

A COMPARATIVE STUDY ON THE MOLLUSCIDICIDAL EFFECT OF WHOLE LEAF  
AND LEAF EXTRACT OF *Blumea balsamifera* Linn. (sambong)  
on *Pomacea* sp. (Golden apple snail)

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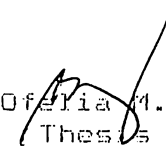
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An Undergraduate Thesis Submitted to  
The Division of Natural Sciences and Mathematics  
College of Arts and Sciences  
University of the Philippines, Manila  
Padre Faura, Manila


A Partial Fulfillment of the Requirements  
for the Degree of  
Bachelor of Science in Biology

May 1990

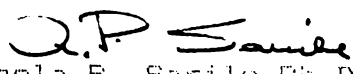
This to certify that this undergraduate thesis entitled "A COMPARATIVE STUDY ON THE MOLLUSCICIDAL EFFECT OF WHOLE LEAF AND LEAF EXTRACT OF *Blumea balsamifera* Linn. (sambong) on *Pomaceae* sp. (Golden apple snail) and submitted by Margaret-Jane S. Cabalum to fulfill part of the requirements for the degree of Bachelor of Science in Biology was approved on May 1990.

  
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## ACKNOWLEDGMENT

To the people whom I haven't given enough thanks...

**Mrs. Ofelia Cuevas**

my thesis adviser, for the guidance and patience

**Mrs. Amelia S. Asencion**

my Bio 198 teacher, for suggesting the topic to work on

**Bureau of Plant and Industry**

for the abundant supply of sambong leaves

**Bioresearch**

for the snails

**Pope Pius XII Ladies Hall**

to the **Sisters**, for the space and voluntary help in looking after the snails when I'm not around

**Faye**

for the company, during trips to different libraries

**Francis**

for the valuable help by providing the computer program for the statistical analysis

**Vanessa**

my partner in crime

**Zards**

for being a true buddy all these years and being there in my times of need

**my sisters and brothers**

whom I share this work with

**Tatay and Nanay**

for the unquestionable and never failing support

**GOD**

for never running out of surprises and blessings

thanks again and again and again.....

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## ABSTRACT

Two hundred seventy golden apple snails (*Pomacea sp.*) were divided into six treatments : I (control), II (leaf feed method) III ( 12.5% leaf extract), IV (25%), V (50%), VI (75%). After 48 hours of testing , it was observed that leaf extract method is a better snail control or molluscicide than the leaf feed method. The most effective leaf extract concentration was 75%. The 50% concentration was not able to kill a significant number of snails, but those that survived have impaired chemoreceptors and muscle fibers.

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## INTRODUCTION

*Pomacea sp.*, invariably called as Golden miracle snail, because of its rapid growth rate; Golden apple snail, because it can grow as big as an apple and golden "kuhol" because it multiplies faster than our native kuhol, is found in abundance in rice fields and irrigation canals nowadays. The Golden apple snail, is a hybrid snail produced after four years of cross breeding and selection work from two species of Ampullaria snails whose native habitat is the Amazon River Basin in South America (Pampanga Agriculture College (PAC), 1985).

An exotic tropical species, it was introduced in the Philippines in 1982 from Taiwan by enterprising individuals and gourmets. It is considered a delicacy in China (Alvarez, 1989). It was intended to be mass cultured in farmed cement tanks, ponds, and other controlled environment (Saxena, de Lara and Justo, Jr., 1987) to increase the protein source of the Filipino people.

The golden snail belongs to Class Gastropoda of the Family Pilidae. The complete taxonomy of the snail based on Morallo-Rejesus et. al. (1988) follows:

Phylum	Mollusca
Class	Gastropoda
Subclass	Prosobranchia
Order	Mesogastropoda
Superfamily	Viviparodea
Family	Pilidae (= Ampullaridae)
Genus	Pomacea (Ampullaria)

It has a golden to chestnut brown colored shell and a creamy white soft flesh. The shell is big with calcareous or corneous operculum. Unlike other species of common marine, freshwater and terrestrial snails, these snails are very active and voracious eaters resulting to their fast growth and reproduction in a comparatively short time. The adult grows as big as the fruit of the apple and multiply ten times faster than the native "kuhol". The sexes are separate or dioecious and the females are egg-laying or oviparous. A female starts to lay eggs at 75 to 90 days. The female lay their eggs in rice stems, grasses, twigs, wall of canals or dikes and any convenient substrate protruding above the water surface. A single gravid female can lay 25 to 300 night above the water or even higher. Eggs hatched after 10 to 15 days and matures for about 100 days depending upon the

temperature. They have a lifespan of two to three years (Madamba and Canaya, 1987).

These snails are quite capable of living out of the water for some time. Their foot is wide and the head carries two lip feelers and two very long main feelers. At the base of the feelers are the eyes on stems. Their well-developed lungs and gills enables them to thrive in 1-inch deep murky water. In study done by PAC, 1985 the snails feed on a wide range of aquatic and terrestrial plants and can thrive in freshwater habitats of adverse conditions because they can take oxygen from the water and the atmosphere. However, these snails are still capable of surviving for several months by burrowing into the soil.

Snail culture of the species, Golden Apple snail, as it is popularly called is being endorsed by private individuals and government agencies such as NSTA, PAC, and MAF. Indeed snail culture of this Golden "kuhol" has economic benefits. Its culture provides another source of protein and at the same time additional income for the people. These snail have 14% high content of minerals and vitamins such as Vitamin C, calcium, manganese, copper, zinc, and iodine. (Matienzo, 1984).

Golden Apple snails are also believed to be a good appetizer and it improves fitness of men. In women, they are

lactogenic when cooked as soup with some vegetables (PAC, 1985). It has also been reported that Romans ate snails to improve digestion while French believed that eating snails assist in the assimilation of alcohol in the human body (Matienzo, 1984).

Aside from being edible, the Golden "kuhol" as they are locally called, could be grinded, and used for poultry and livestock feed. They are excellent bait for fishing and could be used by duck raisers instead of the river snail "suso" which are hard to find when not in season. Golden "kuhol" can be use as feed for fish. (Javier, 1985).

With this economic benefits not to mention its wide range of nutritional value the culture of this snail boomed. Ironically, in the years following its introduction, the snails escaped or spilled from the ponds to the lowland fields via irrigation canals and other waterways which was enhanced by the torrential rains spawned by the typhoons and it became a problem rather than a means to increase the country's protein needs.

Based from press releases issued after its introduction, the Golden snail became a potential pest of rice in 1986 with an initial observed damage of 300 hectares in Luzon. On the same year, the snail damage was then reported in other provinces with undetermined number of infested areas. In

1987, the Golden snail spread to other regions and heavy damage/infestation increased tremendously especially in areas wherein direct seeding is a common practice. In the early part of 1988, the Golden snail was already reported in Mindanao and finally became a national problem.

A recent survey showed that the Golden snail is widespread and has caused significant damage to rice. An estimate of 130,945 hectares of rice field from 386 municipalities were already affected nationwide as of February 1988 and very serious in Cagayan Valley, Occidental Mindoro, Southern Tagalog, Bicol and some parts of Mindanao. In terms hectareage, Region 2 (northeastern part of Luzon) was the most devastated area with more than 80,000 hectares infested. Heavy infestation was reported in the provinces of Isabela, Kalinga Apayao, Occidental Mindoro, Batangas, Sorsogon, Iloilo and Davao del Norte. Of the 57 provinces surveyed only the provinces of Cebu and Negros Oriental showed slight cases of the Golden snail damage (Morallo-Rejesus, B., A.S. Sayaboc and R.C. Joshi, 1985). It was also observed by Morallo-Rejesus and co-workers that the most susceptible to snail damage are the young seedling stage of the rice plant. Aside from destroying newly transplanted rice, it also attacks ornamentals, corn plants (Saxena et al. 1987) and also becoming a threat to citrus and ramie

plantations (Buendia, 1988).

There are recommended strategies to control the Golden snail with due consideration to the hazards it may create on the rice ecosystem. These are biological, mechanical, cultural, and botanicals. Botanical molluscicide will be the focus of this study. A number of plants were discovered that when eaten by the snails causes their death. Snails were also observed to die when exposed or immersed in the extract of these plants.

The objectives of this study are:

1. To compare the effectivity of using fresh leaves as feeds to using leaf extract as molluscicidal agent.
2. To determine the lethal dosage that will kill 50% of the snails (LD<sub>50</sub>).

#### BACKGROUND OF THE SAMBONG PLANT

Sambong is found from northern Luzon to Palawan and Mindanao, in all or most islands and provinces. It is usually common in open grasslands at low and medium altitudes.

The plant is a coarse, tall, erect, halfwoody, strongly aromatic herb which is densely and softly hairy and 1.5 to 3 meters in height. The stems grow up to 2.5 centimeters in diameter. The leaves are elliptic- to oblong-lanceolate, 7 to 20 centimeters long, toothed at the margins, pointed or blunt at the tip, and narrowed to the short petiole, which is often

auricled or appendaged. The flowering heads are stalked, yellow, numerous, terminal, spreading or pyramidal, leafy panicle. The involucre bracts are green, narrow, and hairy. The achenes are 10-ribbed and silky.

The leaves and stem of the sambong plant contain a volatile oil (Ngai camphor oil) which consists of 1-borneol 25%, 1-camphor 75%, a little cineol, limonene, sesquiterpene, alcohol, and phenol phloracetophenon-dimethyl ether.

Sanyal and Ghose (1934) reported that the drug causes contraction of muscular fibers, mucous membranes, and other tissues.

Sambong leaves produce a juice that many Asian countries found to be effective for many disorders. The decoction is used as an antidiarrhetic and antigestralgic. It is also employed in catarrhal affections, and dropped into the eyes for chronic, purulent discharges. Internally, the decoction is both astringent and antihelmentic. It is given for worms and also in dysentery and chronic uterine discharges. The roots of the plant are used locally as a cure for colds. The Javanese and the Chinese use it as an expectorant. In the case of fever a decoction of the leaves is often given, or a decoction of the leaves and roots together (Quisumbing, 1951).

In the Philippines, the use of botanicals has been focused recently not only for insect pest but also for snail control. One of the botanical molluscicide tested was sambong - a very common and accessible plant to the farmers (Rejesus et. al. 1988). Sambong was chosen for this study mainly because it is available at the Bureau of Plant and Industry and literature shows that it has effects on the muscular system. Snails being dependent on their ventral muscular foot for locomotion, would be greatly affected in terms of their movement once exposed to the plant. Though it is desirable to compare the effects of the different botanical molluscicides, there are factors that limits the study. Limiting factors are time, space, and the abundant supply of leaves of the respective plants. The added fact that some of the plants are not easily found in Manila City, requires the study to focus on a plant that is easily accessible near the site of study.

## REVIEW OF LITERATURE

Studies on the biology of the snail, *Pomacea* sp. are few, both on the basic and applied level. Owing to the fact that Golden snail was introduced in the Philippines recently, researches and studies are not that extensive.

Applied studies involves the life span, habits, and the conditions optimal for the growth of the snails. As to the

basic aspect, it reports life cycle, hatching time, laying age of female snails (Saxena, de Lara and Husto Jr. 1987) and the amount of eggs the female snail lays at one time, dependent on their feeds. Observations of oviposition was also reported (Pagulayan, 1985).

A young Egyptian doctor by the name of Dr. Mohammed El-Sawey, was able detect in 1955, that the presence of a wild herb known as damsissa or *Ambrosia maritima* prevents the snail vector of *Schistosoma japonicum* to thrive in the rice paddies. He later observed that the herb infusion causes the mollusk to twist violently out of their shells causing their death. The local version of this wild herb should be found out and tested on Golden snail.

In the Philippines, the different management practices to control or reduce the population of Golden snail in the field fall into five groups. These are the cultural practices, the mechanical control, the biological control, the botanical control and the use of chemical pesticides.

Cultural practices of controlling the Golden snail population involve certain techniques in planting the rice seedlings and preparing the land. Delaying the planting of the rice seedlings in the field by up to 35 days makes them more resistant to attacks from the snails. The Golden snail do not eat old seedlings (Rejesus et al., 1988 and

Rodriguez, 1987). Alternate wetting and drying of the plots after transplanting reduces activity and breeding (Garcia, 1987 and Joshi, 1988).

Mechanical control involves different styles in catching or attracting the snails from the rice paddies. Stacked bamboo sticks can be used to gather the eggs (Joshi, 1988). Adults can be baited by papaya and camote leaves (Joshi and Reyes, 1988), or by placing metal screens in water inlets (Mochida, 1985). A small kangkong plot near or within the field prepared before the rice is planted will also attract the snails (Rodriguez, 1987). Another method is to line the irrigation canals with plastic sheets where the snails can lay and hatch their eggs. Later, the sheets can then be removed and the snails placed in bags (Rodriguez, 1987).

Biological control involves using the Golden snail's natural predators among which are ants, birds, spiders, rats, mites, snakes and other reptiles (Madamba and Camaya, 1987). Moreover, the snail can be eaten by poultry, ducks, pigs, and other livestock.

Several chemicals have been found to be effective in killing these snails. Among the chemicals studied from other countries are: copper sulfate, sodium pentachlorophenate (NAP-PCP), tritenmorph, acetaldehyde tetramer, BHC, thimet, endosulfan, phorate, chlopyrifos, thiodicarb and diazinon

(Garcia, 1987; Sasmal et al., 1984 and Shinha et al., 1986). Locally there are now a number of conventional pesticides available. Among these are, Erestan, Baylucido, Namekill, Brodan, Gusathion A., Thiordan and Machete (Rodriguez, 1987; Rejesus and Sayaboc, 1987). However, pesticides cost as much as P500 to P800 per hectare and proved to be hazardous to man and toxic to paddy fishes and other non-target organisms (Morallo-Rejesus, 1988; Sayaboc and Joshi, 1988).

A safe and economical alternative to the conventional pesticides are botanical pesticides. Aside from being selective agents, they are biodegradable and safe to the other organisms in the field and most especially to man. A number of plants in the Philippines have been found to possess molluscicidal properties. One is the bark of gogo or *Entada phasioloides* (Garcia et al., 1987). Others are the leaves of tubang kamisa or *Droton tigilium* tuba-tuba or *Jatropha curcass* Linn., lagtang or *Menispermum cocculus* Linn., (Agaceta, 1981). The leaves of calamansi proved to be effective in controlling the snails, too (Rejesus et. al., 1988 and Agaceta 1981). Rejesus et. al., (1988) observed that leaves of sambong or *Blumea balsamifera* Linn. were effective molluscicide. He also reported other plants that have molluscicidal properties. These are roots of tubli or *Ferris philippensis*, leaves of makabuhai or *Tinospora*

*rhizophii* and the fruit of the red pepper or *Capsicum frutescens*.

## METHODOLOGY

A total of 270 snails aged one to two months old were divided into 6 groups or treatments namely: I, II, III, IV, V, and VI. Each group contained 45 snails with 15 snails in each replicate. Each group was done in three replicates.

Treatment No. 1 served as the control. The snails were fed with camote leaves instead of sambong. Each set-up was given a fixed amount (5% of total body weight) of fresh leaves per day for two days.

In treatment No. II, the snails were fed with whole fresh leaves of sambong. The amount of leaves fed each day for two days was 5% of their total body weight (see table 1 of Results and Observations).

In treatment III, IV, V, and VI, the snails were soaked for two days in aqueous sambong leaf extract of concentrations 12.5, 25, 50 and 75 per cent, respectively (see table 2 of Results and Observations and Appendix C for preparations).

In all the treatment groups, the snails in each replicate was contained in basins. The snails in each basin were immersed in 20 cm deep of their respective concentrations of extracts. As in the case of the control and

the leaf feed group, the snails were immersed in plain stock water - tap water aged for 2-3 days (Galtsoff et. al., 1957) and fed with camote leaves and whole sambong leaves, respectively.

In the control, camote leaves were used as feeds because it is the usual and the cheapest feeds used for culture of Golden apple snail (Lacaniñao, 1985).

All the snails were starved two days prior to the testing period.

The number of deaths in each treatment group was observed and recorded after 48 hours of testing.

## RESULTS AND OBSERVATIONS

For the leaf method, the calculated amount of leaves fed per set-up each day of the two-day testing period is shown in the table below. The number of snails killed is also indicated.

Table 1. Results of the Leaf Feed Method (Treatment II)

Replicate	No. of snails	Total Body wt. (gm.)	Amount* of leaves fed (gm)	No. Killed
1	15	95	4.8	4
2	15	90	4.5	2
3	15	88.3	4.4	0

\*Amount of leaves fed = 5% of total body weight

Table 2. Snail Distribution and Results of the Leaf Extract Method

Concentration*	No. of replicates	n <sup>1</sup>	N <sup>2</sup>	No. killed
12.5%	3	15	45	4
25%	3	15	45	6
50%	3	15	45	19
75%	3	15	45	31
control	3	15	45	0

\* Dosage is in grams of leaves per liter of water

<sup>1</sup> No. of snails per replicate

<sup>2</sup> Total number of snails per treatment

After two days, the most number of snails killed was in 75% extract with 53.9% corrected mortality. The 50% concentration was not that effective in killing the snails with a 14.5% corrected mortality only. In 25% extract concentration, 0% corrected mortality was computed so as in 12.5%. The control also gave a 0% corrected mortality (see Appendix B)

In the control, it was observed that when the water is not disturbed, the snails freely go out of their shells and cling to the floor and walls of the basins. Any slight disturbance would cause them to retreat into their shells with a closure of the operculum. On the other hand, presence

of food (camote leaves) in the water would induce them out of their shells. The longest time for them to reach newly placed food was observed to be at 5 minutes (Javier,1985).

After the testing period, the basins were siphoned off murky water due to extracts and replaced with clean stock for better observation. To observe for signs of life the different parts (see Plate 3) of the snail like cephalic tentacles and labial palps were watched if extended. Usually the snail responds to presence of food by opening their operculum and slowly extending its labial palps, siphon and tentacles. To induce this reaction, camote leaves were scattered on the surface of the water.

The snails in 75% extract was observed not to respond even after an hour. Those that are considered dead had their operculum slightly open and floating. Some are observed to be starting to rot. Those that are considered alive exhibited very slow movements. They were able to open their operculum very minutely but fails to extend its parts (see Plate 5).

In 50% treatment, the snails were observed to exhibit likewise malfunctions. They were unable to fully extend their ventral foot and if extended were not able to propel the snail effectively. Those that were able to slowly move about took longer time in eating. It took them 45

minutes to start eating, which normally would only take 5 minutes.

In treatments II (leaf feed), I (12.5%), and IV(25%) the snails were not affected much to illicit a significant number of deaths. Mostly responded normally in the presence of food.

Using the  $\text{Chi}^2$  test, the effectivity of treatment II (leaf feed method) and treatment VI (75% leaf extract method) was compared. It was found that the two treatments are not equal and have different effects due to rejection of the null hypothesis (see Appendix A).

Using the bioassay statistical analysis, it was computed that the concentration that would be able to kill 50% of the snails ( $\text{LD}_{50}$ ) is 71.03% (see Appendix B).

## DISCUSSION

The harmful effect of sambong could be traced to its ability to cause the contraction of muscular fibers, mucous membranes, and other tissues (Quisumbing,1951). Since the snails' means of locomotion are their ventral muscular foot (Hickman,1973), an impairment of the "foot's" muscle fibers would greatly affect the movements of the snails,if not totally paralyze it.

A small amount of these extract would not render the snail immobile rather it would enhance its muscle tone due to

the ability of sambong to contract muscles. Constant exposure, on the other hand, to high concentrations of sambong extracts would prove harmful because it would cause the muscle fibers to be at a contracted state that eventually would render the fibers' inability to relax and contract normally.

In comparison with the leaf feed method the leaf extract is more effective in controlling the snails (see Appendix B). One possible reason for this is that a snail will consume only a small amount of leaves in two days, even when starved beforehand. When using a normal feed like camote leaves, the snails will eat only an amount approximately 3 to 5% of their body weight (PAC, 1935). In the experiment, one snail may have a weight of say 32 grams, in two days the snail consumes 1.6 grams of leaves. The volatile oil in the 1.6 grams of leaves is much much less than the 12.5% extract in the experiment where the snails were observed to be normal (see Table 4).

In the actual field this becomes very ineffective since leaf feed method provides a choice for the snails to be exposed or not to the harmful oils of sambong. In the rice field, there is strong possibility that snails would turn to rice seedlings for source of food. In contrast, leaf extract method exposes the snail to a larger concentration of the oils. Also, the oils would pass directly to the blood stream

through the gills, and a large amount can also be absorbed via their epidermis. This is because the snail's circulatory system is open and is completed through sinuses and lacunae, although definite vessels enter and leave the heart. The blood passes through the gills (Hyman, 1967).

In treatment V (50%), the concentration could not be conclusively considered ineffective because those that survived exhibited very slow movements and delayed detection of food.

The snails have microscopic eyes at the base of their feelers, but these function merely to detect light (Hickman, 1973). However, the snail has a paired osphradium at the base of the siphon (see Plate 3). The osphradium which is clothed with a tall epithelium in which may be distinguished some or all of the following cell types: ciliated supporting cells, nonciliated supporting cells, neurosensory cells, mucocytes, other gland cells and pigment containing cells. Osphradia are generally believed to be chemoreceptors that test the composition of the incoming current and the sediments that it contains (Hyman, 1967). Conclusively, the snails in 50% extract based on observations, have impaired osphradia.

However, the snails treated with the 50% extract still had the ability to move upright and cling to walls of the

basin (see Plate 4) . This indicates that their statocysts are still intact. Statocysts are spherical or oval vesicles with fluid filled lumens that contain a single large statolith of few to many smaller bodies called statoconia. The statocysts function to maintain equilibrium (Hickman,1973). Removal of the left statocysts results in rolling on the axis to the right side. Removal of the right statocyst results in rolling to the left side. Swimming is disorganized by removal of both (Hickman,1973).

Few surviving snails in the 75% extract exhibited abnormal balance: floating underside up, inability to cling to surfaces of basin (see plate 5). Such movements indicates impairment of statocysts. The feeble movements may be caused by the action of the extract on the muscle fibers of the ventral muscular foot.

The disadvantages of using leaf extract method are: 1) it is difficult to estimate an exact dose in a vast rice field and 2) very high concentrations may have effects on rice seedlings. Determination of the latter needs further investigation.

#### SUMMARY AND CONCLUSION

The leaf extract method is more effective molluscicide against the Golden apple snail (*Pomacea sp.*) than the leaf feed method. It was found out that the 75% concentration of

the leaf extract was the most effective. The 50% concentration was not enough to kill a significant number of snails, but this treatment impaired the chemoreceptors and slowed the movement of the snails. Using Bio-assay statistical analysis, (see Appendix B). It is determined that the lethal dose that will kill 50% of the snails is 71.03% concentration.

#### RECOMMENDATION

1. Further study to discover more plants that contain molluscicidal agents.
2. More investigations to determine if plant extracts used as molluscicides have any adverse effect on the rice crop.
3. More field studies for the actual field application of the leaf extract method.

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PLATES

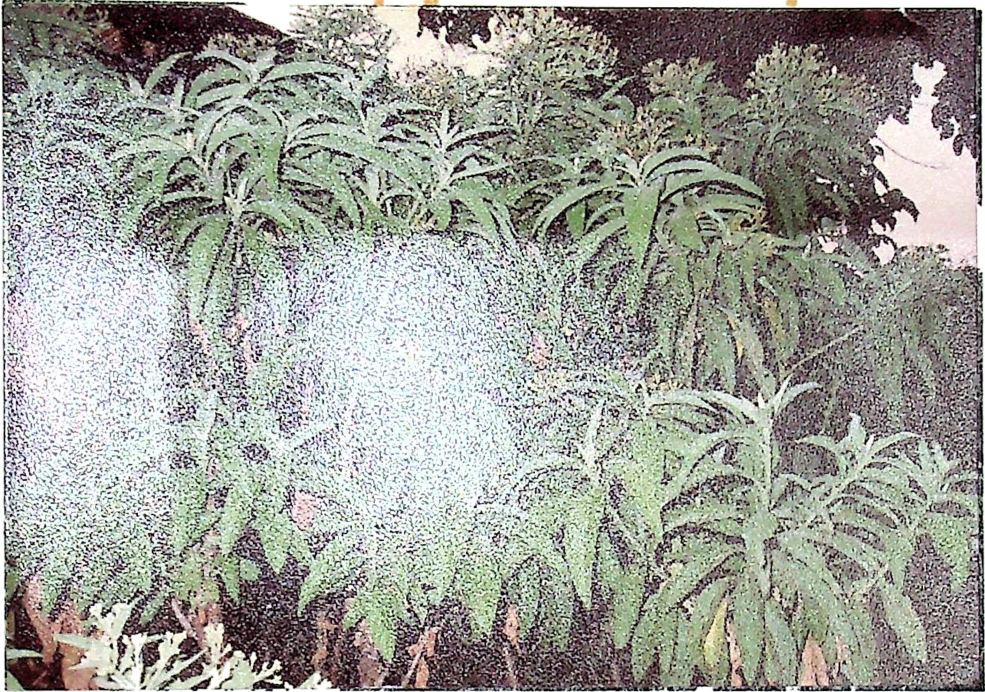


Plate 1. The sambong plant (*Blumes balsamifera*)



Plate 2. The covered basins used for the set-ups.

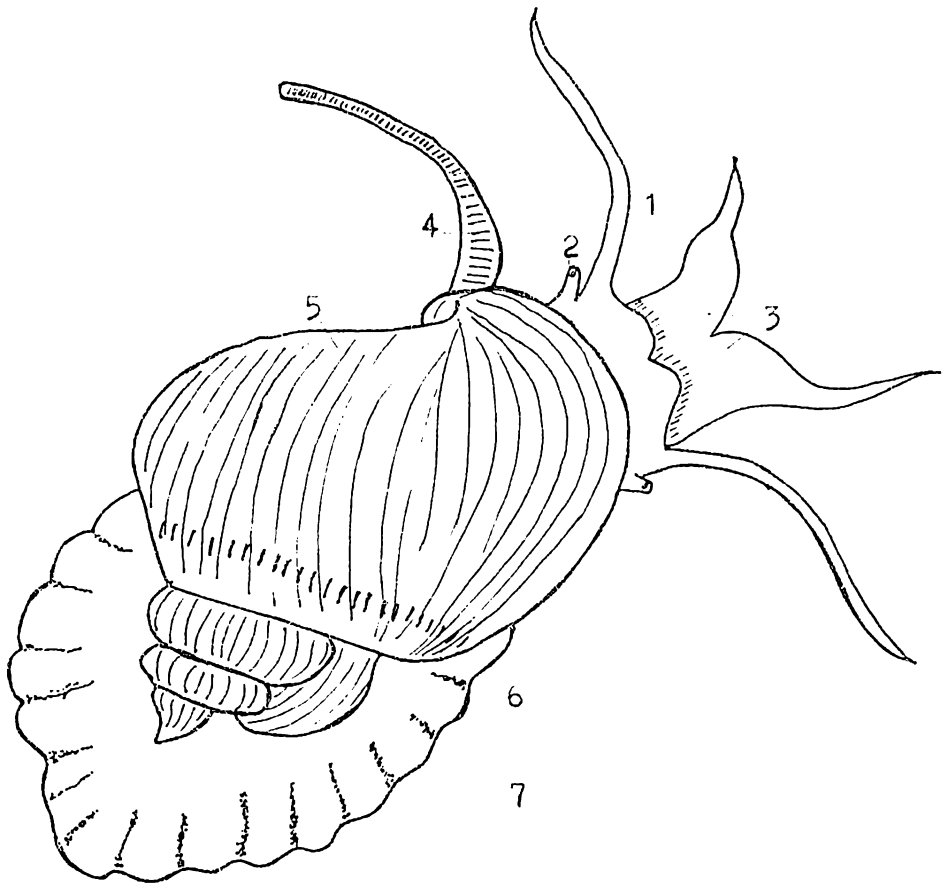


Plate 3. Diagram of *Pomaceae* sp.  
1. Cephalic tentacle, 2. ommatophore,  
3. labial palps, 4. siphon, 5. body whorl  
6. operculum, 7. ventral muscular foot



Plate 4. The Effect of 50% leaf extract method  
(in basin replaced with clean water  
with camote leaves as enticement)



Plate 5. Effect of 75% leaf extract method  
(in basin replaced with clean water  
with camote leaves as enticement)



Plate 6. Effect of Whole leaf feed method  
(in basin replaced with clean water;  
showing two deaths, d)

## Appendix B

### TABLES

Table 3. Number of Snails Killed after 48 hours.

Treatment	1	Trial* 2	3	Average per trial
control	0	0	0	0
leaf feed	4	2	0	2
12.5%	1	3	0	1.3
25%	2	3	3	2.6
50%	7	5	7	6.3
75%	10	10	11	10.3

\* Each trial tested 15 snails

Table 4. Percentage Mortality of the Snails

Treatment	Total Snails treated	% Mortality
control	45	0.0
leaf feed	45	3.0
12.5 %	45	0.0
25%	45	0.0
50%	45	14.5
75%	45	54

## Appendix C

### STATISTICAL ANALYSIS

Chi<sup>2</sup> test to compare the effectiveness of 0% extract (leaf feed) and VI (75% extract).

Table 3. Contingency Table

Treatment	No. of snails killed	No. of survivors	Total number of snails
leaf feed	6 (18.5)	39 (26.5)	45
75%	31 (18.5)	14 (26.5)	45
Total	37	53	90

Note: Figures in parenthesis represent the expected frequencies (E)

$$E = \frac{\text{column total} \times \text{row total}}{\text{grand total}}$$

H<sub>0</sub> : P = P Treatment II is as effective as Treatment VI

H<sub>1</sub> : P ≠ P Treatment II and VI have different effects

Level of significance 0.05

Test Criterion

$$\chi^2 = \frac{(O - E)^2}{E}$$

Critical or rejection region  $\chi^2$  or equal to 3.841

Computation:

$O - E$	$(O - E)^2/E$
6 - 18.5 = -12.5	$(-12.5)^2/18.5 = 8.45$
39 - 26.5 = 12.5	$(12.5)^2/26.5 = 5.90$
31 - 18.5 = 12.5	$(12.5)^2/18.5 = 8.45$
14 - 26.5 = -12.5	$(-12.5)^2/26.5 = 5.90$
0.0	28.70

Conclusion: Reject  $H_0$ . There is a significantly higher number of deaths in Treatment VI (75% leaf extract method) than Treatment II (leaf feed method).

Table 6. Bioassay of the different concentrations

Dose (%)	Mortality corrected	Total treated	No. tested
12.5	0.0	45	4
25.0	0.0	45	6
50.0	14.5	45	
75.0	53.7	45	31

Mortality in the control: 0%

Number of iterations: 12

LD<sub>50</sub> = 71.0296

Level of Confidence: .95

Range: 30.09976 LC 103.130100

Note:

Percent corrected mortality were calculated using Abbott's formula (1925). Probit analysis was done thru available computer. However, manual computation of LD<sub>50</sub> can be done by following 29 basic steps as modified from Finney 1962 (Heinrichs et al., 1981).

## Appendix D

### Preparation of the different leaf extracts

- a) Making the 75% leaf extract  
75 grams fresh leaves per 1000 ml of water. Boil for 15 minutes.
- b) Making the 50% leaf extract  
50 grams fresh leaves per 1000 ml of water. Boil for 15 minutes.
- c) Making the 25% leaf extract  
25 grams fresh leaves per 1000 ml of water. Boil for 15 minutes.
- d) Making the 12.5% leaf extract  
12.5 grams fresh leaves per 1000 ml of water. Boil for 15 minutes.