that reaches 4 to 10 meters in height. The stems are meaty, contain distributed protuberances, and can be up to one centimeter thick. The leaves are thin, oval, 6 to 12 cm long and wide, with a truncate or heart-shaped base, smooth and shiny. Petioles can be anywhere from 3.5 to 6 centimeters long. The racemes arise from the axils of fallen leaves, solitary or in pairs, and are pale green, slender, and 10 to 20 cm long. Flower pedicels are short and pale green. The fruit is 8 millimeters long and is clustered in lengthy clusters. Studies have identified antimalarial, parasiticide, and insecticidal activities, as well as febrifuge, vulnerary, and tonic characteristics (Ahmad et al., 2016).

This study aimed to assess the molluscicidal activity of *A. sativum* and *T. crispa* plants fermented with molasses as an organic snail pesticide, individually and in binary combinations. The key measure utilized to determine the molluscicidal impact was the death rate of *P. canaliculata* in the rice field after applying several extracts (fermented garlic extract, makabuhay plant extract, and garlic-makabuhay extract) in variable concentrations within the given exposure duration.

MATERIALS AND METHODS

A. Collection and identification of plant materials and test organism

A. sativum and *T. crispa* leaves, as well as a specimen of *P. canaliculata*, were obtained in Barangay Lamot 2, Calauan, Laguna. The plants were taxonomically recognized and authenticated at the Botanical Herbarium (Buot et al., 2002) of the Museum of Natural History, University of the Philippines Los Baños, Laguna. On the other hand, the P. canaliculata sample specimen was sent to the UPLB Zoological and Wildlife Museum.

B. Preparation of fermented extract

The garlic and makabuhay plants were cleaned with running tap water to remove dirt and other contaminants. Following the fermentation process, two (2) kilograms of each raw material, the garlic cloves, and the makabuhay plants were finely chopped before plant juice extraction. Two (2) kilograms of molasses were placed in both containers and were mixed thoroughly. The mixture was stored for two weeks at room temperature. The crude extract was filtered to remove the residue. The fermented extracts were diluted in one (1) liter of water to be assayed in the snail.

C. Phytochemical Analysis

This study phase was conducted at the Laboratory Services Division of the Fertilizer and Pesticide Authority (FPA) in Diliman, Quezon City, Philippines. Proper protocols for securing permission to conduct laboratory testing were done. For the presence of the different constituents, 50 mL of the fermented extracts were subjected to qualitative phytochemical analysis. Procedures for the study were done using the Atomic Absorption Spectrometry (AAS) method for iron and the Spectrophotometric process for phosphorous, respectively.

D. Application of fermented organic pesticide

The processed extract contained a controlled mixture on a sprayer that was applied directly to seven hundred twenty (720) golden apple snails (*P. canaliculata*) under four (4) trials for 24-hour exposure. The snails were collected in a fishpond near the rice field, where test plots were prepared. The golden apple snails were put in the setup and were given time to adjust to their new surroundings before using the organic pesticide. Manual counting of dead snails was used, with each group's dead snails being counted after a thorough inspection. If a snail remains inert when probed with a needle or remains retracted within the shell, or if the color of the shell and foot changes to hazy white, it is considered dead (Joshi, 2007). The mortality of snails was recorded using an observational sheet. The summary of the methods is illustrated in Fig. 1.

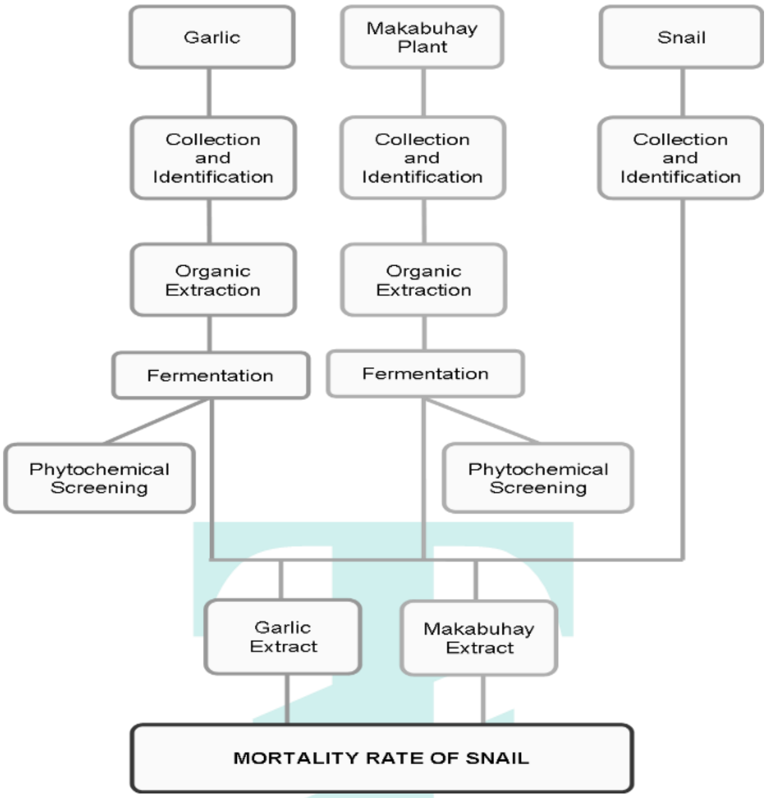

Fig. 1 Flowchart of the course of study

E. Statistical Analysis

One-way ANOVA was employed to identify statistically significant differences in group means and associated processes (Creswell, 2012). This method proved suitable for comparing three samples with various quantities (fermented A. sativum, fermented T. crispa, and Binary Combination). Similarly, post-hoc tests will be conducted to establish which samples are significantly effective as fermented organic insecticide.

RESULTS AND DISCUSSION

Table 1 The mortality rate of *P. canaliculata* in varying concentrations of fermented extracts

Trials	*A. sativum*			*T. crispa*			Binary Combination		
	20%	40%	60%	20%	40%	60%	20%	40%	60%
1st	7	9	9	9	8	10	6	6	10
2nd	5	10	10	8	9	9	5	5	10
3rd	8	9	10	9	10	10	7	9	9
4th	9	10	10	10	10	11	9	10	10
Average Death	7.25	9.50	9.75	9.00	9.25	10.00	6.75	7.50	9.75

N=720 P. canaliculata (20/Trial x 4 Trials x 3 Concentrations x 3 Fermented plant extracts)

Table 1 displays the data acquired to assess the efficacy of the various concentrations used in the small-scale field-testing test plots and the mortality rate for each concentration applied. Seven hundred twenty (720) golden apple snails (*P. canaliculata*) were grouped under four (4) trials of fermented extract assay. The average mortality for each concentration is shown in columns. Findings revealed that the best results were obtained with concentrations of 60 percent with the highest mortality rate among the tested concentrations of each extract (garlic= 9.75; makabuhay= 10.00; garlic-makabuhay= 9.75).

Previous literature also observed these results, which established that plant extracts in greater concentration resulted in higher mortality rates of various snail pests (Chauhan et al., 2011; Shalaby et al., 2011; Prabhakaran et al., 2017). Specifically, several studies pointed to garlic's molluscicidal component, such as allicin, as a factor that could support the mortality rate among snails. Allicin is a chemical created when garlic is crushed or minced before fermentation. Allicin can control other economically important pests, according to Bell et al. (2015). Apart from that, the phytochemical study found that garlic includes bioactive compounds such as triterpenes, steroids, saponins, alkaloids, tannins, and cardiac glycosides, all of which are harmful to snails in earlier studies (Singh & Singh, 2008; Picardal, 2018).

On the other hand, the makabuhay plant, although widely known for its use as a treatment for various diseases, was also suggested to have various biopesticidal activities that could support the molluscicidal activity demonstrated in this study. Dela Torre et al. (2017) used makabuhay as pediculicide ointment and identified physicochemical properties that possibly contributed to its effectiveness, such as moupinamide and physalin I. Another study found that Makabuhay aqueous extract might be a natural larvicidal product that could replace dangerous chemical pesticides. Elanchezhiyan et al. (2015) determined that the crude extract of Makabuhay has a high potential for suppressing the agricultural pest *Spodoptera litura*. Similarly, it was found in the study that the individually prepared makabuhay plant extract in all concentrations tested has induced mortality in snails tested, leaving them swollen and inactive after exposure to the treatment. Though all concentrations induced mortality in snails, the highest concentration (60%) recorded the highest mortality counts.

Looking at the three sets of prepared extract, overall, the makabuhay plant extract in single preparation was found to have recorded the highest mortality in snail specimens. On the other hand, Rao and Singh (2001) claim that synergistic activities in binary plant extract combinations are more harmful to snails than single-treatment preparations. Based on the snail mortality counts, makabuhay extract in a single preparation, given the same concentration, was more effective than the garlic-makabuhay extract binary combination in the current investigation.

An alternative technique to determine the optimal efficacy of one sample, according to McCormack et al. (2011), is to start with a higher dose, wait for a response, and then drop the amount to the lowest effective dose. Some tests may prefer this strategy, but it raises the risk of dosage-related adverse effects, and both the clinician and the patient may be hesitant to reduce the dose once a result is obtained. Overall findings demonstrate that fermented extracts, whether in single or combined preparation, were most effective based on the molluscicidal activity on *P. canaliculata* when administered in higher concentrations (60%).

Table 2 Analysis of variance (ANOVA) on the effectiveness of fermented plant extracts in varying concentrations

		Sum of Squares	df	Mean Square	F	Sig.
A. sativum	Between Groups	15.167	2	7.583	6.500	.018
	Within Groups	10.500	9	1.167		
	Total	25.667	11			
T. crispa	Between Groups	2.167	2	1.083	1.444	.286
	Within Groups	6.750	9	.750		
	Total	8.917	11			
Binary Combination	Between Groups	31.500	2	15.750	14.921	.001
	Within Groups	9.500	9	1.056		
	Total	41.000	11			

Table 2 shows the considerable variation in snail mortality across the multiple concentrations of plant extracts examined. Specifically, it shows a significant difference in the efficacy of A. sativum regarding snail mortality across the different concentrations (p=0.018). Likewise, a significant difference in the effectiveness of the binary combination of garlic-makabuhay fermented extract across the different concentrations applied in the test plots was also observed (p=0.001).

Based on the results, it could be derived that in the case of the single preparation of garlic extract pesticide and combined preparation of garlic-makabuhay extract, effectiveness varies depending on the concentration. The number of snails killed increased as the concentration of the plant extract increased. This was supported by the findings of Taquiling et al. (2015), who found a linear correlation between the number of plant extracts and the mortality rate of snails, i.e., as the concentration of plant extracts used, such as *Harpulia arborea, sandoricum vidalii*, and *Parkia* sp, increases, so does the number of recorded deaths in *P. canaliculata*.

On the other hand, the efficacy of the *T. crispa* plant extract against the *P. canaliculata* did not differ significantly across the three concentrations. This implies that various concentrations of makabuhay extract could potentially kill golden apple snails. Elanchezhiyan (2015) also confirmed the potential use of Makabuhay crude extract as a biocontrol agent against the important agricultural pest *Spodoptera litura.*

According to the Global Invasive Species Program, P. canaliculata has been one of the 100 worst exotic species, according to Joshi (2007). Without accounting for non-crop effects on human health and natural ecosystems, it has resulted in considerable losses to aquatic crops in the Country of up to USD 1200 million annually. It is an environmental concern because resource-strapped farmers use harmful and non-specific agrochemicals to manage this mollusk, further damaging ecosystems, risking farmers' health, and diminishing aquatic biodiversity (Joshi, 2007).

The findings back up Magwenya et al. (2016)'s research on the efficacy of A. sativum as a biopesticide for cotton aphid control (Aphis gossypii). With five treatments, the researchers used a Randomized Complete Block Design (RCBD). Their findings revealed that 85g chopped garlic/9litres water was just as effective as usual Dimethoate 40 EC at controlling aphids, and garlic is recommended as an aphid biocontrol agent in cotton cultivation. Likewise, Rozaq & Sofriani (2009) study stated that pikroretin, alkaloid, starch, glicosida, and soft dammar are all found in *T. crispa*.

Pikroretin's bitter components can kill pests. The efficacy of this fermented organic pesticide may help farmers eliminate *P. canaliculata* with much safer and cost-effective methods that do not harm the environment.

Table 3 Post-Hoc tests on the effectiveness of the fermented organic pesticide

Multiple Comparisons

Fermented Plant Extract		**Mean Difference**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
A. sativum						
20% -	40%	-2.250*	.764	.016	-3.98	-.52
20% -	60%	-2.500*	.764	.010	-4.23	-.77
40% -	60%	-.250	.764	.751	-1.98	1.48
T. crispa						
20% -	40%	-.250	.612	.693	-1.64	1.14
20% -	60%	-1.000	.612	.137	-2.39	.39
40% -	60%	-.750	.612	.252	-2.14	.64
Binary Combination						
20% -	40%	.750	.726	.329	-.89	2.39
20% -	60%	-3.000*	.726	.003	-4.64	-1.36
40% -	60%	-3.750*	.726	.001	-5.39	-2.11

**. The mean difference is significant at the 0.05 level.*

The effectiveness of varying concentrations of the fermented plant extract as a pesticide was compared using the post-hoc tests in table 3. The varying concentrations of fermented *A. sativum* extract revealed that 40% (MD: -2.250; Sig: 0.016) and 60% (MD: -2.500; Sig: 0.010) yielded higher efficacy than the 20% concentration. On the other hand, based on the death rate of *P. canaliculata* in different concentrations, no significant changes in the efficacy of T. crispa plant extract were found. As for the binary combination of the garlic-makabuhay plant extract, 60% (MD: -3.000; -3.750; Sig: 0.003; 0.001) concentration was more effective than the two other concentrations (20% and 40%) of fermented plant extract.

CONCLUSION

Based on the findings of the study, Garlic (*A. sativum*) and Makabuhay (*T. crispa*) fermented organic pesticides were effective for golden apple snails (*P. canaliculata*). The study was carried out by using molasses as the medium for fermentation with three varying concentrations. The efficacy was shown at sixty percent (60%) concentrations, consistent in four (4) trials left for 24 hours. It is suggested that further studies be conducted to identify the bioactive components of the fermented extract produced. Likewise, they may consider testing for binary combinations of garlic and makabuhay with other plant extracts to test for the synergistic effect. Furthermore, the use of sophisticated equipment and facilities for determining the physical and chemical properties of the fermented extract may be considered to characterize them. Lastly, using positive control for further comparison of results is also recommended.

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